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Evaluation and Characterization of *Salmonella* and *Shigella* Species from Abattoir Effluents and Receiving Watersheds in Ikpoba River, Benin City, Nigeria

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Abstract

This study aimed at assessing abattoir effluents and receiving water bodies for the presence of *Salmonella* and *Shigella* species as well as biofilm formation potential and antibiotic susceptibility profile. A total of 24 samples were collected from different sampling points (point of slaughter, discharge point, 200-M upstream and 200-M downstream) between July and December 2018. The samples were evaluated for *Salmonella* and *Shigella* species using standard culture-based techniques and an analytical profile index (API 20E) was used to identify the respective bacteria isolates. Antibiotic resistance profile was determined using the disc diffusion method and biofilm formation was evaluated using the microtitre plate method. The occurrence of *Salmonella* and *Shigella* isolates in this study is as follows: point of slaughter [13(22.03%), 11(24.44%)]; discharge point [22(37.28%), 15(33.33%)]; 200-M upstream [8(13.56%), 7(15.56%)] and 200-M downstream [16(27.12%), 12(26.67%)] respectively. Biofilm formation profile of the *Salmonella* and *Shigella* species in this study is as follows: strong biofilm formation [22(37.29%), 9(20%)]; moderate biofilm formation [20(33.89%), 22(48.89%)]; weak biofilm formation [9(15.25%), 14(31.11%)] and those negative for biofilm formation [8(13.56%), 14(31.11%)] respectively. The resistance profile of *Salmonella* and *Shigella* species for ampicillin [25(42.4%), 12(20.3%)]; amoxicillin-clavulanate [13(22.0%), 5(8.5%)]; azithromycin [27(45.8%), 13(22.0%)]; tetracycline [15(25.4%), 6(10.2%)]; chloramphenicol [31(52.5%), 15(25.4%)]; fosfomycin [27(45.8%), 11(18.6%)] and gentamicin [10(16.9%), 3(5.1%)] respectively. Findings from this study could be used as a baseline study to investigate pathogenic and multi-drug resistant *Salmonella* and *Shigella* isolates in abattoir environments.

1. Introduction

Abattoirs are generally known all over the world to pollute the environment either directly or indirectly from their various activities [1]. Effluent generated from the abattoir is characterized by the presence of various microorganisms which is of public health importance. In Nigeria, the abattoir industry is an important component of livestock production which has been found to provide meat food to over one hundred and fifty million people and employment opportunities for the population [2]. However, the contribution of abattoirs and the associated effects of wastewaters discharged into the environment is rarely considered. Abattoirs are potential sources of enteric pathogens that could possess potential antibiotic resistance genes, which is a serious public health concern [3]. The faecal wastes product from animals remains an important source of contamination in the environment and the food chain. Contamination of the river body and land from abattoir effluents could constitute a serious significant environmental public health hazard [4]. Also, the infections arising from bacteria can be transmitted following a failure of personal hygiene after contact with an infected host. *Salmonella* and S*higella* species are among the microorganism currently under public health surveillance for antimicrobial resistance. An increasing number of primary sources of foodborne pathogens are considered to have been linked to food-producing animals as well as contaminated water sources. Anbessa and Ketema [5] stated that disease occurs as a result of contamination of food by pathogens such as *Salmonella* and *Shigella* is among the major challenges worldwide. Evaluating the existing safety status of foods, including meat and meat products is a proactive measure to reduce the possible risk due to associated foodborne infections. Some abattoirs in developing countries including Nigeria lack basic facilities for the treatment of abattoir effluents and consequently, the disposal of wastewater to both the terrestrial and aquatic environments [6]. This could lead to the transmission of pathogens to humans, the direct outcome of which could lead to diseases including; salmonellosis and shigellosis among others [7]. An understanding of the prevalence, antibiotic resistance pattern and distribution of *Salmonella* and *Shigella* species in abattoir effluents and determining management strategies is fundamental to reducing the risk of high pathogen loads. In this study, we isolated and identify *Salmonella* and *Shigella* pathogens from an abattoir and receiving water bodies as well as biofilm formation potential and antibiotic susceptibility profile. This study aimed at determining the prevalence of *Salmonella* and *Shigella* species from abattoir effluents and receiving water bodies in Ikpoba River, Benin City, Nigeria.

