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Antimicrobial Resistance and Presence of Class 1 Integrons in Salmonella Serovars Isolated from Clinical Cases of Animals and Humans in North Dakota and Uganda

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Abstract

Back ground: Salmonellae are one of the leading causes of food borne illness worldwide and have been used as indicator organisms for studying antimicrobial resistance (AMR) trends. In the United States, Salmonella are among organisms currently under public health surveillance for AMR.

Objectives: The objective of this study was to characterise AMR patterns of Salmonella isolates from animals and humans in North Dakota (ND), and Kampala, Uganda and determine the association between the observed AMR and presence of class 1 and 2 integrons.

Methods: Salmonella isolates were collected from the Veterinary Diagnostic Laboratory (VDL) at North Dakota State University and the North Dakota Department of Health, from 2003 to2008. Additional samples were also retrieved from archives at the Microbiology Department, Faculty of Veterinary Medicine at Makerere University in Kampala, Uganda. AMR profiles were determined using a panel of 15 antimicrobials. Screening for the class 1 and 2 integrons was done using PCR with primers specific for the int1 and int2.

Results: Out of 359 Salmonella isolates tested 36.2% were resistant to at least 2 antimicrobials. The highest resistance frequency was seen against Tetracycline (39.6%) and Streptomycin (34.7 %). A total of 20.7% (57/276) of the ND samples tested positive for presence of class 1 integrons and was significantly associated ($p<0.05$) with AMR to Ampicillin, Kanamycin, Tetracycline and Sulfisoxazole. Of all Ugandan Salmonella isolates tested (94.4% 68/72) were resistant to ≥2 antimicrobials with highest resistance observed against Sulfisoxazole and Trimethoprim-Sulphamethoxazole. Presence of class 1 integron was significantly associated ($p<0.05$) with AMR to Tetracycline and Amoxicillin. DNA sequencing of the class 1 integron variable regions identified several resistance genes including aadA1, dfrA7, and dfrA5 genes. Conclusion: These results signal serious implications for treatment of salmonellosis in both public and animal health.

Keywords: Antimicrobial resistance; Salmonella; Integron 1 North Dakota; Uganda

Introduction

Antimicrobial resistance (AMR) is a natural consequence of infectious agents' adaptation to exposure to antimicrobials used in medicine, food animals, food processing, crop production and the environment [1-4]. There has been a decline in effectiveness of existing antimicrobial agents and thus infections have become more difficult and expensive to treat and epidemics have become harder to control [5, 6, 7]. As a result, in 1996, the United States (US) established The National Antimicrobial Resistance Monitoring System (NARMS), a national public health surveillance system that tracks antibiotic resistance in food borne bacteria [8]. The NARMS program is a partnership between the US Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the US Department of Agriculture (USDA) that monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats, and food animals [8]. NARMS also collaborates with AMR monitoring systems in other countries, to work towards international harmonization of testing and reporting [8]. Salmonella are among the major bacteria currently under surveillance. Salmonella are among organisms currently under public health surveillance for antimicrobial resistance [8].

Salmonella has been reported as one of the leading causes of food borne illness in the US [9] and worldwide [1,6,10]. In the United States of America (US), the major pathogens that have been associated with food borne outbreaks are comprised of viruses, bacteria, parasites, toxins, metals and prions [9]. Of these 7 major food pathogens (Campylobacter jejuni, Clostridium pefrigens, Escherichia coli, Listeria monocytogenes, Salmonella, Staphlococcus aureus and Toxoplasma gondii) are known to cause 3.3-12.3 million cases of food borne illness and up to 3900 deaths, with an estimated total cost of $6.5-$ 34.9 billion (1995 US$) annually [9]. Salmonella is responsible for approximately 1.4 million illnesses, 17,000 hospitalisations and 590 deaths in the US annually [9]. According to Food Net (Food borne Diseases Active Surveillance Network), Salmonella prevalence has consistently remained high in comparison to the other food borne pathogens despite various intervention measures [2]. In 2011 estimates,

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the leading causes of hospitalization were nontyphoidal Salmonella spp. (35%), and the leading causes of death were nontyphoidal Salmonella spp. (28%) [11]. Unfortunately the burden of disease, associated mortality and epidemiology in sub-Saharan Africa is unknown although outbreaks with high case fatality rates are reported to the World Health Organisation [4].

Previous studies have demonstrated the presence of AMR in Salmonella and other bacteria of family Enterobacteriaceace. Often, this resistance is encoded by integrons that occur on plasmids or that are integrated into the bacterial chromosome [12]. Although this AMR genetic mechanism has repeatedly been demonstrated [13, 14] few epidemiological studies [15] have been conducted to quantify the phenotypic resistance that is attributed to these genetic structures. Additionally, there are other genetic mechanisms that contribute to the observed resistance [13] which also need to be characterized. Moreover, few studies have been done in sub Saharan Africa to investigate the role of integrons in AMR acquisition by food borne pathogens.

