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Prevalence of Salmonella Isolated in Cattle, Inva Gene Detection and Antimicrobial Susceptibility Patterns of Isolates.

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ABSTRACT

This study was conducted to investigate the occurrence of Salmonella species in cattle, and determine the serovar distribution in Jos, North Central Nigeria. Antimicrobial susceptibility patterns of isolates and the presence of invA gene were also determined. A total of 712 faecal samples were collected from 17 cattle farms, one control post and one abattoir and screened for Salmonella species according to techniques recommended by ISO 6579 (2002). Isolation rate of 3% was obtained. S. Pullorum, S. Hull, S. Poona and S. Rubislaw were isolated at the rates of 43.5%, 34.8%, 13.0% and 8.7% respectively. Higher isolation rates were recorded in adults (3.2%), females (4%), and White Fulani breeds (3.3%) and on farms (3.3%). Isolation rates of 6.3%, 3.1% and 1.6% were recorded in Jos-East, Jos-North and Jos-South respectively. To the best of our knowledge this is the first report of such Salmonella serotypes in cattle in the study area. This result is significant because S. Pullorum, recorded the highest occurrence. PCR detected the invA gene in 84.2% of isolates. Antimicrobial susceptibility tests showed a low prevalence of resistance probably due to low use of these agents amongst cattle owners in the area. However, 26.1% of isolates manifested resistance to sulphonamide while 4.3% were resistant to colistin as well as streptomycin. There was no record of multiple resistances. In conclusion, there is need to improve biosecurity on farms, discourage housing more than one animal species at a time and improve surveillance to prevent the emergence of new serovars in the area.

Key words: *Salmonella, Cattle, Serovar, White Fulani, invA gene.*

INTRODUCTION

Salmonellosis has a serious economic impact on the cattle industry causing livestock mortality, abortions, reduced milk production and reduced consumer confidence (Jackson et al., 2007). Abattoirs serve as an important means of transmission of this disease. Unfortunately, Nigerian abattoirs have been termed one of the filthiest without an appropriate and coordinated channel of collection and disposal of animal wastes resulting in public health risks (Douglas et al., 2013). Thus, meat and meat products that could have been valuable when properly handled and put in to proper use, turn out to be a public health hazard (David-West, 2005). In addition, biosecurity measures on cattle farms are poorly observed. There is dearth of information on serotypes of Salmonella in cattle, its virulence and antimicrobial susceptibility patterns in Plateau State, Nigeria. Therefore, it is needful to establish an antimicrobial resistance surveillance system which will help provide the most suitable chemo prophylactic and chemotherapeutic regimen for individual cases and different geographic locations (Andoh et al., 2017). This will reduce veterinary costs especially on repeated treatment as well as the pool of resistant bacteria on farms. Controlling Salmonella on farms, abattoirs and control-posts will provide safer beef and beef products. Also, it will enable relevant authorities to re-focus efforts and attention on Salmonella as a disease of cattle to limit geographical spread of unique strains from one location to another.

The distribution of Salmonella serovars changes with place, time, level of production and advancement in farming techniques. The serovars and their mode of distribution is

somewhat the same in developed countries however it changes to a large extent in developing countries. Some serovars continue to dominate for long periods of time; others appear, re-appear or fade out with time. Besides, rapidly growing international trade in food and agricultural produce enhanced the spread between regional boundaries (Niduallah et al., 2017), hence, the need for periodic surveillance.

The objective of this study was to isolate and characterize Salmonella species in local White Fulani, cross-bred and Holstein-Friesian cattle on farms, one major abattoir and a cattle control-post, to detect the presence of invA gene and determine the antimicrobial susceptibility patterns of the isolates.

MATERIALS AND METHODS

Study area

The study area is Jos, Central-Nigeria. Jos is a city in Nigeria's middle belt and is the administrative capital of Plateau State which is located at latitude 80°24'N and longitude 80°32' and 100°38' East. It has a near temperate climate and an average temperature of 13-22°C. It has a popoulation of 816,824 (WPR, 2019). The State is situated in the North Central geopolitical zone of Nigeria and shares common boundaries with Benue, Kaduna, Bauchi, Nassarawa, Taraba and Gombe states. The weather and rocky terrain are very conducive for livestock and arable crops and as such majority of the people are farmers. (Plateau, 2016). Three localities: Jos-North, Jos-South and Jos East were sampled.