2. Methodology

2.1 Sample collection

The abattoir where this study was carried out is located within the Ikpoba slope, a community close to Ikpoba River at Latitude 6 ° 21′0.5′ longitude 5° 38′34.98. Effluent samples were obtained in the morning during the peak activities between 7:00-am and 9:00-am from the abattoir where the animals were slaughtered and the discharge point in the abattoir at a depth of approximately 200 mm. In addition, water samples were collected from adjoining Ikpoba River (200-M upstream and 200-M downstream) at a depth of approximately 500 mm using sterile plastic containers. The samples were immediately conveyed in ice packs to the Applied Microbial Processes & Environmental Health Research Group (AMPEHREG) at the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City Nigeria for analysis within 4 h after collection.

2.2 Isolation of *Salmonella* and *Shigella* species

Samples were serially diluted and 100µl of diluent 10^9 was inoculated using spread plate methods on *Salmonella Shigella* agar (Lab M, Lancashire, United Kingdom). The plates were incubated at 37°C for 18-24 h. After incubation, distinct translucent and colourless colonies that are with or without dark centres were purified sub-cultured on tryptone soy agar (Lab M, Lancashire, United Kingdom) then incubated at 37°C for 18-24 h. Colonies were purified on nutrient agar (Lab M, Lancashire, United Kingdom) and thereafter stored on nutrient agar slants for 4°C until ready for use.

2.3 Identification of *Salmonella* and *Shigella* using Analytical Profile Index (API 20E)

Analytical Profile Index 20E (API 20E) was used to confirm the identity of the *Salmonella* and *Shigella* isolates according to the manufacturer's instructions (bioMerieux, Marcy-l'Étoile, France).

2.3 Phenotypic virulence factors characterization

Haemolytic activity was determined as previously described by [8]. Lipase activity was determined as previously described by [9]. Protease activity was determined as previously described by [10]. Gelatinase production was determined as previously described by [11].

2.4 Biofilm formation assay

Biofilm formation was determined using a microtitre plate method. Biofilm formation was characterized as a negative, weak, moderate or strong producer following methods previously described by [12].

2.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disk diffusion method following the criteria of [13].

3.0 Results and Discussion

3.1 Results

3.1.1 Occurrence of *Salmonella* and *Shigella* species

The percentage frequency occurrence of *Salmonella* species in this study revealed that discharge point has the highest frequency of [22(37.28%)], followed by the point of slaughter [13(22.03%)], while at the 200-M downstream, the occurrence was observed at [16(27.12%)] and the lowest frequency of occurrence was observed at 200-M upstream [8(13.56%)] respectively (Figure 1). The percentage frequency occurrence of *Shigella* species in this study showed that the discharge point has the highest frequency of [15(33.33%)], followed by the point of slaughter at [11(24.44%)], while at 200 M downstream, the occurrence was observed at [12(26.67%)], the lowest frequency was at 200 M upstream [7(15.56%)] respectively (Figure 2).

3.1.2 Biofilm formation profile of *Salmonella* and *Shigella* species

The percentage frequency of biofilm formation profile of *Salmonella* species in this study demonstrated strong biofilm formation [22(37.29%)], moderate biofilm formation [20(33.89%)], weak biofilm formation [9(15.25%)] and negative biofilm formation [8(13.56%)] respectively (Figure 3). The percentage frequency of biofilm formation profile of *Shigella* species in this study was as follows: strong biofilm formation [9(20%)], moderate biofilm formation [22(48.89%)], weak biofilm formation [14(31.11%)] and those negative for biofilm formation [14(31.11%] respectively (Figure 4).

