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ORIGINAL ARTICLE

Plasmid profiles and antibiotic susceptibility patterns of bacteria isolated from abattoirs wastewater within Ilorin, Kwara, Nigeria

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ABSTRACT

Background and Objectives: Waste water from abattoirs could harbour bacteria some of which are pathogenic. Therefore, this study aimed to assess the quality of wastewater from some abattoirs in Ilorin, Nigeria.

Materials and Methods: The counts of viable bacteria, total coliform, faecal coliform, enterococci, *S. aureus*, *P. aeruginosa* and *Salmonella/Shigella* spp. of the wastewater was determined using selective media. The sanitary condition appraisal, antibiotic susceptibility test and plasmid profile of the isolates were assessed using standard methods.

Results: The highest count of viable bacteria and total coliform obtained were 9.0×10^7 and 3.0×10^7 CFU/ml respectively. Faecal coliform and enterococcal count had the same highest value of 3.0×10^5 CFU/ml. The highest count of pathogenic bacteria: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* spp. were 2.5×10^8, 1.9×10^7 and 3.0×10^4 CFU/ml respectively. The abattoirs sanitary scores ranged from 28.6-57.1%. The isolates showed multiple antibiotic resistance (MAR) index ranging from 0.5-1.0. Plasmid curing with 0.1 mg/ml of acridine orange solution led to reduction in the MAR index of most of the Gram negative bacteria. *Pseudomonas stutzeri* was susceptible to all the antibiotics while *Proteus vulgaris* was resistant to all the antibiotics after curing. Most of the Gram negative bacteria isolated belong to the families *Enterobacteriaceae* and *Pseudomonadaceae* while the Gram positive bacteria belong to the families *Staphylococcaceae*, *Enterococcaceae* and *Streptococcaceae*.

Conclusion: It was concluded from this study that wastewaters from the abattoirs were contaminated by bacteria with high MAR index. Most of these bacteria borne their antibiotic resistant factors in their plasmid.

Keywords: Bacteria; Plasmid; Antibiotic resistance; Abattoirs; Wastewaters

INTRODUCTION

Abattoir is a specialized facility where animals are slaughtered and processed to get meat and its products for consumption as food especially by humans (1). Waste products from the abattoirs such as bone meal and blood meal are used in animal feed production while farm yard manures are used as organic manure to improve soil fertility.

In Nigeria, the major animals slaughtered in an abattoir are the cattle, sheep, goat, camel and pig. The Abattoir provides employment opportunities for the teeming population. The number of non-standard abattoirs far outnumbered the few standard ones. Some of the standard abattoirs are found in Lagos, Borno and Nasarawa. Most abattoirs in Nigeria are rarely inspected by veterinarians (2).

Abattoirs uses large quantities of water for washing meat and cleaning processes (3). It has been reported that there are no facilities for treating waste generated in most Nigerian abattoirs; these wastes are either disposed in open dumps or are discharged

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into nearby streams, hence constituting a menace in the environment (4).

Wastes from the abattoirs contain flesh, fur, blood, manure, feather, bones, undigested feed and process water which has a lot of organic constituents (5). The animal blood is released untreated into the flowing stream while the consumable parts of the slaughtered animals are washed directly in the flowing water. The total amount of waste produced per animal slaughtered is approximately 35% of its weight (6).

Bacteriological examination of waste water is a powerful tool in order to foreclose the presence of microorganisms such as pathogenic bacteria that might constitute a health hazard. Microorganisms commonly used as indicators of wastewater quality include: coliforms, faecal streptococci, *Clostridium perfringens* and *Pseudomonas aeruginosa* (7, 8). Contamination of river body and land with abattoir wastes could constitute a significant environmental and health hazard. Abattoir effluents could increase levels of nitrogen, phosphorus, and total solids in receiving water bodies considerably (9).

Abattoir effluent contains several millions of aerobic bacteria and faecal coliform. In addition, there may presence of pathogenic bacteria such as *Salmonella, Streptococcus, Staphylococcus, Escherichia coli* (including serotype O157:H7), *Pseudomonas, Campylobacter jejuni* and *Shigella*. These pathogens may threaten public health by migrating into ground or surface water, or picked up by vectors like animals, birds and arthropods which can help with their dissemination (10).