The objective of this study was to characterize Salmonella isolates from the US (North Dakota) and Uganda (Kampala) based on AMR, presence of integrons and genetic sequencing of the integron gene cassettes.

Materials and Methods

Study design

This was a retrospective case series. Salmonella isolates included in the study were collected either as part of diagnostic procedures for large animal patients or as part of an active hospital surveillance program, and were obtained from the Veterinary Diagnostic Laboratory (VDL) at NDSU (North Dakota State University) and the North Dakota Department of Health (NDDoH) respectively. These isolates had been previously obtained from clinical cases of bovine and human salmonellosis that were presented at the VDL and NDDoH from 2003-2008. All isolates had been cultured and characterised according to methods optimised for Salmonella detection [5,13]. Additionally, archived samples from the Department of Microbiology, at the Faculty of Veterinary Medicine, Makerere University in Kampala Uganda were used. Uganda was chosen as a typical example of a developing nation and also due to the preexisting partnership between the research team at NDSU Department of Veterinary Microbiological Sciences (VMS) and Makerere University Kampala Uganda. Approval to carry out this project was obtained from the NDSU Institutional Review Board and the Uganda National Council of Science and Technology.

Antimicrobial susceptibility testing

Antimicrobial resistance of each Salmonella isolate was determined using a panel of 15 antimicrobials (Sensitire, Trek Diagnostics System, Westlake, Ohio). Each CMV1AGMF plate used for resistance screening contained a full range of minimum inhibitory concentrations (MIC). The panel consisted of 96-well microtitre plate containing different antimicrobials over a wide range of concentrations. The inoculation of the panels was done in accordance with the manufacturer's instructions (Trek Diagnostics). The antimicrobials tested were Amikacin, Amoxicillin/clavulanic acid, Ampicillin, Ceftiofur, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Nalidixic acid, Streptomycin, Sulfizoxazole, Tetracycline, and Trimethoprim / sulfamethoxizole. Antimicrobial resistance was interpreted using Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) standards. For antimicrobial agents without CLSI approved standards, NARMS interpretive criteria were used.

Class 1 and 2 integron detection

Class 1 and 2 integron detection was accomplished by using PCR primers specific for class 1and 2 intergrase; 280–bp and 233bp amplicon respectively. The protocol used was previously described by Miko et al [16]. Screening for the class 1 and 2 integrons was carried out using PCR with primers specific for the int1and 2 [14]. Briefly, in order to extract the DNA, Proteinase K was added to the samples and heated at 94°C for 5 min. Thereafter amplifications were performed in 23 µL 5X of Taq PCR (Polymerase Chain Reaction) Master Mix (Promega), 10 pmol/L each primer, and 2 µl template DNA followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 30 s at 72°C. PCR products were analyzed by gel electrophoresis with 1.5% agarose gels. All PCRs included both positive and negative controls.

DNA purification and sequencing

A representative sample of 24 isolates of Salmonella was selected according to the size (gene profile) of the gene within each isolate and the host from which the isolate was obtained; The single reaction PCR was followed to amplify the Conserved Sequence as previously described by Nde et al., [17]. The amplification products were purified using The Wizard® SV Gel and PCR Clean-Up System according to manufacturer's instruction. Purified DNA was sent to Macrogen USA for sequencing. The sequences were compared with the data in the Gen Bank (http://www.ncbi.nlm.nih.gov/BLAST).

Data analysis

Phenotypic resistance was presented as the percentage of the total isolates tested that were resistant. Descriptive statistics of class 1 integrons detected within Salmonella serotypes were computed using Epi Info version 3.3.2 software (Epi Info TM, U.S Center for Disease Control and Prevention (CDC), Atlanta, GA). The association between the observed resistance and the presence and location of Class 1 integrons in the serovars was determined using Chi-square test of independence as previously described by Khaitsa et al [18]. AMR was coded as absent (0) or present (1) with both the resistant and intermediate isolates considered as resistant. Measures of association computed included Attributable fractions and Odds Ratios.