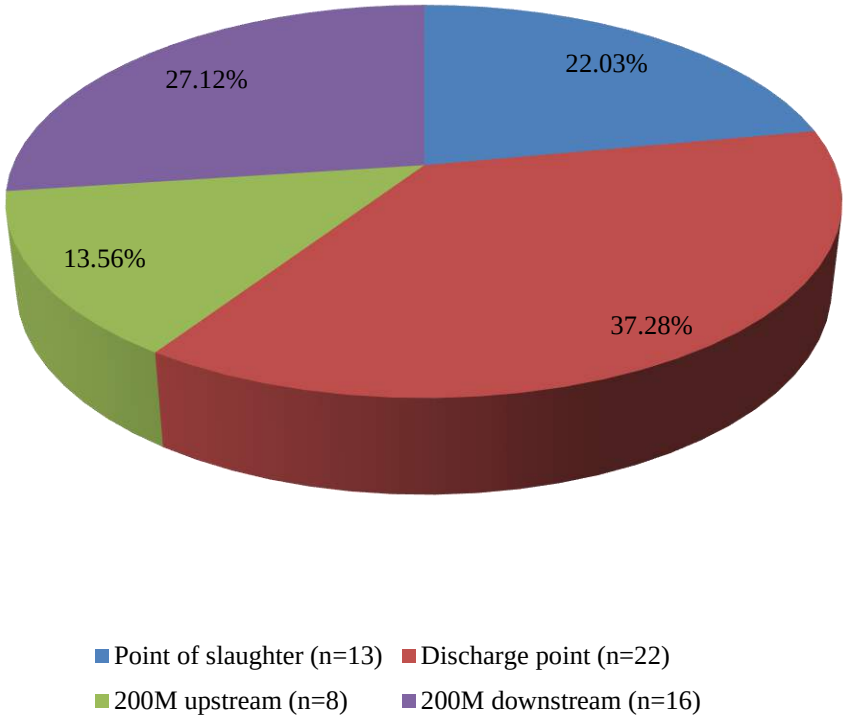

Figure 1. The occurrence of *Salmonella* species from abattoir discharge water and effluent

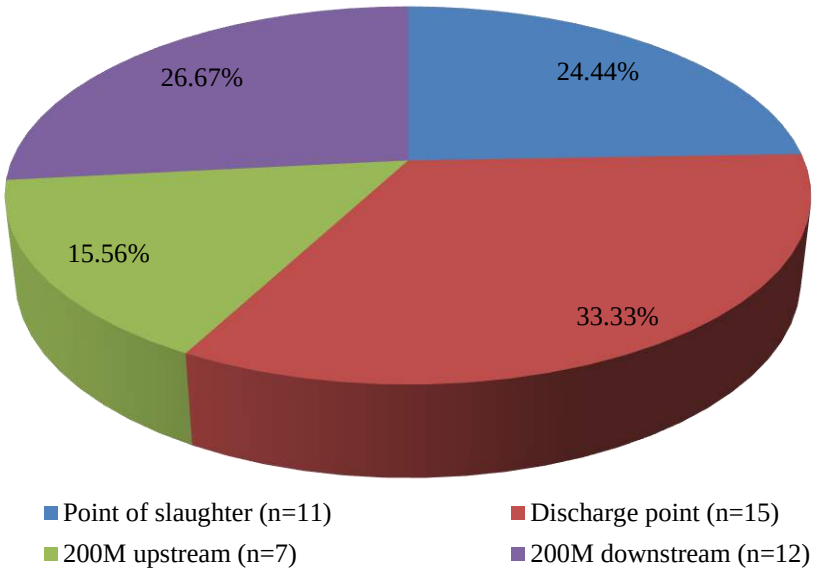

Figure 2. The occurrence of *Shigella* species from abattoir discharge water and effluent