This study will help to provide information on the bacteriological quality of wastewater being discharged into the body of water from the abattoirs in Ilorin, Kwara, Nigeria. The objectives of this research were to determine the viable bacteria, total coliform, faecal coliform and enterococcal counts of wastewater from some abattoirs in Ilorin, Kwara, Nigeria; determine the counts of some pathogenic bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella/Shigella* species in the abattoir wastewater; characterize and identify the bacterial isolates; determine the antibiotic susceptibility pattern of the bacterial isolates; determine the effect of plasmid curing on the resistance pattern of the bacterial isolates; and proffer recommendation on ways of improving the environmental health at the abattoirs.

MATERIALS AND METHODS

Collection of waste water samples at the abattoirs. Water samples were collected in the month of January and March 2018 (2 sampling periods). Two wastewater samples were collected at each abattoir during each sampling periods. A total of 4 different abattoirs were used in this study. Hence, for the 2 sampling periods, a total of 16 wastewater samples were collected from the abattoirs. These abattoirs were: SA_1, Abubakar Saraki Olusola abattoir (small drain); SA_2, Abubakar Saraki Olusola abattoir (main drain); SJ_1, Ojatuntun abattoir (near point); SJ_2, Ojatuntun abattoir (far point, 50 m from SJ_1); SM_1, Mandate Ultramodern market abattoir, (Near point); SM_2, Mandate Ultramodern market abattoir (far point, 50 m away from SM_1); SK_1, KAS ventures, Balogun abattoir, Oloje (First drain); and SK_2, KAS ventures, Balogun abattoir, Oloje (Second drain).

The samples were collected into sterile bottles between 8 to 10 am in the morning. The samples were taken aseptically and were quickly transported in ice chest to the laboratory for analysis (3).

Sanitary survey of the abattoirs. The sanitary survey of the abattoirs was done based on these parameters: presence of potable water in the abattoir, neatness of the floor, floor surface made of concrete, presence of good drainage, skinning of animals not done on bare floor, slaughtering done near the drainage and presence of covered waste disposal bins. Each abattoir was scored either yes or no for each parameter. Then, the sanitary score was obtained for each abattoir by dividing the number of yes scores by the total number of parameters assessed and multiplied the result by one hundred (2).

Determination of bacterial counts of waste water from the abattoirs. The wastewater sample was shaken and serially diluted up to 10^{-6} dilution. Determination of the bacterial count was done using pour plate method. Aliquot (0.1 ml) was taken from each of 10^{-4}, 10^{-5} and 10^{-6} dilutions using different sterile pipettes into the different sterile Petri dishes. Sterile molten nutrient agar was then added to each plate, swirled and allowed to solidify. The plates were incubated at 37°C for 24-48 hours. At the end of the incubation period, the number of bacterial colonies were counted and expressed in CFU/ml (10).

Enumeration of coliforms and enterococci. The total and faecal coliforms were isolated with MacConkey agar and Eosin methylene blue agar respectively. Slanetz and Bartley agar medium was used to isolate enterococci. Aliquot from different dilutions (0.1 ml) was spread on the surface of the different selective media. Coliforms gave red to pink colonies on the MacConkey agar while faecal coliform produced colonies with greenish metallic sheen (10). Colonies which were red or maroon in colour were presumed as enterococci (11).

Isolation and enumeration of pathogenic bacteria. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella/Shigella* spp. were tentatively isolated using Cetrimide agar, Mannitol salt agar (MSA) and Xylose lysine deoxycholate agar (XLD) respectively. *P. aeruginosa* produced green colonies on cetrimide agar; *S. aureus* developed yellow colonies on MSA; and *Salmonella/Shigella* were red with or without black centre on XLD (12-14).

Purification and preservation of isolates. Distinct colonies from the selective media were subcultured on sterile nutrient agar (NA) plates. The pure cultures were then inoculated into sterile NA slants, incubated and stored at 4°C in a refrigerator (15).

Characterization and identification of bacterial isolates. The characterization of the bacterial isolates was based on colonial and cellular morphology as well as biochemical characteristics (15). The Gram negative bacilli were identified using Oxoid Microbact identification kit 24E while the Gram positive bacteria were identified using ABIS advanced bacterial identification software.

