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Citrobacter as a gastrointestinal pathogen, its prevalence and molecular characterization of antimicrobial resistant isolates in food-producing animals in Morogoro, Tanzania

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SUMMARY

Citrobacter is a gastrointestinal commensal of man and animals. The zoonotic *Citrobacter* spp. infection can occur if food products of animal origin are not hygienically handled. Therefore, the prevalence, antimicrobial resistance profile and resistance transmission mechanism of *Citrobacter* spp. in food-producing animals in Tanzania needs to be understood. *Citrobacter* isolates were recovered from 2.4% of the total of 1099 samples from apparently healthy animals. *Citrobacter* isolates were detected in 3.0% and 1.9% of the swine and the cattle samples, respectively. Over 80% of food products contamination with *Citrobacter* isolates originated from slaughtered cattle carcasses just before meat is transported to retail stores. About 62% of the isolates detected were resistant to at least one antimicrobial, whereas, 38.5% of the resistant isolates were exhibiting resistance to three or more antibiotic classes. All 26 presumptive *Citrobacter* isolates were screened for *invA*, *intI1* and *16S rRNA*. None of the isolates carried *invA*. Nearly 19% of the MDR *Citrobacter* isolates were found to carry an *intI1*. All *intI1*-positive isolates contained resistance gene cassettes *dfrA1*, *dfrA7* and *dfrA15*. Detection of resistance gene cassettes in the MDR *Citrobacter* isolates in animals and animal products represents a potential source for horizontal transfer of antimicrobial resistance genes and mobile genetic elements between pathogenic and non-pathogenic bacteria in the microbial population. The findings indicate that animal feces could one of the potential sources for contamination of animal products along the food production chain.

Keywords: MDR *Citrobacter*, Food-producing animals, Antimicrobial resistance, Class 1 integrons, Gene cassettes

INTRODUCTION

Gastrointestinal infection is among the most common infections experienced by humans including the urinary tract and respiratory infections. These infections are among the most common infections in both outpatients as well as hospitalized patients (Sami *et al*., 2017). *Citrobacter* spp. is associated with several infections, such as severe diarrhoea, urinary tract infections and pneumonia in humans with the neonatal meningitis can be fatal with up to 50% mortality rate among infants (Hossain *et al*., 2017).

The members of genus *Citrobacter* are Gram-negative, aerobic or facultative and rod shaped bacteria of the *Enterobacteriaceae* family, widely distributed in water, soil and food (Metri *et al*., 2013). They are known commensals of the intestinal tract of the higher vertebrates including humans and the animals (Liu *et al*., 2016). *Citrobacter* isolates are reported to be the third most common organisms causing urinary tract infections (UTI) in humans after *Escherichia coli* and *Klebsiella* species accounting for nearly 10% of all isolates (Valencia *et al*., 2009).

Detection of *Citrobacter* isolates from hospitalized patients is a common phenomenon. They are emerging as the commonest health care associated multi-drug resistant (MDR) pathogens accounting for 35-40% of the total health care infections, thus posing a serious public health threat (Ranjan and Ranjan, 2013). As reported by Bonadio *et al*. (2001), these organisms are acquired nosocomial pathogens in the health care facilities whose sensitivity to the array of common antimicrobials is dwindling as a result of the antimicrobial resistance due to uncontrolled and indiscriminate antimicrobial usage.

Antimicrobial resistant *Citrobacter* spp. might be introduced into food-producing animals through consumption of contaminated water and feeds, and the microbes may in due course enter the human food supply (Boonyasiri *et al*., 2014). Previous study conducted elsewhere reported a high load of members of *Enterobacteriaceae* family on the carcasses which are a clear indication of failure to realize proper preventive measures in producing safe meat for public consumption (Carrasco *et al*., 2012). The major source of contamination of the cattle carcasses is attributable to poor handling of

fecal matters during evisceration, bacterial load on the equipment used during the slaughter process (Hald *et al*., 2004; Teklu and Negussie, 2011).

The members of the Enterobacteriaceae family such as *Citrobacter* spp. have been reported to acquire antimicrobial resistance through different mechanisms, including mutations in genes and acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer by means of plasmids, transposons, bacteriophages and integrons (von Wintersdorff *et al*., 2016). The growing trend of antimicrobial resistance encountered in human is partly due to acquisition of already resistant pathogenic and commensal bacteria from food-producing animals and the human companion animals (Ohnishi *et al*., 2013).