Results

Antimicrobial susceptibility testing

Overall, Salmonella isolates from North Dakota exhibited the highest antimicrobial resistance towards Tetracycline (39.60%),

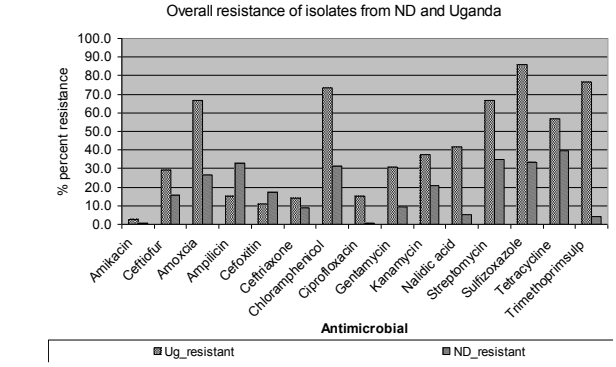

Figure 1: Overall Resistance Profile of *Salmonella* isolates from North Dakota and Uganda tested against National Antimicrobial Resistance Monitoring Systems panel of antimicrobials.

Streptomycin (34.70 %), Sulfisoxazole (33.10%), Ampicillin (32.60%) and Chloramphenicol (31.40%) (Figure1). This pattern was similar to that observed in Salmonella isolates from cattle with the highest resistance observed against Tetracycline (61.0%, 102/170), Streptomycin (54.80%, 94/171) (Table1). Among Salmonella isolates from humans, high antimicrobial resistance (AMR) frequencies were reported against Tetracycline 19.40%, 37/186), Chloramphenicol (16.70%, 31/186) (Table 1). A substantial proportion of the tested Salmonella isolates showed resistance to several antimicrobials within the critically important agents as follows: Streptomycin 34.70

Antimicrobial	North Dakota (% Resistant)		Uganda (%Resistant) N=72	
	Humans (N=186)	Cattle (N=173)	Humans (N=58)	Cattle (N=14)
Amikacin	0	0.58	0.0	16.7
Ceftiofur	4.30	28.07	28.6	41.7
Amoxcia	11.29	42.77	73.7	50.0
Ampicillin	13.44	53.18	16.1	23.7
Cefoxitin	5.92	29.07	8.8	25.0
Ceftriaxone	3.23	17.34	14.8	16.7
Chloramphenicol	16.67	47.37	81.0	50.0
Ciprofloxacin	0	1.16	14.3	27.3
Gentamycin	4.83	13.8	44.2	25.0
Kanamycin	10.21	32.37	49.0	16.7
Nalidixic acid	5.38	4.62	44.9	72.7
Streptomycin	16.37	54.83	77.2	36.4
Sulfizoxazole	16.13	51.45	92.9	83.3
Tetracycline	19.35	61.27	65.4	58.3
Trimethoprimsulp	1.08	7.51	85.7	63.6

Table 1: Overall Resistance Profile of *Salmonella* isolates from North Dakota and Uganda tested against National Antimicrobial Resistance Monitoring Systems panel of antimicrobials.

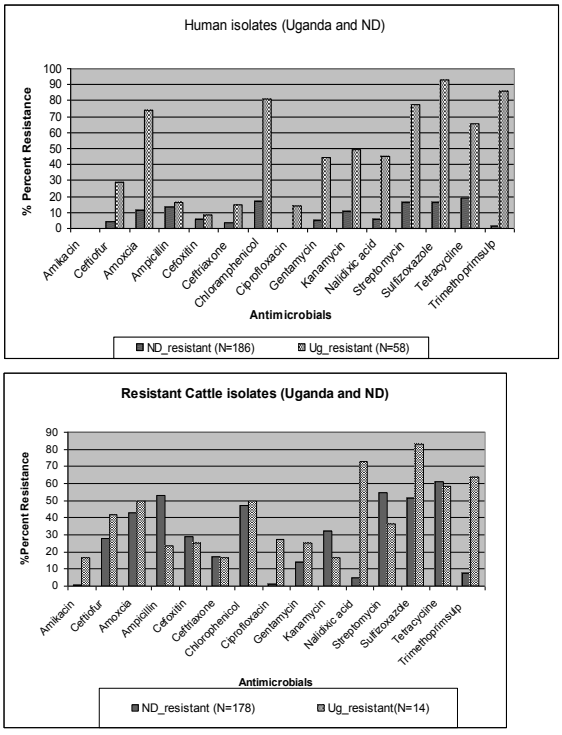

Figure 2: Comparison of Antimicrobial Resistance profiles from cattle and human *Salmonella* isolates from North Dakota and Uganda.