Antibiotic susceptibility test. McFarland standard (0.5) was prepared according to the defined protocol (16). Then, 24 hours old culture of the isolate was inoculated into sterile normal saline and its turbidity matched with the 0.5 McFarland standard. The standardized culture was spread on the surface of sterile set plate of Mueller Hinton agar using sterile swab stick. Antibiotic discs were then placed on the agar and pressed firmly on the surface for efficient activity. The multiple antibiotics employed were manufactured by rapid labs of which set CM-12-NR100 and CM-12-8PR100 was used for the Gram-negative and Gram positive bacteria respectively. However, the vancomycin 30 µg was single disc made by Oxoid. The plates were subsequently incubated for 18-24 hrs before the diameter of zone of inhibition was taken in millimetre using a ruler. The multiple antibiotic discs used and their concentrations were: ceftazidime 30 µg, cefuroxime 30 µg, gentamicin 10 µg, cefixime 5 µg, ofloxacin 5 µg, amoxycillin/clavulanate 30 µg, ciprofloxacin 5 µg, nitrofurantoin 300 µg, ceftriaxone 30 µg, erythromycin 5 µg and cloxacillin 5 µg.

Multiple antibiotic resistance (MAR) index. MAR index was calculated for each isolate; it is the ratio of number of antibiotics to which an organism was resistant to in comparison to the total number of antibiotics to which it was exposed (17).

Plasmid curing of the bacterial isolates. Acridine orange solution (0.1% w/v) was prepared by adding 0.1 g of the acridine orange powder into 100 ml of sterile distilled water. This mixture was subsequently filtered through Millipore filter of 0.45 µm. This solution is equivalent to 1 mg/ml of acridine orange. One millilitre of the acridine orange solution was then dispensed aseptically to each bottle containing 9 ml of sterile nutrient broth. The concentration of acridine orange in each 10 ml broth-acridine solution is 0.1 mg/ml. The bacterial isolates were inoculated into the acridine orange broth and rotated on a shaker at 120 rpm. After 24-48 hours of incubation at 37°C, the organisms were freed from the chemical (acridine orange) by subculturing on sterile nutrient agar slants and incubated at 37°C. Antibiotic susceptibility test was done again for the bacterial isolates that showed resistance to antibiotics prior to the curing (those with MAR index $\geq$0.8) and the changes in resistance pattern was noted. The bacteria that displayed clear changes in resistance pattern after curing were regarded as bearing their resistance factor in the plasmid (18).

Statistical analysis. IBM-SPSS version 20.0 was used to carryout the statistical analysis. Duncan's multiple range test at $\alpha = 0.05$ was used to separate the means and performed one way analysis of variance.

RESULTS

Bacteriological counts of abattoir wastewater. The wastewaters were collected from 2 points

at some distance apart at each abattoir during each sampling period. The samples were collected for 2 sampling periods: January and March 2018. The bacterial count, total coliform, faecal coliform, and enterococcal count for January 2018 sampling period ranged from 6.5×10^6 - 9.0×10^7, 1.0×10^5 - 3.0×10^7, 0 - 3.0×10^5, and 1.0×10^4 - 3.0×10^5 CFU/ml respectively. In addition, the bacterial count, total coliform, faecal coliform, and enterococcal count for March 2018 sampling period ranged from 1.0×10^5 - 1.49×10^7, 1.6×10^6 - 2.8×10^7, 1.0×10^4 - 3.0×10^5, and 0 - 3.0×10^5 CFU/ml respectively (Table 1). The counts of bacteria were not static at each abattoir for each sampling period.

Counts of pathogenic bacteria of the abattoir wastewater. The count of *S. aureus, P. aeruginosa* and *Salmonella* spp. obtained in the month of January 2018 sampling period ranged from 0 to 2.5×10^8, 0 to 4.0×10^6 and 0 to 3.4×10^4 CFU/ml respectively. Furthermore, in the month of March 2018 sampling period, the count of *S. aureus* and *P. aeruginosa* ranged from 0 to 3.0×10^6, and 0 to 1.9×10^7 CFU/ml respectively. *Salmonella* was not isolated from any of the sample in this period (Table 2).