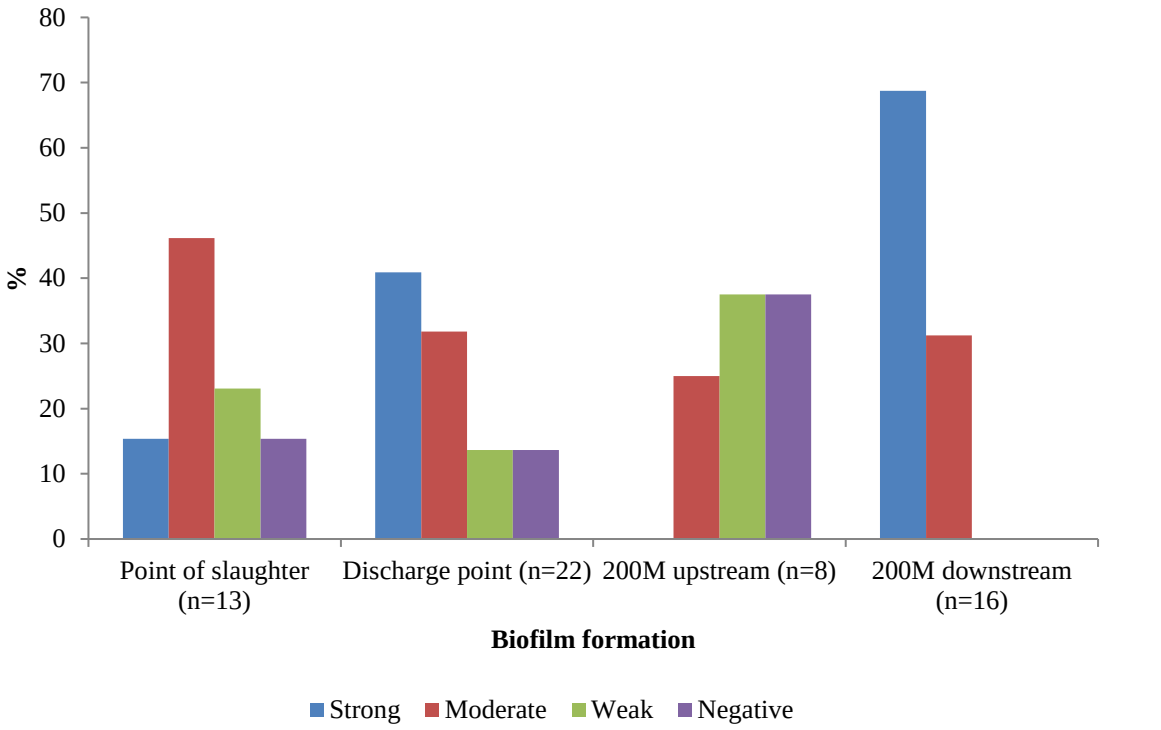

Figure 3. Biofilm formation profiles of *Salmonella* species

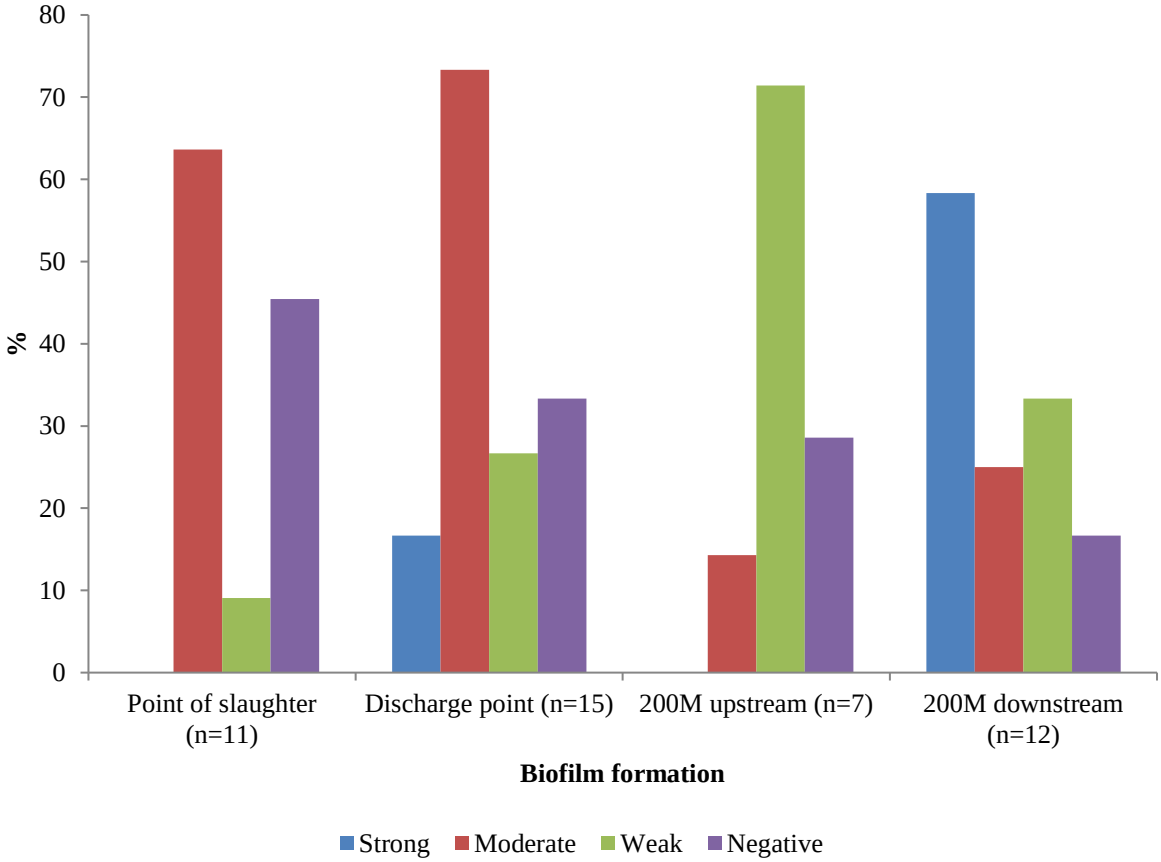

Figure 4. Biofilm formation profiles of *Shigella* species

3.1.3 Frequency of virulence factor formation among *Salmonella* and *Shigella* species

The percentage frequency of virulence factor formation among the isolated *Salmonella* species was as follows: haemolytic activity [44(74.57%)], gelatinase production [41(91.11%)], lipase activity [41(69.49%)] and protease activity [39(86.67%)] respectively (Figure 5). The percentage frequency of virulence factor formation among the isolated *Shigella* species was as follows: haemolytic activity [24(53.33%)], gelatinase production [45(76.27%)], lipase activity [32(71.11%)] and protease activity [39(86.67%)] respectively (Figure 6).

