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Prevalence Of Extended Spectrum Beta-Lactamase Producing Salmonella Spp. Isolated From Abattoir Wastewater Of Keffi, Nasarawa State, Nigeria

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ABSTRACT

A study on the prevalence of extended-spectrum beta-lactamase producing . isolated from abattoir wastewater of Keffi, Nasarawa State, Nigeria was carried out. A total of one hundred and seventy-two (172) abattoir wastewater samples were collected from two abattoirs in Keffi. . were isolated and identified from the samples using standard microbiological methods. Out of 172 samples of abattoir wastewater collected, the overall prevalence of presumptive . was 49 (28.4%). The highest prevalence of . was from abattoir A (30.6%) and the lowest was from abattoir B (25.3%). The isolates from abattoir A were highly resistant to streptomycin (70.9%), but showed less resistance to ceftazidime and cefoxitin (38.7%), ciprofloxacin (35.4%), and gentamicin (29.0%). Multidrug resistance (MDR) profiling revealed that the most frequent MAR index for the isolates from abattoir A was 0.8 (25.8%) and from abattoir B was 0.8 and 0.5 (27.7% each). Phenotypic confirmation identified 7 ESBL producers from the 24 cephalosporin-resistant isolates. Molecular detection of ESBL genes out of a subset of isolates from abattoir A showed a detection rate for bla_{\text{CTX-M}} of 60.0%, while bla_{\text{TEM}} and bla_{\text{SHV}} were 100.0%. For abattoir B, the detection rate for bla_{\text{CTX-M}} was 50.0%, while bla_{\text{TEM}} and bla_{\text{SHV}} were 100.0% at approximately 1500bp. Further large-scale molecular sub-typing studies of these resistance genes should be carried out.

Keywords: Abattoir, Beta-lactamase, Extended-Spectrum, Multidrug-resistant, Wastewater

1.0 INTRODUCTION

Salmonella bacteria are one of the foremost causes of foodborne disease epidemics worldwide (Sharkawy et al., 2017). *Salmonella* spp. are the prominent bacterial pathogens amid other food-borne pathogens responsible for causing gastroenteritis in humans (Sharkawy et al., 2017). Infections caused by *Salmonella* spp. in farm animals has been documented as the leading cause of considerable economic losses worldwide (Jajere, 2019). Globally, about 93.8 million cases and 155,000 deaths are associated with gastroenteritis due to *Salmonella* species every year (Majowicz et al., 2010). Reports revealed that about 85.6% of infections were estimated to be food borne, and infection was associated with many different food types, including beef and beef products (Majowicz et al., 2010).

Abattoir operation could be very beneficial to man; in that, it provides meat for human consumption and other useful by-products, still, it can be very hazardous to public health concerning the waste it generates (Adesemoye et al., 2006). Abattoirs generate large amounts of solid waste and effluents such as rumen contents, blood and wastewater. Abattoirs often have difficulties in disposing of the solid wastes and wastewater in an environmentally acceptable fashion and in many instances untreated rumen contents, blood, and/or other abattoir effluents and wastewater are released into the environment (Odo et al., 2022). The resulting pollution not only causes problems related to odor, flies, and hygiene, but surface and groundwater can be polluted with pathogens (Garedew et al., 2012).

Though the extent of food-borne illness and its severity has been known much more, effort has not been made to overcome the problem (Wabeto et al., 2017). The prevalence of antibiotic resistance in the population is strongly correlated with antibiotic usage (nonspecific usage), as the selection and dissemination of resistant *Salmonella* bacteria are heavily augmented under selective pressure caused by antibiotics. Antibiotic resistant *Salmonella* bacteria arising from abattoir wastewater enter human environments and move about with people and goods thus, creating trans-border resistance (Garedew et al., 2012).