Sanitary survey of the abattoirs. The sanitary scores of the abattoirs were calculated based on some sanitary parameters shown in Table 3. It ranged from 28.6 to 57.1%. The least sanitary score was obtained at Abubakar Saraki Olusola abattoir while the highest were obtained at KAS ventures, Balogun abattoir, Oloje and Ultramodern market abattoir (Table 3). The bacterial loads of the abattoirs were reflection of their sanitary scores. Abattoirs with lower sanitary score had higher bacterial loads.

Characterization and identification of bacterial isolates. The following Gram negative bacteria were identified from the abattoir wastewaters: *Proteus mirabilis, Escherichia coli, Proteus vulgaris, Pseudomonas asymbiotica, Burkholderia cepacia, Klebsiella ozaenae, Erwinia agglomerans, Citrobacter youngae, Citrobacter freundii, Pseudomonas fluorescens, Pasteurella multocida, Burkholderia pseudomallei, Empedobacter brevis, Stenotrophomonas maltophila, Pseudomonas stutzeri* and *Photorhabdus luminescens.* In addition, the Gram positive bacteria isolated were: *Streptococcus hypovaginalis, Enterococcus devriesie, Streptococcus minor, Enterococcus faecium, Aerococcus viridans, Vagococcus lutrae, Streptococcus porci, Staphylococcus cohnii, Staphylococcus aureus, Staphylococcus simulans, Staphylococcus succinus, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.*

Antibiotic susceptibility pattern of bacterial isolates. All the Gram negative bacterial isolates were

Table 1. Counts of bacteria and faecal indicators in the wastewater from the abattoirs in January (J) and March (M) 2018

Sampling points	Count (CFU/ml)							
	BC × 10^5		TC × 10^5		FC × 10^3		EC × 10^4	
	J	M	J	M	J	M	J	M
SA_1	$900^f \pm 40$	$62^c \pm 2$	$300^e \pm 10$	$182^f \pm 5$	$11^b \pm 1$	$40^b \pm 3$	$1^a \pm 0$	$30^d \pm 0$
SA_2	$300^d \pm 30$	$55^{bc} \pm 5$	$1^a \pm 0$	$173^e \pm 4$	$300^d \pm 10$	$250^f \pm 10$	$6^b \pm 0$	$30^d \pm 3$
SJ_1	$170^c \pm 10$	$149^f \pm 10$	$31^b \pm 4$	$280^g \pm 10$	$0^a \pm 0$	$55^c \pm 3$	$2.2^a \pm 0$	$29^d \pm 2$
SJ_2	$600^e \pm 15$	$138^e \pm 8$	$26^b \pm 2$	$105^d \pm 5$	$0^a \pm 0$	$300^g \pm 5$	$2^a \pm 0$	$30^d \pm 2$
SM_1	$125^b \pm 5$	$1^a \pm 0$	$26^b \pm 3$	$16^a \pm 0$	$2^a \pm 0$	$10^a \pm 0$	$15^d \pm 1$	$20^c \pm 2$
SM_2	$300^d \pm 10$	$48^b \pm 3$	$70^c \pm 5$	$28^b \pm 2$	$50^c \pm 3$	$39^b \pm 3$	30e ± 3	$12^b \pm 1$
SK_1	$89^a \pm 5$	$84^d \pm 4$	$106^d \pm 6$	$83^c \pm 3$	$0^a \pm 0$	$200^e \pm 5$	$9.7^c \pm 0$	$0^a \pm 0$
SK_2	$65^a \pm 5$	$58^c \pm 4$	$103^d \pm 5$	$79^c \pm 3$	$0^a \pm 0$	$100^d \pm 3$	$30^e \pm 2$	$0^a \pm 0$

SA_1 =Abubakar Saraki Olusola abattoir (small drain); SA_2 =Abubakar Saraki Olusola abattoir (main drain); SJ_1 = Ojatuntun abattoir (near point); SJ_2 = Ojatuntun abattoir (far point, 50 m from SJ_1); SM_1 = Mandate Ultramodern market abattoir (Near point); SM_2 = Mandate Ultramodern market abattoir (far point, 50 m from SM_1); SK_1 = KAS ventures, Balogun abattoir, Oloje (First drain); SK_2= KAS ventures, Balogun abattoir, Oloje (Second drain); J= January 2018 sampling period; M= March 2018 sampling period; BC= Bacterial count; TC= Total coliform; FC= Faecal coliform; EC= Enterococcal count