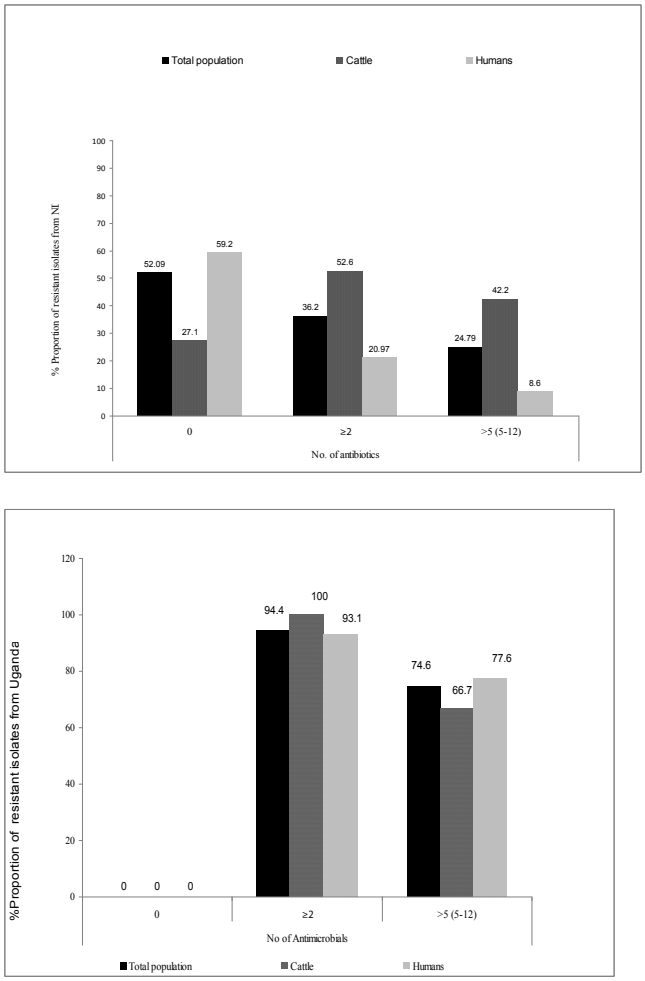

Figure 3: Multidrug Resistance in Salmonella Isolates from North Dakota and Uganda.

(113/326); [54.8% Cattle 85/155 and 16.4% Human 28/171], Ampicillin 31.90% (114/357); [52% Cattle 89/171 and 13.4% Humans 25/186], Amoxicillin-Clavulanic acid 25.80% (92/357) ; [41.5% Cattle 71/171 and 11.3% Humans 21/186] , Ceftiofur 15.70% (56/357) ; [28.10% Cattle 48/171 and 4.30% Humans 8/186]; Ceftriaxone 9.50 % (34/357) [16.4% Cattle 28/171 and 3.20% Humans 6/186] (Figure 2). Among the clinically important antimicrobial subclasses- quinolones (represented by Nalidixic acid) and third-generation cephalosporins (represented by Ceftiofur), 10 (5.40%) of the humans and 7 (4.60%) of the cattle isolates were resistant to Nalidixic acid, a drug in the same class with Ciprofloxacin. Of the 17 Nalidixic acid resistant isolates, 7 (2.4%) also had reduced susceptibilities against ciprofloxacin (MIC >0.25) (2.4%). Also, 56 (15.70%) of all isolates tested were resistant against Ceftiofur, 48 (28.10%) from cattle and 8 (4.30%) from humans (Figure 2).

From Uganda high resistance was seen against Sulfisoxazole (86.10%), Trimethoprim (76.40%), Chloramphenicol (73.60%), Streptomycin (66.70%), Ampicillin (66.70%) and Tetracycline (56.90%) (Figure1 and 2). Some of these drugs fall under the WHO described group of critically important drugs in human medicine. The highest resistance was observed against Sulfisoxazole in cattle (83.3%, 8/12) and humans (91.2%, 52/57) followed by Trimethoprim in humans (85.7%, 48/12) and Nalidixic acid (72.73%, 8/11) in cattle (and 4). Relatively high resistance to Ciprofloxacin (a drug of choice for treatment of

salmonellosis in humans) was seen in cattle-27.30% (3/11) and 14.29% (8/576) human isolates. The lowest resistance was recorded against Amikacin and Ceftriaxone 16.70% (2/12) among cattle isolates and Amikacin 0% (0/56) among human isolates (Table 1).

Multi drug resistance (MDR)

