

Research Article

Occurrence of Antibiotic Resistant Bacteria in Faeces from Abattoir Waste, Processing Water and Products from Dutsin-Ma, Katsina State, Nigeria

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***Corresponding author:** Adesoji AT, Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria**Received:** February 07, 2016; **Accepted:** April 18, 2016; **Published:** April 20, 2016**Abstract**

Background: Antibiotic Resistant Bacteria (ARB) in abattoir waste and products are of public health concern. ARB can shuttle resistance between animal and human population through mobile genetic elements such as plasmid and integron. There is paucity of data to prove ARB occurrence in faeces from abattoir wastes and products in Dutsin-Ma area of Katsina State, Nigeria.

Methodology: One major abattoir in Dutsin-Ma was selected for this study. Samples were therefore, taken from faeces from abattoir wastes, processing water and selected products once per week for three weeks. Microbial qualities were determined by total enterobacteriaceae count and total aerobic bacteria count on Eosin methylene blue agar and Nutrient agar respectively. Thereafter, bacteria were isolated and antibiotic resistant profiles determined by streaking on nutrient agar and disk diffusion method respectively.

Results: In the first fecal sample, highest (8.27 log cfu/ml) and lowest (4.69 log cfu/ml) total aerobic bacteria count were observed. Total enterobacteriaceae results showed that it was only in the first sample that 3.90 and 5.00 log cfu/ml were observed in the processing water and blood samples respectively. We observed 100% resistant to each of amoxicillin, cotrimoxazole and nitrofurantoin among *Pseudomonas* isolated from each of processing water, meat sample, liver samples and effluent respectively. Among the total *E.coli* isolated from the meat sample, 100% resistant to each of amoxicillin, cotrimoxazole, nalidixic acid and augmentin was observed.

Conclusions: Occurrence of ARB from this study proved that samples are reservoir of ARB that could be shuttled between animal and human populace, hence, a public health concern.

Keywords: Faeces; Abattoir products; Antibiotic resistant bacteria; Antibiotics

Background

An abattoir is a special facility designed and licensed for receiving, holding, slaughtering and inspecting meat animals and meat products before release to the public [1]. Nevertheless, slaughtering of livestock continues to increase as a result of the increase in demand for meat and its products [2]. Meat has been and will continue to be an important constituent of human daily meals. This is because it provides proteins and serves as source of energy [3].

However, food-borne pathogens are the leading cause of illness and death in developing countries such as Nigeria costing billions of dollars in medical care and social cost [4]. Contaminated raw meat is one of the main sources of food-borne illness [5,6]. The source of these pathogenic microorganism may be the animals themselves or from outside in particular water used for processing carcasses after slaughtering, the surroundings where these animals are kept as well as the way they are processed after slaughtering [7].

Numerous waste and microorganisms produced during abattoir operation not only pose a significant challenge to effective

environmental management but also are associated with decreased quality of life among animal and human population [8,9]. The waste could also be washed away by surface runoff to contaminate ground and surface waters including market places and streets [9,10].

Antibiotic resistance means that bacteria resist the effect of one or more antibiotics, some bacteria are resistant to antibiotics naturally but bacteria can also acquire resistance [11]. This is because many of the genes involved in these resistances are carried on plasmid, transposons or integron which can act as vectors for disseminating them among bacteria species through transformation, transduction or conjugation [12]. Infections caused by bacteria that are resistant to antibiotics can lead to failure of conventional treatment, longer treatments and death [13]. Resistant bacteria have been reported in effluent, processing water and products from various parts of the world. Nevertheless, no published data have been found reporting these bacteria from any abattoir from Dutsin-Ma town in Katsina State, Nigeria. However, a study like this will help medical practitioners in the town in antibiotic resistant surveillance, thereby, reducing indiscriminate use, prescription and discharge of antibiotics

Table 1: Bacteria isolated from abattoir products, processing water and effluent.