Though the above study may reveal that *Salmonella* spp. are the leading food-borne pathogens that contaminant meat produces and some of the water bodies around the study location, there are only a few reports on the bacteriological assessment, sanitary conditions, and practices in abattoirs in Nigeria. Therefore, this study is

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focused on the prevalence of extended spectrum beta-lactamase producing *Salmonella* Typhi isolated from abattoir waste water of Keffi, Nasarawa State Nigeria.

2.0 METHODS

2.1 Study Area

The study area for this research work was Keffi, Nasarawa State, Nigeria. Keffi is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the capital of Nasarawa State. Keffi is located between latitude 8°5' N of the equator and longitude 7°8' E and situated on an altitude of 850m above sea level (Akwa et al., 2007).

2.2 Sample Size Determination

The sample size was calculated manually using the formula below as earlier described by (Fisher *et al.*, 1998).

$$N = \frac{Z^2 P(1-P)}{d^2}$$

(Note: Standardized formula styling applied for clarity)

Where:

1. N = desired sample size (when the population >10,000);
2. Z = standard normal deviate, usually set at 1.96, which corresponds to a 95% confidence level;
3. P = proportion in the target population, set at 50% (0.5);
4. d = tolerated margin of error.

The proportion was estimated as $p < 0.5$ and $q < 0.5$ for non-infection confidence estimated used would be 95% (≥ 1.5) confidence interval with a degree of accuracy of $d = 0.05$. The design effect of 1 was used. The sample size will be obtained:

$$N = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$$

$$= \frac{0.9604}{0.0025} = 172$$

2.3 Sample Collection

One hundred and seventy-two (172) abattoir samples were collected from different point with a sterile screw sample bottle. The samples were then transported in an ice box to the Microbiology Laboratory, Department of Microbiology Nasarawa State University Keffi for microbiological analysis.

3.0 RESULTS

3.1 Isolation and Identification of *Salmonella* Typhi

The cultural, morphological and biochemical characteristics of *Salmonella* Typhi from abattoir effluent in Keffi, Nigeria is as shown in Table 3.1. Colourless colonies on DCA with black centre and black metallic sheen on BSA which was Gram negative, rod shape, positive on TSI, glucose, maltose and many others were identified as *S*. Typhi.

3.2 Prevalence of *Salmonella* Typhi

Table 3.2 shows the prevalence of *S.* Typhi isolated from abattoirs in Keffi, Nigeria. The overall occurrence of *S.* Typhi was 49 (28.4 %) but was statistically insignificant. The prevalence of isolates in relation to selected abattoirs showed that abattoir A had the highest 31 (30.6%) and lowest was from abattoir B 18 (25.3 %) respectively.

3.3 Antibiotic Resistance Profiles of Presumptive . Isolates from Abattoir Effluents

The antibiotic resistance of *S.* Typhi from abattoir effluent in Keffi, Nigeria is as shown in Table 3.3. The isolates from abattoir A were highly resistant to streptomycin 22 (70.9%) followed by ampicillin 21(67.7%), cefuroxime and co-trimoxazole 20(64.5%), nalidixic Acid 19(61.2 %), Amoxicillin/clavulanic acid18(58.0 %)moderately resistant to ceftazidime 12(38.7%),cefoxitin12(38.7%), ciprofloxacin 11(35.4%) and gentamicin 9(29.0 %). All the percentage resistance values obtained were not statistically significant.

The *S.* Typhi isolated from abattoir B were observed to be moderately resistant to streptomycin 13(72.2%), ampicillin 13(72.2%) followed by co-trimoxazole and cefuroxime 12 (66.6%), Amoxicillin/ clavulanic acid 11(61.1%) and ceftazidime9(50.0%) but a low resistance to Nalidixic acid8(44.4 %), cefoxitin 7(38.8 %), ciprofloxacin6(33.3 %) and gentamicin4(22.2 %) respectively.