Of 359 Salmonella isolates from ND tested, 24.79% (89/359) were resistant to ≥5 antimicrobials while 36.20% (130/359) were resistant to at least 2 (Figure 3). For cattle and human isolates 52.60% (91/173) and 20.97% (39/186), respectively, had resistance to ≥2 antimicrobials while 42.20% (73/173) and 8.60% (16/186), respectively, were resistant to ≥5 antimicrobials (Figure 3). Pan susceptible isolates were 27.1% (66/173) in cattle and 59.2% (121/186) in humans (Figure 3). The most common MDR phenotype among the Salmonella isolates was the classic ACSSuT (Ampicillin, Chloramphenicol, Streptomycin, Sulfisoxazole, Tetracycline) penta-resistance at 29.06% (50/172), followed by the MDR-AmpC (ACSSuT phenotype + resistance to Amoxacillin and Ceftiofur) phenotype with a total of 18.02% (31/172). In cattle, the predominant phenotype was ACSSuT making up to 42.99% (46/107) of the total MDR isolates in cattle followed by resistance to at least MDR-AmpC 21.50% (23/107). In humans the majority of MDR isolates displayed the MDR-AmpC pattern –12.31 % (8/65) followed by the phenotype resistant to Gentamycin, Streptomycin and Sulfisoxazole (7.8 %, 5/65). Of all the MDR-AmpC isolates observed in both cattle and humans, 5 of them had resistance to Nalidixic acid three of which also had resistance to Trimethoprim; 11 of them were resistant to Trimethoprim only (used for the treatment of invasive salmonellosis). Presence of the MDR phenotype ACSSuT or MDR-AmpC was not significantly associated with presence of integron 1 (p value < 0.05). Out of all the multidrug resistant isolates (≥2) only 2 (1.16%) were resistant to Nalidixic acid while 54 (31.40%) were resistant to Ceftiofur. From Uganda, out of 73 Salmonella isolates tested 74.6% (54/73) were resistant to ≥5 antimicrobials while 94.4% (69/74) were resistant to at least 2 (Figure 3). For cattle and human isolates 100% (14/14) and 93.1% (55/59), respectively, had resistance to ≥2 antimicrobials. There were no pan susceptible isolates in both cattle and human isolates from Uganda (Figure 3).

Prevalence of Class 1 and 2 integrons

A total of 20.70% (57/276) of the Salmonella isolates from North Dakota were positive for presence of the integrase 1 gene – indicative of class 1 integron presence. Of these, 26.7% (32/120) were cattle and 16.02% (25/156) were human isolates. Presence of class 1 integron in the Salmonella isolates was significantly associated with antimicrobial

Antimicrobial	Humans Isolates				Animal isolates (cattle)			
	(%)North Dakota [n=186]	(%) Uganda [n=58]	Chi square value	P value	(%)North Dakota [n=172]	(%) Uganda [n=14]	Chi square value	P value
Amikacin	0.0	0.0			0.6	16.7		
Ceftiofur	4.3	28.6	30.07	<0.0001	28.1	41.7	0.71	0.39923
Amoxcia	11.3	73.7	90.25	<0.0001	42.8	50.0	0.28	0.59974
Ampicillin	13.4	16.1	0.16	0.69013	53.2	23.7	5.22	0.02228
Cefoxitin	5.9	8.8	*0.18	0.67208	29.1	25.0	0.08	0.77922
Ceftriaxone	3.2	14.8	*9.54	0.00201	17.3	16.7	0.01	0.93868
Chloramphenicol	16.7	81.0	84.23	<0.0001	47.4	50.0	0.04	0.85131
Ciprofloxacin	0.0	14.3	*22.35	<0.0001	1.2	27.3		
Gentamycin	4.8	44.2	57.54	<0.0001	13.8	25.0	1.2	0.27432
Kanamycin	10.2	49.0	41.18	<0.0001	32.4	16.7	1.22	0.26844
Nalidixic acid	5.4	44.9	57.54	<0.0001	4.6	72.7	*58.99	<0.0001
Streptomycin	16.4	77.2	78.44	<0.0001	54.8	36.4	1.92	0.16598
Sulfizoxazole	16.1	92.9	30.21	<0.0001	51.5	83.3	6.12	0.01334
Tetracycline	19.4	65.4	44.59	<0.0001	61.3	58.3	0.09	0.76068
Trimethoprimsulp	1.1	85.7	191.08	<0.0001	7.5	63.6	34.63	<0.0001

Table 2: A comparison of the proportions in resistance in both human and cattle isolates for panel of antimicrobials.

Antimicrobial	Odds ratio	Lower CI	Upper CI	P-values	Attributable Fraction
Amikacin	Undef	Undef	Undef	0.04	
AMOX/CLA	1.75	0.92	3.33	**0.04**	
Ampicillin	2.78	1.50	5.14	< 0.01	33.84%
Cefoxitin	0.87	0.14	1.20	0.14	
Ceftiofur	0.90	0.39	2.08	0.41	
Ceftriaxone	1.08	0.41	2.78	0.43	
Chloramphenicol	1.02	0.53	1.96	0.47	
Ciprofloxacin	Undef	Undef	Undef	0.01	
Gentamicin	2.21	0.89	5.48	0.27	
Kanamycin	2.56	1.31	5.01	< **0.01**	17.13%
Nalidixic acid	1.55	0.04	4.96	0.48	
Streptomycin	2.36	1.34	4.94	**0.02**	33.07%
Sulfisoxazole	3.13	1.69,	5.82	**< 0.01**	37.26%
Tetracycline	2.12	1.16	3.90	**< 0.01**	29.92%
Trimethoprim	1.39	0.27	7.11	0.34	