Means in the same column with different superscript are significantly different at $p<0.05$

Table 2. Counts of pathogenic bacteria in the wastewater from the abattoirs in January (J) and March (M) 2018

Sampling points	Count (CFU/ml)					
	S. aureus × 10^5		*P. aeruginosa* × 10^5		*Salmonella* spp. × 10^4	
	J	M	J	M	J	M
SA_1	$0^a \pm 0$	$28^d \pm 2$	$0^a \pm 0$	$61^d \pm 5$	$3^b \pm 0$	$0^a \pm 0$
SA_2	$0^a \pm 0$	$7.9^b \pm 0$	$0^a \pm 0$	$190^f \pm 10$	$0^a \pm 0$	$0^a \pm 0$
SJ_1	$26^c \pm 2$	$30^d \pm 2$	$0^a \pm 0$	$27^b \pm 2$	$0^a \pm 0$	$0^a \pm 0$
SJ_2	$2500^d \pm 10$	$0^a \pm 0$	$6^c \pm 1$	$40^c \pm 3$	$0^a \pm 0$	$0^a \pm 0$
SM_1	$16^b \pm 2$	$22^c \pm 2$	$4^{bc} \pm 0$	$190^f \pm 5$	$0^a \pm 0$	$0^a \pm 0$
SM_2	$6.5^a \pm 0$	$30^d \pm 3$	$2ab \pm 0$	$140^e \pm 5$	$0^a \pm 0$	$0^a \pm 0$
SK_1	$0^a \pm 0$	$20^c \pm 2$	$25^d \pm 2$	$0^a \pm 0$	$0^a \pm 0$	$0^a \pm 0$
SK_2	$0^a \pm 0$	$10^b \pm 0$	$40^e \pm 3$	$60^d \pm 3$	$0^a \pm 0$	$0^a \pm 0$

SA_1=Abubakar Saraki Olusola abattoir (small drain); SA_2=Abubakar Saraki Olusola abattoir (main drain); SJ_1= Ojatuntun abattoir (near point); SJ_2= Ojatuntun abattoir (far point, 50 m from SJ_1); SM_1= Mandate Ultramodern market abattoir (Near point); SM_2= Mandate Ultramodern market abattoir (far point, 10 m from SM_1); SK_1= KAS ventures, Balogun abattoir, Oloje (First drain); SK_2= KAS ventures, Balogun abattoir, Oloje (Second drain); J= January 2018 sampling period; M= March 2018 sampling period; BC= Bacterial count; TC= Total coliform; FC= Faecal coliform; EC= Enterococcal count
Means in the same column with different superscript are significantly different at $p<0.05$

Table 3. Sanitary survey and appraisal of the abattoirs

Sampling points	Presence of potable water	Clean floor	Floor surface made of concrete	Good drainage	Skinning of animals not done on bare floor	Slaughtering done near the drainage	Presence of covered waste disposal bin	Sanitary score (%)
SA	Yes	No	Yes	No	No	No	No	28.6
SJ	Yes	No	Yes	No	No	Yes	No	42.9
SM	No	Yes	Yes	Yes	No	Yes	No	57.1
SK	Yes	Yes	Yes	No	Yes	No	No	57.1

SA= Abubakar Saraki Olusola abattoir; SJ= Ojatuntun abattoir; SM= Mandate Ultramodern market abattoir; SK= KAS ventures, Balogun abattoir, Oloje.

resistant to cefuroxime and augmentin. This was followed by *K. ozaenae* and *Erwinia agglomerans* that were only susceptible to ceftazidime and cefuroxime respectively. Ofloxacin, gentamicin, and ciprofloxacin have susceptibility effects on 42.9, 38.1 and 28.6% of the Gram negative bacterial isolates respectively (Table 4). All the Gram positive cocci were resistant to ceftazidime, erythromycin, augmentin, and cloxicillin. Two-third of the Gram positive cocci were vancomycin resistant. The MAR index of all the isolates ranged from 0.5 to 1.0 (Table 5).