Sample collection

A total of 712 samples were collected from 17 cattle farms, 1 abattoir and 1 control post. A cattle control-post is a quarantine station where cattle moving from one region of Nigeria to another are observed for disease before being moved. The farms were selected by random sampling. Three breeds of cattle; local White Fulani, Holstein-Friesian and cross-breeds were sampled. Faecal samples were obtained from the cattle's rectum using sterile polythene bags. Samples were then packed in an insulated box containing icepacks and transported immediately to the laboratory for processing.

Isolation and identification of Salmonella

Salmonella were isolated and identified according to the techniques recommended by the International Organisation for Standardisation (ISO) 6579 (2002). One gram each of the faecal sample was immersed in 9mLs of 0.1% Buffered Peptone Water (BPW) (Oxoid, Basingstoke,U.K) for pre–enrichment using sterile loops. Samples were incubated at 37^{o}C for 18-24 hrs. 1mL of each of the pre-enriched samples were transferred to 9mLs each of Rappaport Vassilliadis (Oxoid, Basingstoke, UK) and Tetrathionate broths (Oxoid, Basingstoke,UK) , this was incubated at 37^{o}C and 42^{o}C respectively for 18-24 hrs. Following incubation, the broths were streaked on MacConkey plates and incubated at 37^{o}C for 18-24 hrs. Non-lactose fermenters were then streaked onto Brilliant-Green Agar (BGA) (Oxoid, Basingstoke,UK) and Xylose Lysine Tergitol$_4$ agar (XLT$_4$) (Oxoid, Basingstoke,UK) plates and incubated at 37^{o}C for 18-24 hrs.

Presumptive *Salmonella* colonies were then transferred to tubes of Triple Sugar Iron (TSI) (Oxoid, Basingstoke,UK) agar using sterile loops and incubated at 37^{o}C for 18-24 hrs. *Salmonella* produced alkaline slant over acid with or without Hydrogen Sulphide (H_2S) and gas, similar to some strains of S. Cholerasuis *S.* Gallinarum, *S.* Typhimurium, *S.* Choleraesuis and *S.* Paratyphi A which do not produce H_2S (Xie et al., 2018). Some isolates did not produce the typical *Salmonella* reactions. Each isolate was also tested for motility, urea hydrolysis, citrate utilization, indole production, lysine decarboxylation and O-nitrophenyl-β-galactopyranoside (ONPG) (Oxoid, Basingstoke, UK) hydrolysis. Isolates which did not hydrolyse ONPG, were negative for urea hydrolysis (urease production), indole negative, motile and positive for lysine decarboxylation was considered *Salmonella*-positive. They were then freeze-dried and shipped to the Italian Reference Laboratory for *Salmonella* (Istituto Zooprofilatico Sperimentale delle Venezie) in Padova, Italy for serotyping (using the microtitre plate method in conjunction with the traditional slide agglutination method)(Fagbamila, 2016**)** and antimicrobial susceptibility tests.

Determination of antimicrobial susceptibility patterns

The antimicrobial susceptibility pattern of *Salmonella* isolates was conducted using a modification of the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). A panel of 16 antimicrobials were used (Oxoid, Basingstoke, UK). These include; colistin (10 µg), sulphamethoxazole + trimethoprim (23.75 µg +

1.75 µg), kanamicin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), amoxicillin + clavulanic acid (20 µg +10 µg), ceftazidine (30 µg), nalidixic acid (30 µg), tetracycline (30 µg), ampicillin (10 µg), streptomycin (10 µg), triple sulfa (0.25 µg), chloramphenicol (30 µg), cephalothin(30 µg), enrofloxacin (5 µg), ciprofloxacin (5µg) (Oxoid, Basingstoke,UK). Each isolate was diluted in sterile saline solution to a 0.5 McFarland standard. The diluted bacterial suspension was transferred onto Müeller Hinton agar plates using sterile swabs. The plates were seeded uniformly by rubbing the swabs against the entire agar surface. Each antimicrobial impregnated disk was applied onto the surface of the inoculated plate by using a sterile disk dispenser. The plates were incubated at 37^{o}C for 18 hours. Interpretation of the growth inhibition zones and classification of isolates as susceptible, intermediate and resistant was done following guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2004). E. coli ATCC 25922 was used as reference strain.