Use of antimicrobials in livestock farming selects for drug-resistant bacteria and such use of antimicrobials creates ideal selective pressures for the propagation of resistant strains. The presence of *dfr* gene cassettes within class 1 integrons is partly an example of potential source of horizontal spread of resistance among bacteria present in different environments, including livestock, where antimicrobials are used for antimicrobial therapy and prophylaxis of food-producing animals (Mathew *et al*., 2007; Prescott, 2008). Therefore, the purpose of this study was to determine the prevalence, antimicrobial resistance profile and resistance transmission mechanism of *Citrobacter* isolates in food-producing animals in Tanzania which are not well understood.

Study area and sample collection

The study was carried out in Morogoro Municipality, Tanzania which lies between latitudes $5^0$7' to 10^0 0^0 S and between longitudes $35^0$6' - $39^0$5' E and at an elevation of 500 - 600 m above sea level and is about 200 km west of Dar es Salaam. The Municipal has a mixture of warm and cool temperatures ranging between 27 - 33.7^{0}C in the dry and warm season and 14.2 - 21.7^{0}C in cool and wet season. In addition, it experiences a sub-humid tropical climate with a bimodal rainfall pattern characterized by two rainfall seasons in a year with a dry season separating the short rains and long rains (Kashoma *et al*., 2015). Samples were collected from households (farms) with cattle and swine and slaughterhouses in Morogoro Municipal from February 2013 to March 2014. Fecal (n = 304), carcass (n = 148) and milk (n = 187) samples from cattle originating from the agro-pastoral communities, as well as from the dairy cattle farms were collected. Other samples from live animals the animal skin, the slaughterhouse personnel and included swine feces (n = 460) from farms with and without mixed farming. All samples were kept in a cool box before transporting to Sokoine University of Agriculture for further processing.

Citrobacter isolation and identification

Isolation of *Citrobacter* isolates in this study followed the same conventional methods as for isolation of *Salmonella* spp. as described previously (Gebreyes *et al*., 2004). This protocol was used for a reason that the two bacteria namely, *Citrobacter* and *Salmonella* spp. have little dissimilarity in terms of the methods of isolation and identification. Briefly, a 10g portion of each fecal sample were pre-enriched in 90 ml of buffered peptone water (BPW; Becton Dickinson, Sparks, MD) and in addition about 90 ml of BPW was added to each Whirl-Pak bag containing individual carcass and floor drag swabs, and incubated at 37°C for 24 h. A 100 µl of each pre-enriched suspension following overnight incubation was added into 9.9 ml of Rappaport-Vassilliadis (RV) enrichment broth (Becton Dickinson, Sparks, MD) and incubated at 42°C for 24 h. Following overnight incubation at 42°C, a 10 µl of each of the enriched suspension was inoculated onto Xylose-lysine deoxycholate (XLD) agar (Himedia, Mumbai, India) plates and incubated at 37°C for 24 h.

Three isolates from colonies with black centres were selected from each positive sample for biochemical tests. Each selected isolate from colonies with black centres were inoculated onto triple sugar iron (TSI) agar (Becton Dickinson, Sparks, MD) slants, Lysine iron agar (LIA) slants (Becton Dickinson, Sparks, MD) and urea broth (Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h. Other additional biochemical tests included, citrate utilization, oxidase and catalase tests. As a result of biochemical indeterminacies, the presumptuous isolates were stored at -80°C until further testing using *invA* Polymerase Chain Reaction (PCR) and *16S rRNA* gene sequencing to discriminate *Salmonella* from *Citrobacter* isolates.

Phenotypic characterisation

Citrobacter isolates were tested for antimicrobial susceptibility to a panel of 14 antimicrobials using Kirby-Bauer disc diffusion method (CLSI, 2002). The antimicrobial agents used and their respective disc potencies were as follows: ampicillin (Am; 10 µg/ml), amoxicillin-clavulanic acid (Ax; 30 µg/ml), amikacin (An; 30 µg/ml), ceftriaxone (Ce; 30 µg/ml), cephalothin (Ch; 30 µg/ml),

chloramphenicol (Cl; 30 µg/ml), ciprofloxacin (CIP; (Km; 30 µg/ml), streptomycin (S; 10 µg/ml), trimethoprim (TMP; 5 µg/ml), sulfisoxazole (Su; 250 µg/ml), and tetracycline (Te; 30 µg/ml). *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. *Citrobacter* isolates showing resistance to three or more antimicrobial agents were classified as multi-drug resistant (MDR) and those isolates with intermediate resistance profiles were considered susceptible.