Plasmid curing of resistant Gram negative bacterial isolates. Table 5 showed the effect of plasmid curing on the antibiotic resistance patterns of the isolates. *Proteus vulgaris* still remained resistant to all the antibiotics while *Pseudomonas stutzeri* was inhibited by all the antibiotics to different extent. The MAR index of all the isolates changed when compared with the MAR index before plasmid curing (Table 6).

DISCUSSION

Some of the bacteria isolated from the wastewater in this study are known to be pathogenic. Their presence may pose great danger to the environment, especially if the effluents are discharged into the rivers without adequate treatment. This can have considerable negative effects on the quality of the receiving water bodies (19).

Table 4. Antibiotic susceptibility patterns and MAR index of Gram negative bacterial isolates

Bacterial isolates	Diameter of zone of inhibition (mm)								MAR
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	index
*Proteus mirabilis**	R	R	R	R	15	R	R	R	0.9
*Escherichia coli** n=1	R	R	R	R	R	R	R	R	1.0
*Proteus vulgaris**	R	R	R	R	R	R	R	R	1.0
*Pseudomonas asymbiotica**	R	R	R	R	R	R	R	R	1.0
Burkholderia cepacia	R	R	R	R	30	R	R	R	0.8
Klebsiella ozaenae	21	R	13	R	R	R	R	R	0.8
Erwinia agglomerans n=1	R	12	22	R	14	R	R	R	0.7
Citrobacter youngae	R	R	20	R	30	R	R	30	0.6
Escherichia coli n=2	R	R	R	R	30	R	R	30	0.8
*Escherichia coli** n=3	R	R	12	R	R	R	R	R	0.9
*Citrobacter freundii**	R	R	R	R	20	R	R	R	0.9
Escherichia coli n=4	R	R	R	R	27	R	R	34	0.8
Myroides odoratus	R	R	22	R	29	R	26	30	0.5
*Pseudomonas fluorescens**	R	R	10	R	R	R	R	R	0.9
Pasteurella multocida	R	R	24	R	R	R	21	R	0.8
Burkholdera pseudomallei	R	R	16	R	23	R	R	27	0.6
*Empedobacter brevis**	R	R	R	R	R	R	R	R	1.0
*Erwinia agglomerans** n=2	R	R	R	R	R	R	R	R	1.0
*Stenotrophomonas maltophila **	R	R	R	R	R	R	R	R	1.0
*Pseudomonas stutzeri **	R	R	R	R	R	R	13	10	0.8
*Photorhabdus luminescens**	R	R	R	R	R	R	R	R	1.0

n= Number of time a bacterium was isolated; R= Resistant; MAR=Multiple antibiotic resistance; CAZ = Ceftazidime 30 µg, CRX = Cefuroxime 30 µg, GEN = Gentamicin 10 µg, CXM = Cefixime 5 µg, OFL = Ofloxacin 5 µg , AUG = Amoxycillin/ Clavulinate 30 µg, CPR = Ciprofloxacin 5 µg, NIT = Nitrofuratoin 300 µg, * = Isolates selected for plasmid curing

Table 5. Antibiotic susceptibility patterns and MAR index of Gram positive bacterial isolates

Bacterial isolates	Diameter of zone of inhibition (mm)									MAR
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	VAN	index
Streptococcus hypovaginalis	R	R	R	8	R	R	R	R	25	0.8
Enterococcus devriesie n=1	R	R	18	R	R	R	25	R	22	0.7
Streptococcus minor	R	15	20	16	R	R	20	R	R	0.6
Enterococcus faecium	R	R	10	R	R	R	25	R	R	0.8
Aerococcus viridans n=1	R	R	12	R	R	R	R	R	R	0.9
Vagococcus lutae	R	R	16	17	R	R	25	R	R	0.7
Enterococcus devriesie n=2	R	R	R	12	R	R	19	R	R	0.8
Aerococcus viridans n=2	R	R	21	13	R	R	13	R	R	0.7
Streptococcus porci	R	R	13	15	R	R	21	R	R	0.7
Staphylococcus cohnii	R	R	16	19	R	R	R	R	R	0.8
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	1.0
Staphylococcus simulans	R	R	18	15	R	R	28	R	R	0.7
Staphylococcus succinus	R	R	18	16	R	R	10	R	20	0.6
Staphylococcus haemolyticus	R	R	17	16	R	R	24	R	20	0.6
Staphylococcus saprophyticus	R	R	11	15	R	R	R	R	20	0.7