Table 3.1: Cultural, Morphological And Biochemical Characteristics Of Salmonella Typhi From Abattoir Effluent

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics											Inference
	Gram stain	Mophology	Oxd	Ur	Ct	TSI	Ind	Mr	VP	Glu	Lac	Mal	Mann	Suc	

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Colourless colonies on DCA and black metallic sheen on BSA	-	rod shape	-	-	-	+	-	-	-	+	-	+	+	-	*S.* Typhi

KEY; DCA=Deoxycholate agar; BSA= Bismuth sulphide agar; -=Negative; +=positive; Oxd=Oxidase; Ur=Urease; Ct=Citrate; TSI=Triple Sugar Iron; Mr=Methyl red; VP=Voges-Proskauer; Glu=Glucose; Lac=Lactose; Mal=Maltose; Mann=Mannitol; Suc=Sucrose; Xyl=Xylose; Lys=Lysin

Table 3.2 : Prevalence Of Salmonella Typhi From Abattoir Effluent In Keffi

Abattoirs	No. of samples	No. of (%) *S.*Typhi
A	101	31(30.6)
B	71	18(25.3)
Total	172	49 (28.4)

Key; A= Cow section, B= Goat section,X^2=0.3274, *P* value = 0.5671

Table 3.3: Antibiotic Resistance Profiles Of Presumptive . Isolates From Abattoir Effluents

Antibiotics	Disc content (µg)	Resistance (%) (n=49)	
		A (n=31)	B (n=18)
Amoxicillin/clavulanicacid (AMC)	30	18 (58.0)	11(61.1)
Ceftazidime (CAZ)	30	12 (38.7)	9(50.0)
Cefuroxime (CTX)	30	20(64.5)	12(66.6)
Cefoxitin (FOX)	30	12 (38.7)	7(38.8)
Ciprofloxacin (CIP)	5	11 (35.4)	6(33.3)
Gentamicin (CN)	10	9 (29.0)	4(22.2)
Ampicillin(AMP)	30	21 (67.7)	13(72.2)
Streptomycin (S)	30	22 (70.9)	13(72.2)
Co-trimoxazole (SXT)	25	20 (64.5)	12(66.6)
Nalixidic acid (NA)	30	19 (61.2)	8(44.4)

Key; A= Cow section, B= Goat section,X2=23.4027, P-value = 0.0540

3.4 Antibiotic Resistance Profile

The antibiotic resistance profile of *S.* Typhi isolates is as shown in Table 4.4. The highest phenotype resistance profile in the isolates from abattoir A were; CTX-CAZ-S-SXT-AMC-FOX and CXT-CAZ-S-SXT-AMC-NA-CN-C-AMP with the prevalence of 4(12.9 %) and the lowest were CTX-CAZ-AMC-CIP- AMP-CXT-CAZ-S-SXT-AMC-NA-AMP and CXT-CAZ-S-SXT-AMC-NA-CN-CIP-NAwith the prevalence of 1(3.2%).

However, in abattoir B, the most frequently occurring phenotypic resistance was CTX-CAZ-AMC-CIP-AMP (16.6 %) while the lowest wasCTX-CAZ-AMC-NA-CTX-CAZ-S-SXT-AMC- FOX- CTX-CAZ-SXT-AMC-NA-CIP-CN- FOXand CXT-CAZ-S-SXT-AMC-NA-CN-CIP-NA 1(5.5%) respectively. All the percentage prevalence of the various phenotypic resistances were found to be statistically non-significant.

3.5 Multiple Antibiotic Resistance (MAR) Index

The MAR index of *S.* Typhi isolates is as given in Table 4.5. All MAR isolates had a MAR index of ≥0.4 and the frequent MAR index in the isolates from abattoir A was 0.8 (25.8%)butfrom abattoir B 0.8 and 0.5 (27.7 %)were the most frequent.