Determination of *16S rRNA* and *invA* genes in Presumptuous isolates

Isolates were tested for the carriage of *invA* gene (*Salmonella* invasion gene) using a PCR. Absence of *invA* gene suggests that the isolates are tentatively *Citrobacter* isolates until tested for *16S rRNA* gene. Briefly, the isolates were inoculated onto tryptic soy agar (TSA) plates and incubated at 37°C for 24 h. The genomic DNA was extracted using the Qiagen DNeasy tissue kit according to the manufacturer's instructions (Qiagen Ambion, Austin, TX, USA). Primers used to amplify the *invA* gene included Forward (5'-TCGTCATTCCATTACCTACC-3') and Reverse (5'-AAACGTTGAAAAACTGAGGA-3'). The thermocycling conditions included Hot Start *Taq* activation at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and amplification was done in 35 cycles. The reaction mixture was kept at 72°C for 10 min after the final cycle (Hoorfar *et al*., 2000). A total of 26 presumptuous *Citrobacter* isolates were selected for *16S rRNA* sequencing. Primers used for amplification of the *16S rRNA* included 27F (5'-AGAGTTTGAT**YM**TGGCTCAG-3') and 907R (5'-CCGTCAATTC**M**TTTGAGTTT-3') (Mao *et al*., 2012). The PCR amplification conditions were initial denaturation at 95°C for 4 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and then the amplification cycle was repeated for a further 35 cycles and final extension was done at 72°C for 7 min. Ten microlitres of the PCR product of each isolate tested was electrophoresed on a 1% agarose gel stained with 5 µl of 10-mg/ml ethidium bromide for 1 h at 120 V using 0.5X Tris borate-EDTA (TBE) as running buffer. A 1-kb Plus DNA ladder was used as a molecular size marker.

Detection of class 1 integron and resistance gene cassettes

5 µg/ml), gentamicin (Gm; 10 µg/ml), kanamycin The presence of class 1 integron and gene cassettes integrated between conserved segments (5'-3'CS) of class 1 integrons were detected by PCR. Primers used for amplification of the *IntI1* included IntI1-F (5'- GCCTTGCTGTTCTTCTACGG-3') and IntI1-R (5'-GATGCCTGCTTGTTCTACGG-3') (Levesque *et al*., 1995) and those for conserved segments included 5'CS (5'-GGCATCCAAGCAGCAAG-3') and 3'CS (5'-AAGCAGACTTGACCTGA-3') (Ploy *et al*., 2000). The PCR temperature profile included Hot Start *Taq* activation at 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, and then a final extension step at 72^0C for 7 min (Lindstedt *et al*., 2003). About 10 µl of PCR product of each isolate tested was electrophoresed on a 1% agarose gel stained with 5 µl of 10-mg/ml ethidium bromide for 1 h at 120 V using 0.5X Tris borate-EDTA (TBE) as running buffer. A 1-kb Plus DNA ladder was used as a molecular size marker.

***16S rRNA* and Gene cassettes sequencing**

The PCR products generated for sequencing of *16S rRNA* and variable regions of gene cassettes of class 1 integrons were purified using ExoSAP-IT PCR clean-up method. Briefly, a 5 µl of each of the post-PCR reaction products and a 2 µl of ExoSAP-IT reagent (Miles Road, Cleveland, OH) were mixed together, followed by incubation at 37°C for 15 min and 80°C for 15 min. Following clean-up, a 10 µl of each purified PCR products were pre-mixed separately in the same tube with 5 µl of 5 pMol/µl of each sequencing primers. The pre-mixing and the submission were done according to the organization guidelines (GENEWIZ, South Plainfield, NJ).