3.1.3 The resistance profile of isolated *Salmonella* and *Shigella* species

The resistance profile of *Salmonella* species in this study was as follows: ampicillin [25(42.4%)], amoxicillin-clavulanate [5(8.5%)], azithromycin [27(45.8%)], tetracycline [15(25.4%)], chloramphenicol [31(52.5%)], fosfomycin [27(45.8%)], gentamicin [10(16.9%)] respectively (Table 1). The resistance profile of *Shigella* species in this study was as follows: ampicillin [12(20.3%)], amoxicillin-clavulanate [13(22.0%)], azithromycin [13(22.0%)], tetracycline [6(10.2%)], chloramphenicol [15(25.4%)], fosfomycin [11(18.6%)], gentamicin [3(5.1%)] respectively (Table 2).

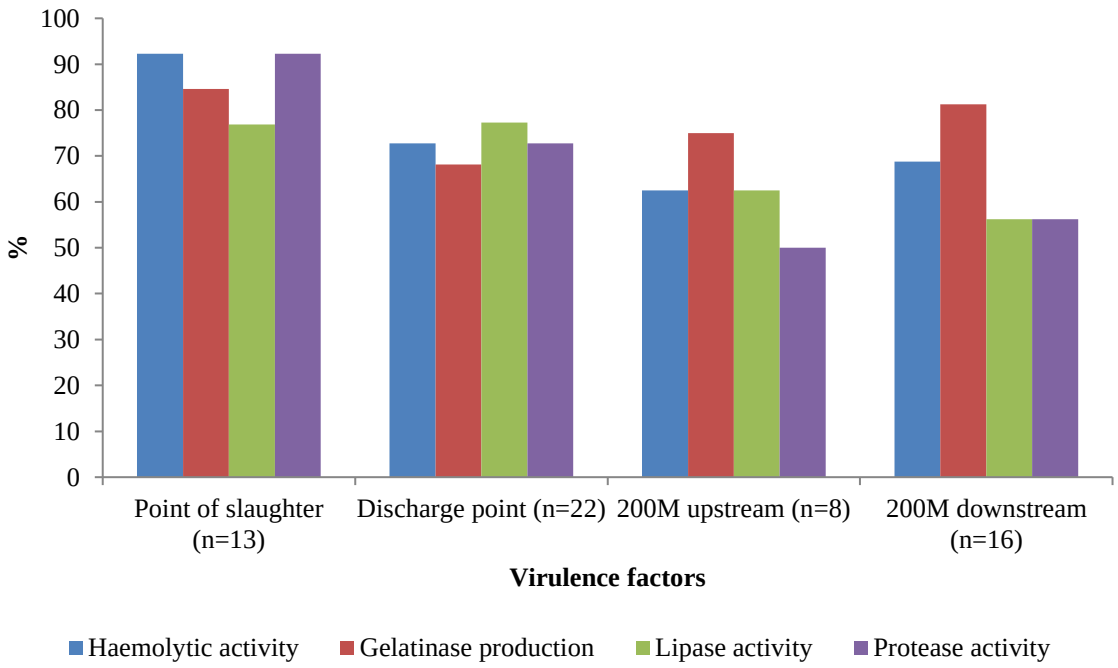

Figure 5. Distribution of *Salmonella* species virulence factors

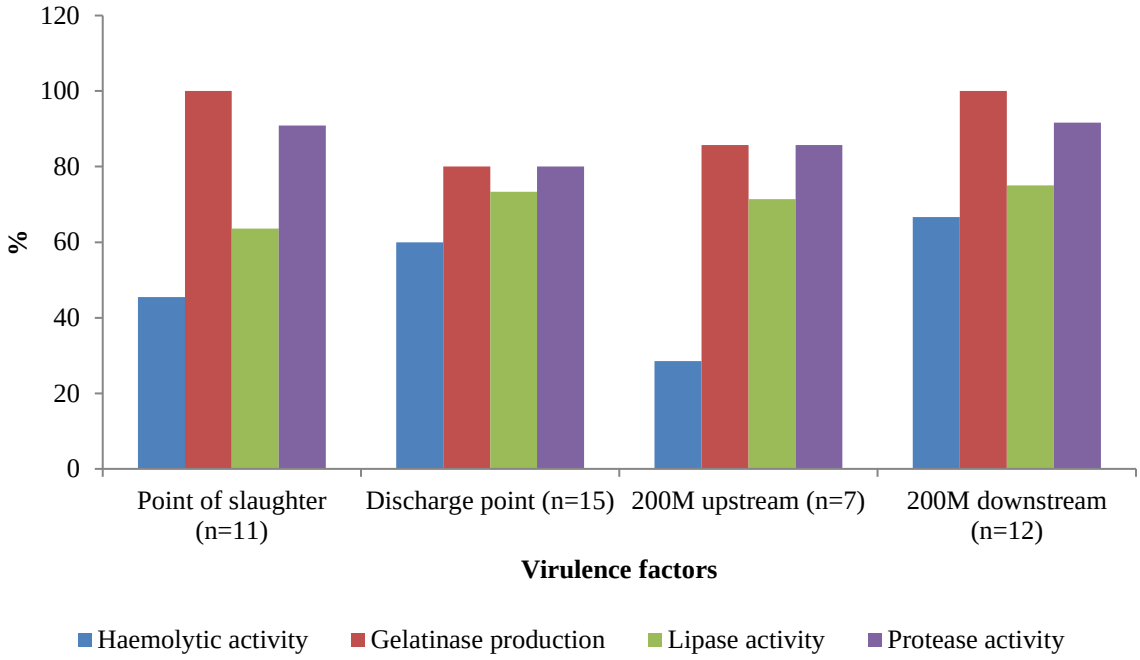

Figure 6. Distribution of *Shigella* species virulence factors

3.2 Discussion

Personal and environmental hygienic practices have been a major factor influencing the proliferation and dissemination of pathogenic microorganisms. In animal husbandry, the prevalence of diseases associated with *Salmonella* and *Shigella* has been on an increase due to poor sanitary conditions [14]. This study observed the presence of *Salmonella* in most of the points sampled and the highest frequency rate of 37.28% was observed at the point of effluent discharge. Winfield and Groisman [15] have earlier affirmed that *Salmonella* is an environmentally persistent pathogen and it can survive and proliferate in diverse environments. The prevalence of *Salmonella* observed in this study (37.28%) is however higher than the 33.3% and 19.5% prevalence reported by [16] and [17] respectively. Similarly, there has been a report of *Salmonella* spp. occurrence from receiving water bodies and vegetables irrigated with wastewaters from abattoir to be 12.3% and 13.2% respectively [18]. This study equally detected the presence of *Shigella* in all the samples analyzed, with the highest occurrence rate of 33.33% recorded in samples obtained at the point of effluent discharge. The higher prevalence of *Salmonella* and *Shigella* at the point of effluent discharge could be attributed to a high concentration of untreated waste materials in comparison to other sampling points. High bacterial count in abattoir wastewater has earlier been attributed to a high content of whole blood which serves as a nutrient medium that enhances microbial growth [19]. The least prevalence of *Salmonella* and *Shigella* was observed in effluents collected at the point of slaughter. The lower prevalence could be attributed to the fact that the abattoir workers periodically wash off the slaughtering area with detergents after the slaughtering process and waste discharge which however might not have been effective. This agrees with the report of [20] which states that detergent and other surfactants positively correlate with the inhibition of the bacterial population.

Table 1: Distribution of antibiotic susceptibility profile of *Salmonella* species