3.6 Phenotypic Detection of Extended Spectrum B-Lactamase

The ESBL production from cephalosporin resistant *S.* Typhi isolates is as given in Table 4.6. Out of 16 cephalosporin resistant *S.* Typhi from abattoir A, 5 (31.3%) were ESBL producers and from abattoir B out of 8 cephalosporin resistant *S.* Typhi2 (40.0 %) were ESBL producers.

3.7 Prevalence of Extended Spectrum β -Lactamase Resistance Genes

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The prevalence of ESBL resistance genes from *S.* Typhi producing ESBL is as shown in Table 4.7. Out of 5 isolates from abattoir A the percentage detection of *blaCTX*-M was (60.0%), *blaTEM* and *blaSHV* were 100.0%.while from abattoir B the percentage detection of *blaCTX*-M was (50.0%), *blaTEM* and *blaSHV* were 100.0%as shown in Plate 4.1 to 4.3 respectively.

Table 3.4: Antibiotic Resistance Phenotypeprofile Of *Salmonella* Typhi From Abattoir Effluent In Keffi

Antibiotic resistance profile	Total (%)Isolates		Total
	A (n= 31)	B (n=18)	49
CTX-CAZ-AMC-NA	2(9.6)	1 (5.5)	3(6.1)
CTX-CAZ-AMC-NA- FOX	3 (9.6)	0 (0.0)	3(6.1)
CTX-CAZ-AMC-CIP-AMP	1 (3.2)	3 (16.6)	4(8.1)
CTX-CAZ-AMC-NA-AMP	3 (9.6)	2 (11.1)	5(10.2)
CTX-CAZ-S-SXT-AMC- FOX	4 (12.9)	1 (5.5)	5(10.2)
CXT-CAZ-S-SXT-AMC-NA-AMP	1 (3.2)	2 (11.1)	3(6.1)
CXT-CAZ-SXT-AMC-NA-CIP- FOX	2 (6.4)	0(0.0)	2(4.0)
CTX-CAZ-S-SXT-AMC-CN-NA- FOX	3(9.6)	2 (11.1)	5(10.2)
CTX-CAZ-SXT-AMC-NA-CIP-CN- FOX	2 (9.6)	1(5.5)	3(6.1)
CXT-CAZ-S-SXT-AMC-NA-CN-AMP	3 (9.6)	2 (11.1)	5(10.2)
CXT-CAZ-S-SXT-AMC-NA-CN-C-AMP	4 (12.9)	1 (5.5)	5(10.2)
CXT-CAZ-S-SXT-AMC-NA-CN-CIP-NA	1 (3.2)	1 (5.5)	2(4.0)
CTX-CAZ-S-SXT-AMC-NA-CN-CIP- FOX	2 (9.6)	2 (11.1)	4(8.1)

KEY; CTX= Ceftaxime; CAZ= Cefdtazidime; AMC=Amoxycillin/Clavulanic acid; AMP= Ampicillin, CIP= Ciprofloxacin; NA=Nalidixic acid; S=Sterptomycin, CN= Gentamicin, SXT=Sulphamethoxazole/Trimethopri; FOX = Cefoxitin

X^2=5.6250, P-value = 0.5841

Table 3.5 : Multiple Antibiotics Resistance (Mar) Index Of *Salmonella* Typhi From Abattoir Effluent In Keffi

No. of antibiotic resistance to (a)	No. of antibiotics tested (b)	MAR index (a/b)	Frequency (%) A (n=31)	B (n=18)	Total 49
10	10	1.0	0(0.0)	0(0.0)	0(0.0)
9	10	0.9	7 (22.5)	4(22.2)	11(22.4)
8	10	0.8	8 (25.8)	5(27.7)	13(26.5)
7	10	0.7	3 (9.6)	2(11.1)	5(10.2)
6	10	0.6	5 (16.1)	3(16.6)	8(16.3)
5	10	0.5	7 (22.5)	5(27.7)	12(24.4)
4	10	0.4	2 (6.4)	1(5.5)	3(6.1)
3	10	0.3	0 (0.0)	0(0.0)	0(0.0)
2	10	0.2	0 (0.0)	0(0.0)	0(0.0)
1	10	0.1	0 (0.0)	0(0.0)	0(0.0)