DNA sequences analysis

All DNA reverse sequences were converted to match the complement DNA forward sequences using the online Reverse complement software (www.bioinformatics.org/sms/rev_comp.html). The two DNA sequences for each isolates namely, forward and the reverse complement) were aligned using ClustalW2 (www.ebi.ac.uk/clustalw) and trimmed to obtain the consensus DNA sequences. The consensus nucleotide sequences were chimera checked using online DECIPHER software (Wright *et al*., 2012). None of the 28 nucleotide sequences deciphered chimeras, were compared with available databases using the GenBank BLASTN to determine approximate phylogenetic affiliations (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic relationships were inferred by MEGA

6.0 (Tamura *et al*., 2013) using the Neighbor-Joining (N-J) method and the Kimura 2-P model of sequence evolution (Kimura 1980; Saitou and Nei, 1987).

Statistical analysis

Data were entered and validated in Microsoft excel 2007 (Ms Corp., Redmond, WA, USA) and analyzed using MedCalc® Version 12.7.1.0 (8400 Ostend Belgium). Descriptive statistics were computed to determine the prevalence of *Salmonella* from different animal species and sample types. In addition, the MedCalc® was used to compare the frequency of antimicrobial resistance of *Salmonella* spp. recovered from different animal species and sample types. A value of $P \leq 0.05$ was considered significant.

RESULTS

Prevalence of *Citrobacter* isolates in Food-producing Animals

Citrobacter isolates were detected from 26 (2.4%) of the total of 1099 feces samples from apparently healthy animals and animal products. The results showed that *Citrobacter* isolates were detected in 3.0% (14 of 460) and 1.9% (12 of 639) of the swine and cattle, respectively. A total of 2 *Citrobacter* isolates (0.7%, 2 of 304), 2 (1.1%, 2 of 187) and 8 (5.4%, 8 of 148) were recovered from the cattle feces, milk samples and the dressed cattle carcasses of Tanzania short-horned Zebu at the slaughterhouses, respectively. *Citrobacter* isolates recovery from swine and cattle samples was not significantly different between swine versus cattle group ($P = 0.3754$).

The proportion of *Citrobacter* isolates detected from cattle fecal samples was significantly lower than in the animal products (milk and carcass) (% difference = 8%, 95% CI, 4.2 to 11.9; $P = 0.0001$). The findings have shown that the two-thirds (66.7%, 8 of 12) of the detected *Citrobacter* isolates originated from the animal products. The odds of recovering *Citrobacter* isolates from animal products were 4.65 times higher than if the isolates were recovered from the cattle fecal samples (95% Confidence Interval [CI] for an odds ratio [OR] of 1.001 to 21.377, $P= 0.049$), indicating that one of the major sources of the contamination of the milk and cattle carcasses is partly attributable to poor handling of fecal matters during evisceration, bacteria on the animal skin, the personnel and the equipment used during milking and slaughter process.

Antimicrobial resistance and detection of resistance cassettes in class 1 integrons

According to the disc diffusion, the most common antimicrobial resistance was to cephalothin (46.2%), followed by amoxicillin-clavulanic acid (34.6%), ampicillin (26.9%), tetracycline (19.2%), trimethoprim (15.4%), streptomycin (15.4%), and sulfisoxazole (15.4%). There was no antimicrobial resistance found to amikacin, kanamycin, gentamycin, ciprofloxacin, chloramphenicol, ceftriaxone and ceftiofur. Of the 16 (61.5%, 16 of 26) *Citrobacter* isolates resistant to one or more antimicrobials, 50% (8 of 16) and 31.3% (5 of 16), 12.5% (2 of 16) and 6.3% (1 of 16) of the isolates were detected from the swine feces, dressed cattle carcass swabs, cattle feces and milk, respectively.

Over one-third (37.5%, 6 of 16) of the resistant *Citrobacter* isolates were detected from the animal products (carcasses and milk), 38.5% (10 of 26) of the isolates were pansusceptible and 38.5% (10 of 26) of the isolates were MDR *Citrobacter*. In addition, about 3 of 16 (18.8 %) of the resistant *Citrobacter* isolates amplified *intI1* gene and 100% (3/3) of *intI1*-positive isolates contained resistance gene cassettes known as *dfrA1-orfC*, *dfrA7* and *dfrA15* of size 1250 bp, 800 bp and 700 bp, respectively. The MDR *Citrobacter* isolates which contained the cassettes *dfrA1-orfC*, *dfrA7* and *dfrA15* showed the resistance types AmAxCfSuTeTMP, AmCfSSuTeTMP and AxCfSuTMP, respectively (Table 1).