Detection of invA gene

The DNA from the bacteria was extracted using ZR Fungal/Bacterial DNA MiniPrep^{TM} Extraction kit (Zymo, South Africa) according to manufacturer's instructions. The *inv*A gene was targeted and primers (Fermentas, Frankfurt, Germany) produced to target this region were used:

- 5'-GTG AAA TTA TCG CCA CGT TCGGGCAA-3'Forward
- 5'-TCA TCG CAC CGT CAA AGG AAC C-3'Reverse

PCR incubations were performed using a DNA Thermal cycler (Applied Biosystems, California, USA). The PCR mixture was contained in 200µl thin-walled PCR tubes each containing DNA (10.0µl), DNAse water (10.0µl), *inv*A1 (Forward) (2.5µl), *inv*A2 (Reverse) (2.5µl), Dream Taq PCR Master Mix (25.0µl) containing ready to use Dream Taq DNA polymerase, buffer, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP). Amplification was carried out under the following conditions: Initial denaturation at 95^{o}C for 2 mins**;** denaturation at 95^{o}C for 30 s; primer annealing at 53^{0}C for 30 s; primer extension at 72^{o}C for 1 min for 35 cycles and final extension at 72^{o}C for 7mins. The PCR amplicons were resolved on 1.5% agarose in Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide (0.5µg/ml). Ten µl of the PCR product from each of the tubes was mixed with 2µl of 6X buffer and electrophoresed along with 50bp DNA molecular weight marker (Gene Ruler, MBI Fermenters) at a constant 80V for 45 mins in 1X TAE buffer. Amplified product was viewed and documented under UV light by gel documentation equipment. (Syngene, Bangalore, India)

RESULTS

Of the 712 samples collected 21 (2.95%) were *Salmonella* positive. *Salmonella* was isolated in all 3 localities sampled. Jos-East had an isolation rate of 6.29% (11 of 175), Jos North had 5% (5 of 98) and Jos South had an isolation rate of 1.14% (5 of 439) *Salmonella* was isolated on farms with an isolation rate of 3.32% (19 of 21) and from abattoir with 2.5% (2 of 21). No isolation was recorded at the cattle control-post. The isolation rate in the local White Fulani breed was 3.3% (20 of 609), 1% (1 of 100) in the Holstein Friesian and no isolation was recorded for the cross breeds (0 of 3). Age wise, adult cattle had a higher isolation rate of 3.21% (9 of 592) while the calves recorded an isolation rate of 1.67 % (2 of 120). Female cattle recorded an isolation rate of 3.5% (19 of 548) while the male recorded 2% (2 of 164)

Four serotypes; *S*. Pullorum, S. Hull, S. Poona and S. Rubislaw were isolated (Table I). Two samples showed mixed infection i.e more *S.* Hull and *S.* Poona were isolated from one sample while S. Hull and S. Pullorum were isolated from another.

All 23 isolates were susceptible to sulphamethoxazole + trimethoprim, kanamicin, gentamicin, cefotaxime, amoxicillin/clavulanic acid, nalidixic acid, tetracycline, ampicillin, streptomycin, ceftiofur, cephalothin, enrofloxacin, ciprofloxacin and chloramphenicol (Table II). However 4.35% (1) were resistant to colistin, another 4.35% (1) were resistant to streptomycin while 26.1% (6) were resistant to sulphonamide (Table II). From Jos-East and Jos-South, 4.35%(1) each showed resistance to colistin and sulphonamide while from Jos-North 1 4.35%(1) showed resistance to streptomycin and (21.7%)(5) showed resistance to sulphonamide.

Nineteen (19) of the isolates were subjected to PCR, to detect i*nv*A gene which was detected in 84.2% (16) of the isolates as shown in Table III and illustrated in Plate I and II.

DISCUSSION

Salmonella was isolated in all 3 localities sampled but Jos-East had the highest isolation rate of 6.29% (11 of 175). This was significant (P<0.05). The isolation rate in the local White Fulani breed was 3.3% (20 of 609); this was higher than in other breeds, although it was not significant (P >0.05). Age wise, adult cattle had a higher isolation rate of 3.21% (9 of 592). It was not significant however (P >0.05). Female cattle also had a higher isolation rate of 3.5% (19 of 548). This was not significant either (P>0.05).

In the present study, 2.95% of cattle sampled were positive for *Salmonella*. The low isolation rate of *Salmonella* obtained in this study indicates a low *Salmonella* infection rate which might be due to single sampling per animal, a low carrier rate in cattle in the study area or possibly due to methods of sampling and isolation.