KEY; A= Cow section, B= Goat section

X^2=8.3771, P-value = 0.8688

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Table 3.6: Phenotypic Detection Of Extended Spectrum B-Lactamase Production In Cephalosporin Resistant *Salmonella* Typhi From Abattoir Effluent In Keffi

Abattoir	No. of isolates	No. (%) ESBL production
A	16	5 (31.2)
B	8	2 (25.0)
Total	24	7 (29.1)

KEY; ESBL; Extended spectrum β-lactamase
X^2=0.0562, P value = 0.8125

Table 3.7: Prevalence Of Extended Spectrum Beta-Lactamase Resistance Genes In *Salmonella* Typhi From Abattoir Effluent In Keffi

ESBL genes	No. (%) *S.* Typhi			
	No. isolate A	No. detected	No. isolate B	No. detected
blaTEM	5	3 (60.0)	2	1(50.0)
blaCTX-M	5	5 (100)	2	2(100)
blaSHV	5	5 (100)	2	2(100)

X^2=0.6078, P-value = 0.9963

4.0 DISCUSSION

The isolation of Salmonella Typhi from the two abattoirs in the study area was not surprising and this finding is an indication that the bacteria responsible for typhoid fever can be transmitted from abattoir effluent. The percentage prevalence of the isolates observed in the study was higher than 35.7% reported by Ohiemi et al. (2021) in Abuja, 40.0% reported by Babatunde et al. (2022) in Ilorin but less than 71.0% and 72.5% reported by Alemu and Zewde (2012) in Bahir Dar, Ethiopia. The presence of $S.$ Typhi in the two abattoirs effluent may be attributed to faecal contaminations or contact of carcass product with contaminated surfaces such as abattoir floors, hides and skin, or butchers' equipment which are washed off by using water (Ohiemi et al., 2021). The prevalence of $S.$ Typhi observed in this study was statistically insignificant respectively and this implies that the abattoirs effluent in the study areas does not affect isolation rate but indicate the level of Salmonella Typhi contamination in the abattoirs, contaminations to the meat and other surfaces in the abattoirs which allowed high level of faecal. The presences of Salmonella in food of animal origin pose serious risk to public health (Babatunde et al., 2022). In addition, Salmonella has been reported to cause serious economic loss to public through morbidity and mortality of affected individuals (Ibrahim et al., 2022). The presences of Salmonella Typhi in the abattoirs effluent that found it way into water body is of great danger to the communities that uses the river or stream as sources of their drinking water or for farming because it will lead to outbreak of Salmonellosis in such communities (Ohiemi et al., 2021). The high percentage resistance of the $S.$ Typhi isolated from abattoir effluent to some antibiotics namely; streptomycin (70.9%), ampicillin (72.7 %), Cefuroxime and co-trimoxazole (64.5%) as recorded in this study was not expected and this may be due to abuse or inappropriate use of the antibiotics by adding antibiotics made for human to animal feeds as growth enhancers (Ochiai et al., 2008). The percentage resistance of the $S.$ Typhi isolated from abattoir effluent to streptomycin (72.2%), co-trimoxazole (66.6%) and amoxicillin/clavulanic acid (61.1%) as observed in this study was similar to the study reported by Olawale et al. (2020) in Ilorin who reported 92.1% resistance to amoxicillin/clavulanic acid, 81.5 % to streptomycin and 72.1% to co-trimoxazole but in disagreement to study reported by Olawale et al. (2020) who recorded 100.0% resistance to amoxicillin/clavulanic, co-trimoxazole and streptomycin. The difference in the pattern of resistance may be attributed to location and also the movement of the animals from one location to another was the antibiotics have been abuse before they are being slaughtered in the study area (Olawale et al., 2020). The moderate resistance of ceftazidime and cefoxitin (38.7%), ciprofloxacin (33.3 %) and gentamicin (22.2 %) recorded in this study from the two abattoir in Keffi was expected mostly the gentamicin and ceftazidime because these antibiotics are in injection form which make it difficult of people to abuse it by adding it to animal feed or using to treat the animals. This is similar to studies reported by Garedew et al. (2012), Ayandele et al. (2020), and Ohiemi et al. (2021); these authors reported low resistance (20.1%) of gentamicin and (18.8%) ceftazidime against $S.$ Typhi isolated from abattoir effluent. Infections caused by multidrug resistant (MDR) pathogens are associated with the risks of inadequate empirical antimicrobial therapy on one hand and of unnecessary broad-spectrum coverage on the other hand. However, infections caused by MDR pathogens remains highly challenging (Rhee et al., 2020). The occurrence of MDR resistant isolates observed in this study was also similar with the study reported by Babatunde et al.