n= Number of time a bacterium was isolated; R= Resistant; MAR=Multiple antibiotic resistance; CAZ = Ceftazidime 30 µg, CRX = Cefuroxime 30 µg, GEN = Gentamicin 10 µg, CTR = Ceftrixone 30 µg, ERY = Erythromycin 5 µg, CXC = Cloxicillin 5 µg, OFL = Ofloxacin 5 µg , AUG = Amoxycillin/Clavulinate 30 µg, VAN = Vancomycin 30 µg

Table 6. Antibiotic susceptibility patterns and MAR index of plasmid cured Gram negative bacterial isolates

Bacterial isolates	Diameter of zone of inhibition (mm)								MAR
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	index
Proteus mirabilis	20	R	R	R	12	R	R	11	0.6
Escherichia coli n=1	18	R	R	R	15	R	19	R	0.6
Proteus vulgaris	R	R	R	R	R	R	R	R	1.0
Pseudomonas asymbiotica	R	R	23	10	25	8	R	R	0.5
Escherichia coli n=3	R	15	17	R	28	R	12	24	0.4
Citrobacter freundii	19	R	17	R	15	R	R	12	0.5
Pseudomponas fluorescens	R	R	15	14	20	21	10	17	0.3
Empedobacter brevis	24	R	20	R	26	R	R	22	0.5
Erwinia agglomerans	25	R	18	16	24	R	12	25	0.3
Stenotrophomonas maltophila	20	R	25	R	R	R	16	12	0.5
Pseudonas stutzeri	26	26	18	22	26	26	26	26	0.0
Photorhabdus luminescens	20	R	18	R	26	R	R	22	0.5

CAZ = Ceftazidime (30 µg), CRX = Cefuroxime (30 µg), GEN = Gentamicin (10 µg), CXM = Cefixime (5 µg), OFL = Ofloxacin (5 µg), AUG = Augmentin (30 µg), NIT = Nitrofurantoin (300 µg), CPR = Ciprofloxacin (5 µg), R = Resistant

The highest bacterial count of 9.0×10^7 CFU/ml obtained in this study was higher than 4.9×10^7 CFU/ml obtained by Adebowale et al. (20) from abattoir effluent in Abeokuta, Nigeria. In a similar study, coliform and faecal streptococcal counts which ranged from 5.6×10^4 - 6.9×10^4 and 4.3×10^4 - 8.8×10^4 CFU/ml was obtained (4). In this study higher coliform and enterococcal count of 3.0×10^7 and 3.0×10^5 CFU/ml were obtained respectively. Statistical analyses revealed that there is significant difference in the bacteriological parameters of the wastewaters from the abattoirs (Tables 1, 2).

The Gram negative bacteria isolated in this study were *Proteus mirabilis, Escherichia coli, Proteus vulgaris, Pseudomonas asymbiotica, Burhkolderia cepacia, Klebsiella ozaenae, Erwinia agglomerans, Citrobacter youngae, Citrobacter freundii, Myroides odoratus, Pseudomonas fluorescens, Pasteurella multocida, Burkholderia pseudomallei, Empedobacter brevis, Stenotrophomonas maltophila, Pseudomonas stutzeri* and *Photorhabdus luminescens* while the Gram positive bacteria were *Streptococcus hypovaginalis, Enterococcus devriesie, Streptococcus minor, Enterococcus faecium, Aerococcus viridans, Vagococcus lutrae, Sreptococcus porci, Staphylococcus cohnii, Staphylococcus aureus, Staphylococcus simulans, Staphylococcus succinus, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.* These organisms belong to the family *Enterobacteriaceae, Pseudomonadaceae, Streptococcaceae, Staphylococcaceae* and *Enterococcaceae.* In a study of effluent from abattoir, *E. coli, Bacillus* sp., *Klebsiella* sp., *Enterococcus* sp., *Proteus, Pseudomonas, Salmonella* and *Staphylococcus* was isolated by Adebowale et al. in 2016 (20).