Sample	<i>E.coli</i> (% with respect to source)	<i>Salmonella</i> (% with respect to source)	<i>Klebsiella</i> (% with respect to source)	<i>Pseudomonas</i> (% with respect to source)	<i>Proteus</i> (% with respect to source)	TOTAL (%)
Processing Water	2(50.00)	ABS	ABS	2(50.00)	ABS	4(5.71)
Meat	4(57.00)	2(29.00)	ABS	1(14.00)	ABS	7(10.00)
Liver	12(76.00)	1(6.00)	1(6.00)	1(6.00)	1(6.00)	16(22.85)
Blood	3(30.00)	ABS	5(50.00)	ABS	2(20.00)	10(14.28)
Feces	8(80.00)	1(10.00)	ABS	1(10.00)	ABS	10(14.28)
Effluent	17(74.00)	ABS	2(9.00)	1(4.00)	3(13.00)	23(32.85)
Grand total	46(65.71)	4(5.71)	8(11.42)	6(8.57)	6(8.57)	70

Code: ABS: Absent.

into the environment. Therefore, this study aimed at determination of microbial quality and isolation of antibiotic resistant bacteria from waste, processing water and products from a major abattoir in Dutsin-Ma LGA.

Materials and Methods

Site description

Mayanka abattoir in Dutsin-Ma town is a major abattoir located in Dutsin-Ma local government area of Katsina State, Nigeria was selected for this study. This abattoir has a slaughter rate of two cattle, thirty goats and sheep per week. Wastes from slaughtering processes are usually washed into the drainage system without prior treatment. Dutsin-Ma is the head quarter of the Local Government Area (LGA). It is located on latitude and longitude (12°27'18"N7°29'29"E). The LGA has an area of 527 km² and a population of 169,671 as at the 2006 census [14]. The inhabitants of the Local Government are predominantly Hausa and Fulani by tribe and their main occupation is farming and animal rearing.

Sample collection

Six samples were collected from meat, liver from eviscerated carcass, faces, water for carcass processing, blood from carcasses and liquid effluent from abattoir outflow of this abattoir. Ten grams and 20ml of each solid and liquid samples respectively were collected. A total of 24 samples were collected by obtaining samples from each of the six samples once per week for four weeks. It is should be noted that we collected samples only once per week because the abattoir is located inside the local market, and they only slaughter on the market day i.e. once per week. This, means the abattoir is only operational once per week. All samples were maintained at temperature of 4°C in an ice pack to prevent the multiplication of endogenous microbes. Afterwards, they were transported to the Microbiology Laboratory of the Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria for further analysis.

Determination of microbial quality, isolation of bacteria and storage of bacteria from samples

Microbial quality was determined by pour plate technique by serial dilution which involves measuring 1ml of liquid sample or 1 gram of solid sample into 9ml of sterile distilled water. Serial dilution was carried out to between 10³ to 10⁴ dilution factors in order to obtain countable bacteria colonies on the agar plates. Samples were then mixed by shaking before plating on appropriate media. Total plate counts were determined by plating out with a sterile pipette

1ml of the diluted samples from 10⁻² and 10⁻⁴ into sterile Petri discs. Afterwards, sterile Nutrient Agar (NA) that had already been sterilized in an autoclave at 121°C for 15 minutes and cooled to 55°C in a water bath were then poured into the plate and allowed to set. Plates were then incubated in inverted position in an incubator at 37°C for between 24 and 48hrs. Colonies developed on agar plates were counted with a colony counter. Similar steps were repeated for total enterobacteriaceae Eosin Methylene Blue (EMB) agar. Colonies with different morphologies were observed on the plates and streaked out on Nutrient Agar plate for purification and isolation. Colonies were then stored at 4°C on Nutrient Agar (NA) slants for further identification and characterization.

Bacteria characterization and identification

These were determined by gram staining as well as appropriate biochemical tests according to [15,16].

Determination of antibiotic resistant profile of isolates

Sensitivity to antibiotics was determined by the agar diffusion technique recommended by the CLSI (Clinical Laboratory Standards Institute) [17] on Mueller-Hinton agar (Oxoid) using the following antibiotic impregnated disks (Abtek Biologicals Ltd): amoxicillin (25ug), cotrimoxazole (25ug), nitrofurantoin (300ug), gentamicin (10ug), nalidixic Acid (30ug), ofloxacin (30ug), augmentin (30ug) and tetracycline (30ug). Results were classified as sensitive, resistant and intermediate while multidrug resistant bacteria were selected based on their resistance to over three classes of antibiotics.