(2022). The occurrence of MDR $S.$ Typhi isolated from abattoir effluent observed in this study was lower than 81.3% and 80.9% reported by Raufu et al. (2021) and Abimiku et al. (2019). The occurrence of this MDR isolates may have public health concern, since the MDR organism are known to cause infections that are difficult to be treated and may likely result to high morbidity, mortality and economic burden to the people (Ngwai et al., 2014; Abimiku et al., 2019). The findings of this study show that not all cephalosporin resistant $S.$ Typhi isolated from abattoir effluent was ESBL producers and this suggest that the resistance of the isolates to cephalosporin may be due to other mechanism of resistance other than ESBL production such as modification of the target site of cephalosporin (Seyedjavadi et al., 2016). The prevalence of ESBL producers for this current study (29.1%) is in disagreement with the 80.0% reported by Kavai et al. (2018) and the 81.6% reported by Ibrahim et al. (2022) who worked on poultry products. The prevalence of the ESBL resistance genes such as blaTEM, blaCTX-M, and blaSHV in ESBL producing isolates is an indication that the genes are responsible for the expression of the enzymes that inactivate cephalosporin antibiotic observed in this study. The occurrence of the ESBL resistance genes observed in this study is in agreement with the study earlier described by Ngwai et al. (2014) and Ibrahim et al. (2022). However, the detection of ESBL genes in the study area is a threat to public health and will negatively affect treatment outcomes in our healthcare settings in the study area. The more the resistance genes on one $S.$ Typhi isolate, the more resistant the $S.$ Typhi will be against different antimicrobials that will be used to treat the infection caused by $S.$ Typhi isolated in the study area.

5.0 CONCLUSION

The *S.* Typhi isolated from abattoir effluent in Keffi was low (28.4 %). The isolates from abattoir A were highly resistant to streptomycin, ampicillin, cefurazime and co-trimoxazole, nalidixic Acid Amoxicillin/ clavulanic acid but less resistant to ceftazidime and cefoxitin, ciprofloxacin and gentamicin. The isolates from abattoir B were highly resistant to streptomycin and ampicillin, co-trimoxazole and cefurazime but less resistant to nalidixic Acid, cefoxitin, ciprofloxacin and gentamicin. The isolates from abattoir effluent in Keffi some were cephalosporin resistant isolates and were ESBL producers and *blaTEM* (100%) and *blaSHV* (100 %) gene were commonly ESBL genes detected.

6.0 RECOMMENDATIONS

From the findings of this study the following are recommended,

i. There is need to introduce biological sewage treatment of effluent from the two abattoir before discharging into the environment to avoid infection caused by *S.* Typhi
ii. All meat products from the study area should be well cook to avoid infection caused by *S.* Typhi
iii. Abattoir houses should be clean with water and disinfectants after each slaughtering of animal to reduce or remove the fecal matter from the animal.

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