Table 3: Association of Antimicrobial Resistance and Presence of Class 1 Integron among *Salmonella* isolates from North Dakota.

resistance to: Ampicillin (OR 2.78; CI 1.50, 5.14; p-value Fishers exact <0.001); Kanamycin (OR 2.56; CI 1.31, 5.01; p-value Fishers exact < 0.001); Tetracycline (OR 2.12; CI 1.16, 3.90; p-value Fishers exact 0.02) , Streptomycin (OR 2.58; CI 1.34, 4.94; p-value Fishers exact < 0.02) and Sulfisoxazole (OR 3.132; CI 1.69, 5.82; p-value < 0.001) (Table 2). Of the samples from Uganda, a total of 45.80% (33/72) tested positive for presence of integrase 1 gene. Of these, 45.80% (27/59) were human and 46.20% (6/13) were cattle isolates. Out of a subset of 30 isolates from Uganda 3 (10%) of them tested positive for integron 2. There were higher proportions (47.9%, 34/72) of integron positive MDR Salmonella isolates from Uganda compared to those from ND (29.85%, 40/134). Presence of class 1 integron was significantly associated with AMR to Tetracycline (OR 5.94, CI 1.85, 19.09; p-value < 0.001) and Amoxicillin (OR 4.41; CI 1.442, 13.497, p-value < 0.01) (Table 3).

Association of class 1 integron to the observed antimicrobial resistance

Up to 32.35% (22/68) of MDR isolates (>5 antimicrobials) had the integrase 1 gene. Of these 17 (30.90%) were from cattle and 5 (38.50%) from humans. An attributable fraction (AF) and significant associations were computed to quantify role of class 1 integron in MDR Salmonella. Significant (P-value < 0.01) AF values for the isolates from ND were recorded against: Ampicillin 33.84%; Sulfizoxazole 37.26 %; Streptomycin 33.07%; Kanamycin 17.13%; Tetracycline 29.92%. Among the isolates from Uganda 36.31% of resistance towards Amoxicillin and 65.20% of Tetracycline was attributed to presence of class 1 integron (Table 2).

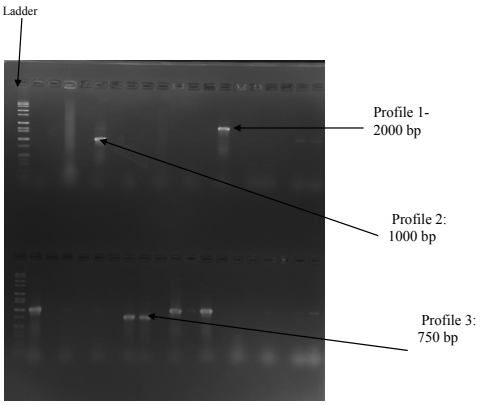

Figure 4: DNA Sequencing Results; 1.5% Agarose Gel showing different sized Integron 1 Gene Cassette amplicons. Amplification of isolates was done using their conserved sequences (CS). MW: Hi-Lo TM DNA Ladder was used.

DNA sequencing

After amplification the most frequently encountered profile had a 1000 bp followed by a 750 bp amplicon (Figure 4). Only 63.33% (57/90) of Integrase 1(Int 1) positive Salmonella isolates from North Dakota contained the integron conserved sequence in their integration site. In order to determine the content of the variable regions the detected amplicons were subjected to DNA sequencing. Among these North Dakota isolates 2 gene cassette profiles were detected (1000 bp and 750 bp). Sequencing of the 1000 bp amplicon identified mainly the aadA family of genes including; aadA1 which confer resistance to Streptomycin and Spectinomycin; additionally acetyltransferase (aac(6')-Ib-cr) which confers resistance to Amikacin, Tobramycin and Kanamycin was also identified; however, the 750 bp mainly contained the dfrA1 gene. Among the isolates from Uganda all 3 gene cassette profiles were detected (Figure 4). In one isolate two different profiles were identified. The identified cassettes were aadA1 which confer resistance to Streptomycin and Spectinomycin; dihydrofolate reductase dfrA7, dfrA5, dfrA1 which confer resistance to Trimethoprim and aminoglycoside acetyltransferase, (aac(6')-Ib-cr) which confers resistance to Amikacin, Tobramycin and Kanamycin, the most common profile had a combination of more than one of these genes.