Results

In this study, a total of 70 bacteria were isolated, highest (32.85%) and lowest were from effluent and processing water respectively. Five Gram-negative bacteria genera were identified in all the sampled points. They include: *E. coli*, *Salmonella*, *Klebsiella*, *Pseudomonas* and *Proteus* (Table 1). *E. coli* (17/46) and *Klebsiella* (5/8) were frequently isolated from effluent and blood respectively. However, (Tables 2 and 3) showed the total aerobic and enterobacteriaceae count respectively. Highest (8.27 log cfu/ml) total aerobic bacteria was observed in the meat sample during the first sampling while lowest count of 4.69 log cfu/ml was observed in the feces (Table 2). The count in the meat and feces samples decreased to 5.30 log cfu/ml and 3.04 log cfu/ml respectively in the third sample. Total enterobacteriaceae results showed that it was only during the first sampling that 3.90 and 5.00 log cfu/ml was observed in the processing water and blood respectively. No count was observed in the second and third samples.

Table 2: Total aerobic bacteria count of processing water, Abattoir products and effluents.

Sample	1 st Sample (log CFU/ML)	2 nd Sample (log CFU/ML)	3 rd Sample (log CFU/ML)
Processing water	5.81	7.39	1.04
Meat	8.27	3.77	5.30
Liver	7.68	5.90	6.07
Blood	5.51	9.25	7.36
Feces	4.69	2.62	3.04
Effluent	5.47	7.32	2.44

Table 3: Total enterobacteriaceae count in processing water, abattoir products and effluents in log cfu/ml.

Sample	1 st Sample (log CFU/ML)	2 nd Sample (log CFU/ML)	3 rd Sample (log CFU/ML)
Processing water	3.90	NG	NG
Meat	2.30	3.30	NG
Liver	NG	2.60	NG
Blood	5.00	NG	NG
Feces	NG	NG	4.00
Effluent	1.60	6.30	5.60

Code: NG: No growth.

Table 4: Percentage antibiotic resistance of bacteria from processing water, abattoir products and effluent.

Sample/ Bacteria	Percentage bacteria resistant to ANTIBIOTIC								TOTAL
	AMX	COT	NIT	GEN	NAL	OFL	AUG	TET	
Processing water									
<i>E.coli</i>	100	50	50	0	0	0	50	0	2
<i>Pseudomonas spp</i>	100	100	100	0	100	0	100	100	2
Meat									
<i>E.coli</i>	100	100	0	0	100	0	100	50	4
<i>Salmonella spp</i>	50	50	0	0	50	0	50	50	2
<i>Pseudomonas spp</i>	100	100	100	0	100	0	100	0	1
Liver									
<i>E.coli</i>	83	92	0	0	16	0	25	33	12
<i>Salmonella spp</i>	0	0	0	0	0	0	0	0	1
<i>Klebsiella spp</i>	100	100	100	0	0	0	0	100	1
<i>Pseudomonas spp</i>	100	100	100	0	0	0	0	100	1
<i>Proteus spp</i>	100	100	100	0	0	0	100	100	1
Blood									
<i>E.coli</i>	33	66	33	0	0	0	0	33	3
<i>Klebsiella spp</i>	100	60	60	60	80	0	20	20	5
<i>Proteus spp</i>	100	50	100	0	50	0	100	0	2
Feces									
<i>E.coli</i>	33	88	0	0	0	0	0	0	9
<i>Salmonella spp</i>	0	0	0	0	0	0	0	0	1
<i>Pseudomonas spp</i>	100	100	100	0	100	0	0	100	1
Effluent									
<i>E.coli</i>	47	82	11	5	11	0	5	52	17
<i>Pseudomonas spp</i>	100	100	100	0	100	0	100	0	1
<i>Klebsiella spp</i>	100	100	100	0	100	0	100	100	1
<i>Proteus spp</i>	66	66	66	0	33	0	66	33	3

Code: **AMX:** Amoxicillin; **COT:** Cotrimoxazole; **NIT:** Nitrofurantoin; **GEN:** Gentamicin; **NAL:** Nalidixic Acid; **OFL:** Ofloxacin; **AUG:** Augmentin; **TET:** Tetracycline.