Research on faecal shedding of *Salmonella* in cattle especially in the study area is scarce. However, *Salmonella* surveys as regards faecal carriage in cattle in Nigeria vary in prevalence ranging from 1.5% from rectal swabs of 809 cattle in Kaduna, Nigeria (Kwaga et al.(1984), 3.0% from rectal swabs of 1146 cattle in Zaria, Nigeria (Olayemi,1978) to 5.5% obtained by Collard and Sen (1956) from faeces and lymph nodes of cattle in Ibadan, Nigeria.

In comparison, other African studies like that of Molla et al. (2003) in Ethiopia recorded a lower prevalence of 1.9% from faecal samples of slaughter cattle (Molla et al., 2003). On the other hand, Addis *et al*. (2011) in the same country established a higher prevalence where 10.76% (21/195) of dairy cattle tested were positive for *Salmonella* and out of which 71.4% (15/21) were of faecal origin.

Beyond Africa however, lower prevalences were established by McEvoy et al. (2003) who obtained a faecal carriage of 2% (5 of 250) from cattle in an Irish abbatoir. Equally, Madden et al. (2006) from faeces of 200 beef cattle at a Northern Ireland abbattoir arrived at a prevalence of 3% similar with this study.

While higher prevalence rates were established in some other continents like Australia where Fegan *et al.* (2004) recorded fecal shedding of *Salmonella* in 9% of 155 feedlot cattle in contrast to 4.5% of grass-fed cattle, though no significant difference between the results was observed. In the U.S however, Khaitsa *et al.* (2007) in North Dakota also noticed a hike in fecal shedding from 0.7% (on arrival) to 62% after seven months most likely associated with *Salmonellae* that were resistant to the winter and resurfaced in the spring. Other authors like Cummings et al. (2010) in New York arrived at a prevalence of 42.1% (120 of 285). While a much higher prevalence was reported by Edrington et al. (2008) in U.S which recorded 96% in August but decreased to 19% in October. According to (Rhoades *et al*. (2009) the prevalence of *Salmonella* follows a similar pattern with approximately 1.3%, 3% and 60% in chilled carcasses, faecal samples and hides respectively. Though, research has shown that prevalence of pathogens in cattle differ from one study to another. Some of the determinants e.g. animal, methods of rearing, age, season, feed, collection methods, sample types, etc. all contribute to the difference in outcome for each study. However, there may be other contributing factors which are yet to be discovered

As mentioned above *Salmonella* was isolated in all the three localities sampled with a higher isolation rate in Jos-East. The higher isolation rate could be attributed to the movement of high populations of cattle through the area which is a major cattle route especially for nomadic herdsmen. Based on site, *Salmonella* was most frequently isolated on farms as compared to the abattoir. No isolation was made in the control-post. The lower isolation rate in the abattoir is likely due to the lowered gastric acidity in the animals from starvation during movement to the abattoir. The isolation rate in the local White Fulani breed was higher than in Holstein-Friesian and cross breeds, although it was not significant (P>0.05). Female cattle recorded a higher isolation rate. This can be associated with the larger number of female cattle in the study area as compared to males. However, this rate was not significant (P>0.05). Age wise, the adult cattle had a higher isolation rate. This is perhaps due to the higher adult cattle population sampled. However, it was not significant (P>0.05).

Salmonella Pullorum had the highest occurrence rate compared to other serotypes which is unusual since S. Pullorum is avian host specific. This observation could stem from the haphazard grazing habit of cattle which could extend to poultry farms/surroundings or paths which poultry have crossed. Furthermore, other routes of infection spring up when poultry wastes find

its way into water bodies which are sources of drinking water for livestock, contaminates their pasture or is used for irrigation of their forage crops (Orji *et al*., 2005). Some researchers have reported that animals other than cattle are associated with the *Salmonella* cycle of various serovars. These include an association between various bird species contaminating feeds and the transmission of *Salmonella* to cattle. Wild birds may play a role in transmission of *Salmonella* to cattle and other domestic animals though they are not considered a main source of infection for livestock; instead the source of infection is from feed contamination and recycling among farm animals. (Coulson *et al*., 1983). Furthermore, these birds are highly mobile and migratory travelling over long distances thereby serving as potential disease vectors (Elmberg *et al*., 2017). They are susceptible to salmonellosis and get infected through carrion feeding e.g. in vultures or through consumption of contaminated water. They transmit this infection to other animals through faecal contamination of their grazing pasture, feed or water (Tizard, 2004).