Discussion

This study supports previous reports [19-23] that antimicrobial resistance in Salmonella is both a human and veterinary problem. This is further backed by reports from the USDA [24] which state that approximately 25% of small feedlot cattle operations and 70% of large feedlot operations use antimicrobials in their feed. In ND, observed resistance against Tetracycline, Streptomycin, Sulfisoxazole, Ampicillin and Chloramphenicol was in tandem with, although slightly lower than, reports from four other state veterinary diagnostic laboratories in the US [22]. Also, Salmonella isolates from ND showed lower resistance towards Tetracycline, Streptomycin, Sulfisoxazole, Ampicillin and Chloramphenicol compared to isolates from Uganda where greatest resistance was towards Tetracycline, Streptomycin, Sulfisoxazole,

Antimicrobial	Odds ratio	Lower CI	Upper CI	P-Values	Attributable Fraction
Amikacin	4.14	0.4893	22.58	0.2185	
AMOX/CLA	4.41	1.44	13.50	0.0048	**36.31%**
Ampicillin	1.766	0.62	5.03	0.013	
Cefoxitin	1.12	0.32	22.46	0.52	
Ceftiofur	1.10	0.25	4.82	0.61	
Ceftriaxone	2.0	0.58	7.88	0.26	
Chloramphenicol	1.38	0.46	4.16	0.27	
Ciprofloxacin	0.74	0.21	2.62	0.42	
Gentamicin	2.10	0.69	6.03	0.09	
Kanamycin	0.84	0.32	2.44	0.47	
Nalidixic acid	0.93	0.12	7.09	0.62	
Streptomycin	2.56	0.91	8.39	0.06	
Sulfisoxazole	5.50	0.61	49.80	0.11	
Tetracycline	5.94	1.85	19.09	< 0.01	**69.23**
Trimethoprim	3.60	0.88	14.75	0.40	

Table 4: Association of Antimicrobial Resistance and Presence of Class 1 Integron among *Salmonella* isolates from Uganda.

Ampicillin and Chloramphenicol. This difference could be attributed to the easy access to antimicrobials by the general public in Uganda as compared to the US caused by poor prescription and drug adherence in Uganda [25].

Other studies have reported that in Africa, multidrug-resistant non-typhoidal salmonellae (NTS) are one of the leading causes of morbidity and high mortality in children less than 5 years of age [26]. In a study conducted in Nairobi, Kenya [26] the majority of NTS obtained from cases were Salmonella enterica serotype Typhimurium (106 out of 193; 54.9%) and Salmonella enterica serotype Enteritidis (64; 33.2%), a significant proportion (34.2%) of which were resistant to three or more antibiotics, including ampicillin, tetracycline, cotrimoxazole and chloramphenicol. In this study [26] 23.4% of NTS were fully susceptible to all 10 antibiotics that were tested while not a single isolate from Uganda was pan susceptible. Resistance observed in Salmonella isolates from Uganda reflected levels previously recorded among isolates from food animal species in Uganda [25]. Among cattle isolates resistance was high to drugs commonly used in Uganda; Tetracycline, Penicillin, Trimethoprim, Ampicillin, and Chloramphenicol, and less resistant to antibiotics less commonly used in the animal industry in Uganda including Amikacin, Ciprofloxacin, Ceftiofur, Cefoxitin [25]. Similarly this trend was also seen among human isolates from Uganda where low resistance was seen against Ciprofloxacin and Cefoxitin. However, high resistance was recorded against Chloromphenical, Trimethoprim, Gentamicin and Tetracycline as reported before [20] possibly due to management of bacteraemia among clinical cases in Kampala. The high resistance against Gentamicin had not been reported before, and is of concern because Gentamicin is currently recommended in combination with Ampicillin for the management of presumed bacteraemia in severely malnourished children [20].

In the US, third-generation cephalosporins (such as Ceftriaxone) and fluoroquinolones (such as Ciprofloxacin) are choice drugs for the treatment of Salmonella infections in humans [27]. The emergence of isolates resistant to Nalidixic acid with reduced susceptibilities to Ciprofloxacin is of great concern given the possibility of treatment failures [27]. Moreover the AMR patterns observed revealed higher resistance to Nalidixic acid among humans compared to cattle possibly due to flouroquinolone use in the treatment of invasive salmonellosis in adults which might have led to cross resistance [28]. High resistance of Salmonella isolates from cattle in the US to beta -lactam antimicrobials was observed .While this could be attributed to the occurrence of multiple drug resistant isolates, the specific use of some of these drugs in animal medicine, such as Ceftiofur (FDA approved for the treatment of bovine respiratory diseases) [1] could explain this phenomenon. This could also explain the considerable resistance observed against Ceftriaxone a drug in the same class with Ceftiofur (cross resistance) which is not used in animal medicine but is indicated for treatment of invasive salmonellosis in children [27]. Relatively low resistance to these set of drugs, Ceftriaxone, Cefoxitin, Ampicillin, Ciprofloxacin from the isolates from Uganda could be attributed to low access and the high cost of these drugs as previously reported [20,25]. Similarly the higher resistance against Kanamycin in the cattle isolates from ND could be attributed to cross resistance to Neomycin used in cattle for the control of E. coli associated morbidity and mortality. Interestingly, in Uganda resistance levels against Kanamycin in cattle were low which was contrary to what was observed in ND isolates and could possibly be due to low access to the drug in Uganda.