Characterization of *16S rRNA* of *Citrobacter* isolates

Using MEGA 6 software, the phylogenetic affiliations of the *Citrobacter* isolates [n = 25], from cattle and swine sample types and a few members of the *Enterobacteriaceae* family as out-group members (n = 8) (*Salmonella* Typhimurium strain LT2 [NC003197.1], *Enterobacter asburiae* strain LF7a [NC015968.1], *Citrobacter rodentium* strain ICC168 [NC013716.1], *Salmonella* Anatum strain 315 [JQ694223.1] and *Escherichia coli* strain UMN026 [NC011751.1] from the GenBank and two *Salmonella* spp. from a parallel study were inferred. Based on *16S rRNA* sequence analysis, *Citrobacter* isolates and out-group members formed two distinct clades. All *Citrobacter* isolates (n = 26) in the first clade further aggregated to form two clusters. *Citrobacter* isolates (n = 14) were shown to cluster

to one of the two cluster at 99% sequence similarity index and the eleven other *Citrobacter* isolates (n = 11) with the out-group members [*Salmonella* Anatum, E. *coli* strain UMN026 (NC011751.1)] at 64% similarity index. The other members of the *Enterobacteriaceae* family used in this study for comparison were found to cluster as out-growers forming the second clade.

Table 1. Sequencing of resistant cassettes and 16S rRNA of Citrobacter isolates

Sample ID	Animal	Sample type	R- profile	Accession number	Gene sequenced
C.16746	Swine	Feces	Pansusceptible	KM986871	*16S rRNA*
C.16704	Swine	Feces	Pansusceptible	KM986865	*16S rRNA*
C.16741	Swine	Feces	Pansusceptible	KM986851	*16S rRNA*
C.16744	Swine	Feces	S	KM986852	*16S rRNA*
C.16699	Swine	Feces	AmCfSSuTeTMP	KM823525	*dfrA7*
C.16700	Swine	Feces	AmCfTe	KM986863	*16S rRNA*
C.16708	Swine	Feces	Pansusceptible	KM986872	*16S rRNA*
C.16756	Bovine	Carcass swab	Pansusceptible	KM986870	*16S rRNA*
C.16806	Bovine	Carcass swab	TMP	KM986855	*16S rRNA*
C.16694	Bovine	Carcass swab	AmAxCf	KM986848	*16S rRNA*
C.16702	Bovine	Carcass swab	AxCf	KM986849	*16S rRNA*
C.16693	Bovine	Carcass swab	Pansusceptible	KM986854	*16S rRNA*
C.16715	Bovine	Carcass swab	Pansusceptible	KM986856	*16S rRNA*
C.16790	Bovine	Carcass swab	AmAxCf	KM986859	*16S rRNA*
C.16791	Bovine	Carcass swab	Ax	KM986858	*16S rRNA*
C.16748	Bovine	Feces	AxCfSuTMP	KM823524	*dfrA15*
C.16696	Swine	Feces	Cf	KM986861	*16S rRNA*
C.16739	Swine	Feces	TeSSu	KM986850	*16S rRNA*
C.16679	Swine	Feces	AmAxCfS	KM986847	*16S rRNA*
C.16712	Bovine	Feces	AmAxCf	KM986853	*16S rRNA*
C.16697	Swine	Feces	Pansusceptible	KM986862	*16S rRNA*
C.16774	Swine	Feces	AxCfTe	KM986868	*16S rRNA*
C.16819	Swine	Feces	AmAxCfSuTeTMP	KM823521	*dfrA1*
C.16720	Swine	Feces	Pansusceptible	KM986860	*16S rRNA*
C.16775	Bovine	Milk	Cf	KM986869	*16S rRNA*
C.16778	Bovine	Milk	Pansusceptible	KM986867	*16S rRNA*

Antimicrobials: Ax, amoxacillin-clavulanic acid; Am, ampicillin; Cl, chloramphenicol; CIP, Ciprofloxacin; An, amikacin; Gm, gentamycin; Km, kanamycin; S, streptomycin; Su, sulfisoxasole; TMP, trimethoprim; Te, tetracycline; XLN, ceftiofur; Ce, ceftriaxone; Cf, cephalothin

DISCUSSION

Antimicrobial resistance is becoming one of the global devastating event ever recorded in human history (MacGowan and Macnaughton 2013). It poses a serious public health threat worldwide. The level of occurrence of resistant foodborne pathogens of the *Enterobacteriaceae* family including the *Citrobacter* isolates is increasingly high in developing countries. Since the *Citrobacter* isolates are gastrointestinal commensals of the food-producing animals they can contaminate food products of animal origin if are not hygienically handled (Nayar *et al*., 2014). Thus, the emergence of multidrug-resistant (MDR) foodborne pathogen such as *Citrobacter* isolates in food-producing animals is of public health concern because of the risk of transfer of antimicrobial resistant isolates or the resistance determinants to consumers through the food chain (Baquero *et al*., 2008).