Salmonella Gallinarum and *S.* Pullorum are host specific to avian species (Eswarappa *et al*., 2009) and are known to be of minor importance to public health (Shivaprasad, 2000), although the genome of the latter is continually evolving, which could increase the hosts it affects in future (Liu *et al*., 2002). The serotype is known to exhibit genomic plasticity hence it is not unusual that *Salmonella* Pullorum has been reported in pigs, cattle, cats, dogs, foxes, mink, rabbits, guinea pigs, laboratory and wild rats, chinchillas and chimpanzees(Centre for Food Security and Public Health, 2009).

The other serotypes, S. Hull, S. Poona and S. Rubislaw were isolated in a decreasing order of 34.8%, 13.04%, and 8.70% respectively. Reports of *S.* Poona and *S.* Rubislaw have been made in Nigeria from commercial chicken layer farms (Fagbamila *et al*., 2017). Likewise, Adesiyun et al. (1989) in Zaria, Nigeria isolated 2 serotypes similar with this study i.e *S.* Hull and *S.* Rubislaw. Others were *S.* Dublin, *S.* Widemarsh, *S.* Handoff, *S.* Mpouto, *S.* Wilhelmsburg and *S.* Stanleyville.

However, different serotypes have been observed by other authors in Nigeria.These include those of Kwaga et al. (1984) who recorded the following serotypes: *S.* Bergen (most prevalent) *S.* Cough, *S.* Chester, *S.* Southbank, *S.* Dublin and *S.* Langford from cattle in Nigeria. The same author in 1985 from lymph nodes, raw beef and whole carcasses discovered serotypes like *S.* Saintpaul, *S.* Stanleyville, *S.* Infantis, *S.* Livingstone, *S.* Waycross, *S.* Ealing, *S.* Widemarsh, *S.* Ikayi, *S.* Tilene, *S.* Eppendorf, amongst others (Kwaga,1985). In contrast, Stevens *et al*. (2006) in Senegal, isolated *S.* Bredeney, *S.* Corvallis, *S.* Kentucky, *S.* Muenster and *S.* Waycross from beef whereas in an Ethiopian survey carried out in cattle at the abattoir, *S.* Braenderup, *S.* Dublin and *S.* Saintpaul prevailed (Molla *et al*., 2006).

The serotypes in this study except S. Pullorum may be the cause of foodborne outbreaks, in particular from fruits, vegetables and spices contaminated by *Salmonella* from feral reptiles or other animals. This may explain recent outbreaks of *S.* Poona in the USA (Mølbak *et al*., 2002). Another survey in the United States detected *S.* Montevideo, *S.* Typhimurium, *S.* Muenster and *S.* Kentucky from beef (Schlosse *et al*., 2006). While McEvoy *et al*. (2003) isolated *S.* Dublin, *S.* Angona and *S.* Typhimurium definitive type (DT104) from rumen and faecal samples of cattle in an Irish abattoir.

Much differently, the following serotypes were confirmed by Edrington et al, (2008); *S.* Kentucky, *S.* Montevideo, *S.* Senftenberg, *S.* Livingstone, *S.* Newport, *S.* Brandenberg, *S.* Cubana, *S.* Mbandaka amongst others. While the research by Cummings et al. (2010) revealed that *S.* Cerro was the most frequent serovar among herds that had at least one laboratory confirmed clinical case. Other serovars were *S.* Kentucky (14.0%, 101 of 723), *S.* Typhimurium (including the Copenhagen variant; 9.4%, 68 of 723), and *S.* Newport (5.9%, 43 of 723). From the studies above *S.* Dublin and *S.* Kentucky are the most frequently isolated serotypes different from those found in this study. However, the serotypes obtained in this study may reflect the serotypes present in this environment although, if more colonies per sample had been picked, we might have found more serotypes, masked by dominant ones. In this work however, single isolated colonies were randomly selected among characteristic ones for confirmation.