Unlike in humans, Chloramphenicol resistance in cattle was associated with presence of the penta-resistance phenotype-ACSSuT (Ampicillin, Chloramphenicol, Streptomycin, Sulfisoxazole and Tetracycline). This could be attributed to the prohibition of Chloramphenicol in food animals by the FDA because of its potential to induce aplastic anaemia in humans [29], whereas its use in human medicine still continues, for infections where other antimicrobials are not effective or contraindicated; sustained use of a drug could result in selection of resistance genes among commensal and pathogenic bacteria. Results from this study indicated a difference in the antimicrobial susceptibility of Salmonellae in different hosts, and from different geographical regions. Cattle isolates displayed a higher resistance than those from humans in North Dakota; the opposite was true for the isolates from Uganda possibly because the ND cattle isolates were from were clinical cases. Resistance patterns observed were similar to those seen among clinical NTS isolates from the region [30]. Selective pressure could also results in the proliferation and dissemination of such drug resistant strains [3,31]. Among the ND isolates the multi-drug resistant ACSSuT phenotype was the predominant phenotype as previously reported [29]. This phenotype has been linked to the emergence and spread of the multi drug resistant S. Typhimurium DT-104 [13]. The majority (75%, 21/28) of the MDR-AmpC isolates from the US were recovered from cattle. This is in agreement with previous reports [32] of its recovery only from diseased cattle. This finding has significant implications both in human and animal medicine [32]. In this study, 11(39%) of the MDR-AmpC isolates were also resistant to Trimethoprim-Sulfamethoxazole, 3 (11%) were also resistant to Nalidixic acid while 2 (7%) were resistant to Nalidixic acid only. Resistance against Nalidixic acid is a marker for the emergence of flouroquinolone resistance or reduced susceptibilities. Higher resistance against Ciprofloxacin and Nalidixic acid among the isolates from Uganda, especially the humans could possibly be attributed to the easy access to and questionable handling of these drugs [25].

The high incidence of integrons reported from the Salmonella isolates tested points toward their role in the spread of resistant genes; previous studies [23,33,30] have reported similar prevalence of class 1 integrons in Salmonella. Also, in this study we reported significant associations between resistance to several antimicrobials and presence of class 1 integrons. According to our study, class 1 integron explained a sizeable proportion of the multidrug resistant profiles observed. However not all MDR isolates had presence of integrons. Up to 51.4% (37/72) and 70% (251/359) of multi drug resistant Salmonella isolates from Uganda and ND, respectively, did not have class 1 integrons further confirming the presence of other mechanisms that mediate the observed resistance. This was supported by the observed attributable fractions (AF) of < 100% indicating that other mechanisms that mediate the observed resistance existed. Additionally,the high frequency of dfra1 (Trimethoprim) and aadA1 genes (Streptomycin) was not a surprise due to previous reports in the literature [34]. We observed that for the ND isolates, resistance against Streptomycin and Trimethoprim was largely mediated by presence of class 1 integron as had been earlier reported [9]. It is possible that Class 2 integrons also contributed to the carriage and dissemination of antimicrobial resistance genes in Uganda. Further research could focus on quantifying this association by estimating AFs for other mechanisms that code for AMR in Salmonella isolates.

Among Salmonella isolates from Uganda that were subjected to DNA sequencing, one isolate depicted substantial similarity (91%) to the Salmonella enterica subsp. enterica serovar Typhimurium plasmid pSLT-BT that was identified in Malawi and Kenya. This isolate was implicated in an epidemic of multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa [6]. This isolate had several resistant genes including aadA1 and dfrA1gene. Although this study provided useful information on AMR and possible

mediating mechanisms in Salmonella isolates from Uganda and ND, its widespread application was limited because archived samples were used therefore information on prior use of antimicrobials and previous history of hospitalization, which may be associated with bacterial resistance, was not established. .

Conclusion

These data indicated high AMR among antimicrobials widely used in veterinary and human medicine with several Salmonella isolates exhibiting multidrug resistance. AMR was observed against drugs whose veterinary use is restricted, implying possible horizontal transmission. To the best of our knowledge this was the second account of the role of integron 1 among Salmonellae in Uganda. These results signal serious implications in the treatment of salmonellosis in both public and animal health and underscore the need for further research into mechanisms that mediate antimicrobial resistance among Salmonellae.

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