Antibiotics	Point of slaughter (*n*=13)			Discharge point (*n*=22)			200 M upstream (*n*=8)			200 M downstream (*n*=16)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
AMP	7(53.9)	2(15.4)	4(30.8)	9(40.9)	2(9.1)	11(50)	1(12.5)	2(25)	5(62.5)	8(50)	0(0)	8(50)
AMC	2(15.4)	5(38.5)	6(46.2)	6(27.3)	9(40.9)	7(31.8)	0(0)	0(0)	8(100)	5(31.3)	2(12.5)	9(56.3)
AZM	5(38.5)	3(23.1)	5(38.5)	12(54.5)	7(31.8)	3(13.6)	2(25)	1(12.5)	5(62.5)	8(50)	2(12.5)	6(37.5)
TET	3(23.1)	1(7.7)	9(69.2)	7(31.8)	5(22.7)	10(45.5)	0(0)	0(0)	8(100)	5(31.3)	6(37.5)	5(31.3)
CIP	0(0)	0(0)	13(100)	2(9.1)	6(27.3)	14(63.6)	0(0)	0(0)	8(100)	4(25)	2(12.5)	10(62.5)
SXT	1(7.7)	2(15.4)	10(76.9)	5(22.7)	5(22.7)	12(54.5)	0(0)	2(25)	6(75)	7(43.8)	3(18.8)	6(37.5)
CHL	5(38.5)	2(15.4)	6(46.2)	13(59.1)	6(27.3)	3(13.6)	1(12.5)	3(37.5)	4(50)	12(75)	4(25)	0(0)
FOS	3(23.1)	3(23.1)	7(53.8)	11(50)	7(31.8)	4(18.2)	2(25)	2(25)	4(50)	11(68.8)	3(18.8)	2(12.5)
NIT	0(0)	0(0)	13(100)	0(0)	0(0)	22(100)	0(0)	0(0)	8(100)	0(0)	2(12.5)	14(87.5)
GEN	0(0)	3(23.1)	10(76.9)	5(22.7)	6(27.3)	11(50)	0(0)	0(0)	8(100)	5(31.3)	3(18.8)	8(50)

Legend: AMP: Ampicillin (10µg), AMC: Amoxicillin-clavulanate (20/10µg), AZM: Azithromycin (15µg), TET: Tetracycline (30µg), CIP: Ciprofloxacin (5µg), SXT: Trimethoprim-sulfamethoxazole (1.25/23.75µg), CHL: Chloramphenicol (30µg), FOS: Fosfomycin (200µg), NIT: Nitrofurantoin (300µg), GEN: Gentamicin (10µg), R: Resistant, I: Intermediate, S: Sensitive

Table 2: Distribution of antibiotic susceptibility profile of *Shigella* species

Antibiotics	Point of slaughter (*n*=11)			Discharge point (*n*=15)			200 M upstream (*n*=7)			200 M downstream (*n*=12)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
AMP	3(27.3)	0(0)	8(72.7)	4(26.7)	0(0)	11(73.3)	1(14.3)	0(0)	6(85.7)	4(33.3)	3(25)	5(41.7)
AMC	1(9.1)	1(9.1)	9(81.8)	2(13.3)	0(0)	13(86.7)	0(0)	0(0)	7(100)	2(16.7)	2(16.7)	8(66.7)
AZM	4(36.4)	1(9.1)	6(54.6)	3(20)	1(6.7)	11(73.3)	2(28.6)	1(14.3)	4(57.1)	4(33.3)	1(8.3)	7(58.3)
TET	2(18.2)	0(0)	9(81.8)	2(13.3)	1(6.7)	12(80)	0(0)	0(0)	7(100)	2(16.7)	1(8.3)	9(75)
CIP	0(0)	0(0)	11(100)	0(0)	0(0)	15(100)	0(0)	0(0)	7(100)	1(8.3)	1(8.3)	10(83.3)
SXT	1(9.1)	0(0)	10(90.9)	0(0)	1(6.7)	14(93.3)	0(0)	0(0)	7(100)	2(16.7)	0(0)	10(83.3)
CHL	3(27.3)	1(9.1)	7(63.6)	6(40)	4(26.7)	5(33.3)	2(28.6)	1(14.3)	4(57.1)	4(33.3)	3(25)	5(41.7)
FOS	4(36.4)	1(9.1)	6(54.6)	3(20)	0(0)	12(80)	1(14.3)	1(14.3)	5(71.4)	3(25)	2(16.7)	7(58.3)
NIT	0(0)	0(0)	11(100)	0(0)	0(0)	15(100)	0(0)	0(0)	7(100)	0(0)	2(17.7)	10(83.3)
GEN	1(9.1)	0(0)	10(100)	0(0)	0(0)	15(100)	0(0)	0(0)	7(100)	2(16.7)	1(8.3)	9(75)

Legend: AMP: Ampicillin (10µg), AMC: Amoxicillin-clavulanate (20/10µg), AZM: Azithromycin (15µg), TET: Tetracycline (30µg), CIP: Ciprofloxacin (5µg), SXT: Trimethoprim-sulfamethoxazole (1.25/23.75µg), CHL: Chloramphenicol (30µg), FOS: Fosfomycin (200µg), NIT: Nitrofurantoin (300µg), GEN: Gentamicin (10µg), R: Resistant, I: Intermediate, S: Sensitive