Majority of the isolates were susceptible to the panel of antimicrobials. They were susceptible to sulphamethoxazole + trimethoprim, streptomycin, kanamycin, gentamicin, amoxicillin/clavulanic acid, nalidixic acid, tetracycline, ampicillin, ceftiofur, cefotaxime,

cephalothin, enrofloxacin, ciprofloxacin and chloramphenicol. However, resistance to sulphonamide was detected followed by colistin and streptomycin. Development of resistance to sulphonamides can be as a result of the indiscriminate use of the antimicrobials in animal husbandry in the area. In comparison, another similar study in the area by Bata *et al*. (2015) established that the antibiotic sensitivity pattern of the isolates from beef and quail eggs in Jos, Nigeria showed varying degrees of sensitivity. The isolates were moderately sensitive to Ciprofloxacin, Sulphademethoxazole, Chloramphenicol and Gentamycin recording the highest sensitivity. The isolates were completely resistant to Tetracycline, Neomycin, Oxacillin and Erythromycin (Bata *et al*., 2015). Similarly, Adesiyun et al. (1989) discovered 87% (20), 35% (8) and 35% (8) isolates were resistant to streptomycin, neomycin and tetracycline respectively, while 26% (6), 22% (5) and 13% (3) isolates were not susceptible to gentamicin, ampicillin and chloramphenicol respectively.

Other African studies like the work done by Stevens et al. 2006 in Senegal, discovered a large proportion of the isolates were resistant to nitrofurans 111 (62.4%), but less to streptomycin 53 (21.5%) and lesser to sulfamethoxazol. 35 (14.7%.). Two were resistant to spectinomycin, 2 to chloramphenicol, 1 to tetracycline and 1 to pefloxacin.While, Addis, 2011 in Ethiopia established a 100% sensitivity to ciprofloxacin followed by cotrimoxazole and chloramphenicol with 93.3% and 66.66% resistance to streptomycin. There was 83% resistance to two or more antimicrobials. Though, Alemayehu *et al.* (2003) reported that 52% of *Salmonella* isolated at a slaughterhouse in Ethiopia from beef were resistant to at least three antimicrobials.

Considering other regions, Cummings et al, (2010) in U.S recorded that among herds that had at least one laboratory-confirmed clinical case, 6.4% (47 of 734) were MDR and 79.0% were susceptible to all the antimicrobials which included ampicillin, ceftiofur, chlortetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulfadimethoxine and trimethoprim+ sulfamethoxazole).

However, Edrington et al, (2008) in U.S recorded that the isolates were susceptible to all antimicrobials examined with the exception of spectinomycin. In Ireland on the other hand, *Salmonella* isolated from meat products were 100% resistant to rifampicin, 92% resistant to tetracycline, 86.3% to oxytetracycline, 86.3% to sulphamethoxazole and 80.9% to streptomycin (Duffy *et al*., 1999).

Considering other regions, Alemayehu *et al.* (2003) reported that 52% of *Salmonella* isolated at a slaughterhouse in Ethiopia from beef were resistant to at least three antimicrobials. In the United States, 84% of the *Salmonella* isolates from retail meats were resistant to at least one antimicrobial and 53% to at least three antimicrobials. *Salmonella* isolated from meat products in Ireland were 100% resistant to rifampicin, 92% resistant to tetracycline, 86.3% to oxytetracycline, 86.3% to sulphamethoxazole and 80.9% to streptomycin (Duffy *et al*., 1999). By comparison therefore, the strains isolated from the study area showed a low level of resistance to commonly used antimicrobials.

Among the serotypes, only *S.* Poona manifested complete resistance. This resistance was exhibited to sulphonamide and streptomycin. *S.* Pullorum showed intermediate resistance to sulphonamide and colistin while *S.* Hull equally manifested an intermediate resistance to sulphonamide. Among the localities, Jos-North recorded a higher resistance rate. This resistance may have been due to the availability of drugs in this locality being the centre of the study area where economic activities are highest. There may also be an abuse of antimicrobials in the area which is a common practice by quacks.

Molecular studies revealed that, of the 19 isolates tested, *inv*A gene was detected in 16 (84.21%). The *inv*A gene has been widely used as the target gene for identifying *Salmonella* by real-time PCR (Malorny *et al*., 2007) and is important in the pathogenesis of *Salmonella* (Everest *et al*., 1999). The inability to detect the gene in some isolates is probably due to mutation which may result in a compromise of its invasiveness.

Conclusion

This study demonstrated a low *Salmonella* occurrence rate in cattle in the study area but there is evidence of increasing resistance. There is need for biosecurity on cattle farms placing emphasis on the feed, water, hygiene, waste disposal and quarantine of new and sick animals. It is necessary to discourage the

housing of more than one animal species at a time. There is a need to improve surveillance especially at borders paying attention to diseased animals to avoid spread and introduction of new serotypes into other areas. Information on *Salmonella* serotypes, antimicrobial susceptibilities and virulence characteristics need to be passed to human medical practitioners to aid in isolation and control of *Salmonella* in humans.

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