Table 5: Phenotypes of Multidrug resistant bacteria.

Source	Bacteria	Resistant Phenotype
Processing water	<i>Pseudomonas</i> spp	AMX, COT, NIT, NAL, AUG, TET
	<i>Pseudomonas</i> spp	AMX, COT, NIT, NAL, AUG, TET
Meat		
	<i>Salmonella</i> spp	AMX, COT, NAL, AUG, TET
	<i>E.coli</i>	AMX, COT, NAL, AUG
	<i>E.coli</i>	AMX, COT, NAL, AUG
	<i>E.coli</i>	AMX, COT, NAL, AUG, TET
	<i>E.coli</i>	AMX, COT, NIT, NAL, AUG, TET
	<i>Pseudomonas</i> spp	AMX, COT, NAL, AUG
Liver		
	<i>Klebsiella</i> spp	AMX, NIT, AUG
	<i>Klebsiella</i> spp	AMX, COT, NIT, NAL, TET
	<i>Klebsiella</i> spp	AMX, COT, NIT, NAL, TET
	<i>Klebsiella</i> spp	AMX, NIT, TET
	<i>Proteus</i> spp	AMX, NIT, AUG
	<i>Proteus</i> spp	AMX, COT, NIT, NAL, AUG
	<i>E.coli</i>	AMX, COT, NIT,
Effluent		
	<i>Klebsiella</i> spp	AMX, COT, NIT, NAL, AUG
	<i>Klebsiella</i> spp	AMX, COT, NIT, NAL, AUG, TET
	<i>E.coli</i>	AMX, NIT, NAL
	<i>E.coli</i>	AMX, COT, AUG
	<i>E.coli</i>	COT, GEN, TET
	<i>E.coli</i>	AMX, NIT, NAL
	<i>Proteus</i> spp	AMX, COT, NAL
	<i>Proteus</i> spp	AMX, COT, NIT, NAL, AUG, TET
	<i>Pseudomonas</i> spp	AMX, COT, NIT, NAL, AUG
Feces		
	<i>Pseudomonas</i> spp	AMX, COT, NIT, NAL, TET

Code: AMX: Amoxicillin; COT: Cotrimoxazole; NIT: Nitrofurantoin; GEN: Gentamicin; NAL: Nalidixic acid; OFL: Ofloxacin; AUG: Augmentin; TET: Tetracycline.

Percentages of antibiotic resistant bacteria were shown on (Table 4). We observed 100% resistant to each of amoxicillin, cotrimoxazole and nitrofurantoin among *Pseudomonas* isolated from each of the processing water, meat sample, liver samples and effluent respectively. Among the total *E.coli* isolated from the meat samples 100% resistant to each of amoxicillin, cotrimoxazole, nalidixic acid and augmentin was observed. Lower resistant to these same antibiotics i.e. 47%, 82%, 11%, 5% respectively was observed among *E.coli* from the effluent. Multi-Drug Resistant (MDR) bacteria isolates and their corresponding phenotypes. The only MDR bacteria observed from the feces and processing water is *Pseudomonas* spp while the MDR bacteria isolated from the effluent include *Klebsiella*, *E.coli* and *Proteus*. Resistant to amoxicillin is most common among these MDR bacteria. One MDR *Salmonella* spp was isolated from meat sample and it showed resistant to amoxicillin, cotrimoxazole, nalidixic acid, augmentin and tetracycline (Table 5).