The presence of *Staphylococcus* spp. in the wastewater from the abattoirs could come from the meat during slaughtering, floor surface of the abattoirs, beef processing, and the people handling the meats. The skin, mouth, spitting, and sneezing activities of the people in the abattoirs could contaminate the meat and the environment with *S. aureus* (21, 22).

The sanitary survey of the different abattoirs revealed that most of the public abattoirs were not well taken care of; it was observed that all the processes of slaughtering, skinning, evisceration and splitting the carcasses into quarters were done on dirty and unhygienic floor. This will predispose the meat to microbial contamination. The wastewater from the washing of meat as well as from the abattoir floor surfaces were channel through pipes into the nearby stream or drainage. Three of the abattoirs (75%) discharged their wastewater into the drainage while only one (25%) discharged its waste into nearby stream. Some authors have reported poor sanitary conditions of abattoirs and indiscriminate discharge of wastewater from the abattoirs (21, 23).

Bacteria belonging to the family *Pseudomonadaceae* are spoilage organisms of meat. Meat can be spoiled quickly under aerobic condition by psychro-

philic species such as *Pseudomonas fluorescens.* In this study, 3 species of *Burkholderia* and 2 species of *Pseudomonas* were isolated. *Pseudomonas putida* was isolated by Neboh et al. in their study of effluent from an abattoir at Ijebu-Igbo, Ogun, Nigeria (3).

Enterobacteriaceae family isolated in this study include *E. coli, Proteus* spp., *Citrobacter* spp., *Klebsiella, Erwinia* and *Photorhabdus luminescens. Photorhabdus luminescens* are normal gut microbiota of animals. However, they have been implicated to cause a wide range of respiratory tract, blood stream, central nervous system, urinary tract and epiglottis infection. Soft tissue infections have been reported in patients due to *Photorhabdus* species (24). *Photorhabdus luminescens* is a lethal pathogen of insects. The organism secretes enzymes which break down the body of an infected insect and bioconvert it to nutrients which can be used by both bacteria and nematode (25). Coliform bacteria concentration in the abattoir wastewater exceeded the recommended limit of 400 MPN/100 ml for effluents being discharged into water bodies (10).

Before plasmid curing, all the Gram negative bacterial isolates were resistant to augmentin and cefixime while 95.2% were resistant to ceftazidime and cefuroxime. Furthermore, 57.1, 61.9 and 71.4% of the Gram negative bacteria were resistant to ofloxacin, gentamicin and ciprofloxacin respectively. All the Gram positive bacteria were resistant to ceftazidime, erythromycin, augmentin and cloxacillin. Most of the Gram positive bacteria (93.3%) were resistant to cefuroxime. Two-third of the isolates were resistant to vancomyin. Gentamicin, ceftrixone and ofloxacin was able to inhibit the growth of 80, 73.3 and 66.7% of the isolates respectively.

The antibiotic resistance of some of the Gram negative bacteria isolated in this study were lost after plasmid curing indicating that they were plasmid mediated. However, *Proteus vulgaris* did not show change in antibiotic resistance profile after plasmid curing. This depicts that its resistance factor was borne on the chromosome. It has been reported that bacteria that have undergone plasmid curing could lost resistance to 75% of the initially tested antibiotics (26). In this study, the plasmid curing was done with acridine-orange. Acridine orange is one of the examples of intercalating agents. Its mechanism of action is through preferential inhibition of plasmid replication of the bacterial cells (27).

This study also confirms the widespread resistance to commonly used antibiotics in both human and animal health. The public health significance of these findings is that antibiotic resistant bacteria in the abattoir effluents may colonize human population via contamination of the meat, vegetable irrigated with the contaminated water, and other usage of the contaminated waste water.

CONCLUSION

It is concluded from this study that waste water from the abattoirs contain pathogenic bacteria most of which demonstrated multiple antibiotic resistance patterns. The resistant factor of some of the isolates were plasmid mediated.

The governments at local and state levels should collaborate to ensure that the standard of abattoirs be upgraded. Modern abattoirs with the good facilities should be constructed. The indiscriminate use of antibiotic in the feed and water of the livestock should be discourage in order to prevent the transfer of the genes responsible for antibiotic resistance from animals to man. The abattoir wastewater should be treated before being discharge into the water body.

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