The high prevalence of *Salmonella* and *Shigella* observed in this study is an indication of unhygienic slaughtering procedures employed by the butchers and also the indiscriminate discharge of animal and human wastes into the environment. Although the detection of *Shigella* in this study cannot entirely be attributed to animal waste since livestock is not a usual host of *Shigella*, there is the possibility of faecal contamination from abattoir workers and other individuals residing in the abattoir environment. This corroborates with previous studies which acknowledged that humans and other primates are the conventional hosts of *Shigella*, however, reported the detection of *Shigella* in monkeys, cows, pigs, chickens and other animals [21, 22, 23, 24].

The percentage frequency of biofilm formation profile of *Salmonella* species in this study for strong biofilm formation, moderate biofilm formation, weak biofilm formation and negative biofilm formation was 22(37.29%), 20(33.89%), 9(15.25%) and 8(13.56%) respectively. The highest frequency of strong biofilm formers was observed in samples collected from the downstream (68.75%), followed by the discharge point (40.91%). The highest frequency of moderate biofilm formers was observed in samples collected from the point of slaughter (46.15%). The percentage frequency of biofilm formation profile of *Shigella* species in this showed that study [9(20%)], 22(48.89%)], [14(31.11%)] and [14(31.11%] demonstrated strong biofilm, moderate biofilm, weak biofilm and negative biofilm formation respectively. The highest frequency of strong biofilm (58.33%) was observed in the downstream isolates. Strong biofilm formation was not observed in the sample collected from point of slaughter and upstream respectively. The difference in biofilm formation potential of the isolates used in this study could be ascribed to the difference in nutrient availability, varied incubation temperature, the static and dynamic nature of the environment, coupled with species diversity [25].

Virulence factors including hemolytic activity, gelatinase production, lipase activity and protease activity were observed in this study. These virulence factors are of a health concern as there has been a report of a positive correlation between virulence factors and pathogenicity [26]. Similarly, other studies have affirmed that gelatinase enhances the ability of the microorganism to penetrate the cell membrane of potential hosts cell leading to a disease process [27, 28].

In this study, *Salmonella* species and *Shigella* species isolated from different sampling points were found to demonstrate variable resistance patterns to the antibiotics tested. The highest resistance demonstrated by *Salmonella* species was against chloramphenicol (52.5%). This is higher than the resistance reported in studies of [29], [30] and [31] which reported chloramphenicol resistance of 18.48%, 5.1% and 11% respectively. Similarly, the highest resistance demonstrated by *Shigella* species in this study was against chloramphenicol (25.4%) while the least resistance was against gentamicin (5.1%). Contrarily, other studies have reported a significantly higher resistance of *Shigella* species to chloramphenicol [31]. It was stated in [32] that chloramphenicol resistance in bacteria can be attributed to enzymatic inactivation of nonfluorinated phenicols by chloramphenicol acetyltransferase genes. The variation in resistance rates in these studies could be attributed to the difference in antibiotics usage, the season of research and the location of study. The resistance to multiple antibiotics observed in this study could be due to the uncontrolled availability of these antimicrobial agents among drug vendors, which leads to misuse in both the human and animal populations. The extensive usage of antibiotics by farmers in animal production for preventing bacterial infection and growth promotion has been reported [33]. There is the possibility of horizontal resistant gene transfer from contaminated food products to the human when consumed [34]. Because of this, the presence of these antimicrobial-resistant bacteria in nearby water bodies could also result in the horizontal transfer of resistance genes to humans that ingest the contaminated water.

Conclusion

The contamination of abattoir environments with pathogenic microorganisms is a menace that should be curbed considering the subsequent health risks associated with it. The implementation of an effective surveillance system to ensure the relocation of abattoirs close to water bodies and the enforcement of rules that averts the establishment of new abattoirs in locations close to receiving waters could control existing and prospective contamination arising from effluents. Routine monitoring of slaughtering conditions, promotion of hygienic practices and the construction of a standard pretreatment system to ensure the treatment of effluents before discharge is recommended. Antimicrobial resistance can be curtailed with a credible campaign emphasizing the vital importance associated with the prudent intake of antibiotics by humans and in animal husbandry.

Conflicts of interest/Competing interest: There was no conflict of interest among the authors.

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