Discussion

In this study, we observed the presence of enterobacteriaceae count (3.90 log cfu/ml) in the processing water only in the first sample. This could directly be attributed to the fact that the water source was obtained from poorly constructed wells that are prone to fecal contamination from water sellers, who supply water in plastics containers. Therefore, this may be the source of contamination observed in meat sample which showed 2.30 log cfu/ml enterobacteriaceae counts during this sample period. Two samples met the recommended zero *E.coli* counts in water used for washing carcasses, which is also drinking water standard [18]. However, high enterobacteriaceae count (i.e 3.30 and 2.60 log cfu/ml) was observed in the second sample respectively. This could be as a result of lack of standard operating procedures and poor hygienic practices such as flaying, eviscerations, and splitting of carcass on the floor as observed during sampling in this abattoir. There is even tendency of feces from the carcass, mixing with the meat, thereby contamination of the meat. This is also similar to the observation of Bello et al. [19] in some other abattoir in Northwestern Nigeria. However, samples that showed no enterobacteriaceae count did not show sterility as count were observed on the agar used for total plate count in all samples (Table 2).

In Nigeria, many abattoirs dispose their effluents directly into streams and rivers without any form of treatment and the slaughtered animals are washed by the same water [20]. This is the case in most abattoirs in Nigeria and we observed similarity during sampling. Therefore, the occurrence of high total bacteria count and enterobacteriaceae count in the effluent from this abattoir implies a lot to public health. This could result in outbreaks of *E.coli* infection as observed by Nelson. [21], Millard et al. [22] and Cieslak et al. [23]. However, among bacteria isolated from effluent from this study *E.coli* showed the highest occurrence. This definitely signifies high concentration of fecal discharge. The total bacteria count in this study also exceeded the recommended limit for the discharge of effluents into water bodies and land application in Nigeria [24].

Moreover, in less developed nation like Nigeria, water bodies like river are used for drinking, bathing, washing, watering of animals, watering of crops and other domestic purposes. Hence, a high impact on the public health of users [25]. Other bacteria isolated from wastes and abattoir products from these studies include *Pseudomonas*, *Salmonella*, *Klebsiella* and *Proteus*. Most of these bacteria have been implicated as pathogenic organisms [26,27].

It has been reported that antibiotics are widely used in food animal production for therapy and prevention of bacterial infection for growth promotion [28]. Studies have also shown that 50-90% of drugs administered to farm animals are excreted into the environment either un-metabolized or as metabolic intermediates which even though inactive, may undergo transformation to the active form in the environment [29]. Our field work observation as discussed early observed discharge of fecal material from the processing carcasses being released as effluent in the environment. This can consist of a lot of un-metabolized antibiotic residue which Kummerer [29]. Also reported can persist as residue in waste, soil, food and water with a number of consequences. One of the consequences may be high isolation of Multi-Drug Resistant (MDR) *Klebsiella*, *E.coli*,

Proteus and *Pseudomonas* from effluent. Similar trend was also observed among bacteria isolated from products meant for consumer consumption from this abattoir like meat, liver, and even blood. High resistant to amoxicillin, cotrimoxazole, Nitrofurantoin was observed among these bacteria.

The occurrence of these MDR bacteria could pose three major public health risks to consumers of these products according to Piddock [30]. (a). Ingestion of products contaminated with MDR bacteria could cause infection which requires antibiotic therapy and therapy can then be compromised due to resistant strain. However, it should be noted that even if the products are cooked, it will not destroy genes responsible for the resistance. These bacteria can only be destroyed. (b). Resistant non-pathogenic bacteria are selected in animals which are transferred to human via consumption of contaminated food products and resistance genes are subsequently transferred to other bacteria in the gut through mobile genetic element such a plasmid, integrons and gene cassette, insertion sequence and transposon. (c). Antibiotics which may remain as residues in animal products such as meat, liver and blood can also lead to the selection of resistant bacteria in the consumer of the product.

Conclusion

It was therefore, established from the finding of this study that products and effluents from this abattoir could be a source of dissemination of antibiotic resistant bacteria between animal and human population. Hence, effort must be made by public health workers to stem this trend to prevent public health implication.

Authors' Contributions

AAT and AF planned this study. AF performed the experiment under the supervision of AAT. AAT prepared the manuscript while IJS made significant contribution to the manuscript preparation. All authors read through the manuscript and approved it.

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