Although *Citrobacter* spp. are less commonly isolated, they are emerging as a common nosocomial multidrug-resistant (MDR) pathogens in Tanzania. In a study conducted by Mshana *et al*. (2009), *Citrobacter* spp. were reported at a prevalence of 1%, whereas in this study, the *Citrobacter* isolates were detected at a prevalence of 2.4% and a bit higher prevalence than that reported by Ayoyi *et al*. (2017) from pregnant women. Other previous studies in Tanzania and East African region have also reported the prevalence of *Citrobacter* isolates in range of 1-2.5% in humans (Nabbugodi *et al*., 2015; Sekharan *et al*., 2017). This study reports the higher rates of prevalence of *Citrobacter* isolates in the animal products [milk (1.1%) and the dressed cattle carcasses (5.4%)] than in the cattle feces (0.7%). These higher prevalence of *Citrobacter* isolates on the cattle carcasses and milk than in the feces is a clear indication of failure to observe high levels of hygiene in producing safe meat and milk for public consumption. The major source of contamination of the dressed cattle carcasses could be attributed to contaminated slaughterhouse floor, improper handling of fecal

matters during evisceration, bacterial load on the animal skin, the personnel and the equipment used Arguello *et al*., 2012). Thus, the strict preventive measures need to be instituted to limit possible contamination of the milk and the dressed carcasses by foodborne pathogens (Funk *et al*., 2001; Kich *et al*., 2011).

Detection of class 1 integrons carrying resistance gene cassettes, namely: *dfrA1-orfC*, *dfrA7* and *dfrA15* of size 1250 bp, 800 bp and 700 bp from *Citrobacter* isolates is certainly one of the very indispensable findings in the current study. The *Citrobacter* isolates from which the three resistance gene cassettes were detected were multi-drug resistant (MDR) isolates containing the resistance types AmAxCfSuTeTMP, AmCfSSuTeTMP and AxCfSuTMP. The involvement of resistance gene cassettes are first reports from *Citrobacter* in food-producing animals in Tanzania. The involvement of class 1 integrons in carrying other resistance gene cassettes for horizontal transmission of resistance genes in bacteria was also reported in MDR *S*. Kentucky in Tanzania. This *aac(3)-Id–aadA7* gene, contained a single cassette array of 1500 bp which was shown to be transmitted by the class 1 integron-mediated MDR *S*. Kentucky (Sato *et al*, 2009). Detection of class 1 integrons in Tanzania was also reported from *Escherichia coli* and *S. enterica* subsp. *arizonae* from a new flock of lesser flamingoes imported from Tanzania to Hiroshima Zoological Park, Japan (Sato *et al*., 2009).

Nearly 62% of the *Citrobacter* isolates detected from the food-producing animals were resistant isolates and almost 40% of the isolates were MDR isolates implying that the magnitude of the antimicrobial resistance in *Citrobacter* isolates is increasingly high and the general public should be concerned of the resistant foodborne pathogens in the food chain. MDR *Citrobacter* spp. is becoming an importance nosocomial pathogen in health care settings. Any entry of such pathogens in immunocompromised and elderly persons, children may lead to life threatening conditions and the treatment options in many cases fail (Scallan *et al*., 2011; Kozak *et al*., 2014; Guerra *et al*., 2016.

Conclusions

In summary, this study was able to show the level of contamination of food products and the possible attributable causes for such contamination in the food products (dressed cattle carcasses and the milk) if no strict preventive measures are taken to limit the entry of foodborne pathogens in the food chain. This study also reports the involvement of class 1 integron in food products of animal origin and its

(Hald *et al*., 2003; Teklu and Negussie, 2011;

significance in horizontal transmission of resistance genes among the pathogenic and non-pathogenic bacteria in the animal body or in the environment.

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