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Molecular detection of Campylobacter species from human and cattle faecal samples in Kilosa District, Tanzania

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Abstract

A growing number of *Campylobacter* species other than *C. jejuni* and *C. coli* have been considered as emerging human and animal pathogens but their contribution to human gastroenteritis is poorly documented. This study aimed at detecting *Campylobacter* species from human and cattle faecal samples in Kilosa District, Tanzania using molecular techniques without culture. Seventy (70) faecal samples were collected from five diarrheic and 65 non-diarrheic human patients attending Kilosa District Hospital in Tanzania from July to October 2019. During the same period, 30 faecal samples were also collected from healthy cattle in the same district. Genus and species identification of *Campylobacter* was conducted on the samples using molecular techniques [the polymerase chain reaction (PCR) and 16S rRNA sequencing]. Phylogenetic analysis was carried out by comparison of the 16S rRNA gene sequences to reference strains by the Neighbor-Joining method in MEGA X. *Campylobacter* species detection rate by PCR was 65.7% (46/70) and 20% (6/30) in humans and cattle, respectively. There were five human diarrheic cases, four of which were positive for *Campylobacter* and of these, two were children ≤15 years of age. In humans, 16S rRNA sequencing revealed that *C. concisus* was the most predominant species occurring at a frequency of 37.8% (14/37), followed by uncultured *Campylobacter* spp. 24.3% (9/37) and *C. hominis* 21.6% (8/37). The least represented species were *C. jejuni* and *C. lanienae*, all occurring at 2.7% (1/37). In cattle, five (100%) sequenced PCR products matched with *C. lanienae*. Phylogenetic analysis revealed that with the exception of *C. lanienae*, 16S rRNA sequences of *Campylobacter* species were closely related to the reference strains used (Percent identity: 90.51-96.56%). Based on our findings, we recommend that molecular techniques, mainly PCR be adopted for the direct detection of *Campylobacter* species during laboratory screening and surveillance studies.

Keywords: *Campylobacter, molecular diagnosis, polymerase chain reaction, sequencing, gastroenteritis, humans, cattle, Tanzania*

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Introduction

Campylobacter species, one of the zoonotic pathogens causing gastroenteritis, are responsible for 96 million cases of diarrhoea in humans each year (Ruiz-Palacios, 2007; Havelaar *et al.,* 2013). *Campylobacter* has also been reported to cause infertility in cattle and abortions in sheep, goats, and cattle (Sahin *et al.,* 2017). Poultry, mainly chickens, are considered as the primary reservoir of *Campylobacter* but various domestic and wild animals have also been reported as potential sources of *Campylobacter* (Gahamanyi *et al.,* 2021). Apart from the animal reservoirs, it is likely that the natural environment (soil and water) plays a key role in transmission, either directly to humans or indirectly via farm animals (Bronowski *et al.,* 2014). The incidence of human cases of campylobacteriosis has been increasing in both developed and developing countries throughout the world (Kaakoush *et al.,* 2015; Sulaiman *et al.,* 2020). However, *Campylobacter* infections have been considered as hyperendemic in most of the low and middle-income countries (LMICs) (Coker *et al.,* 2002) and the major risk factors include eating contaminated poultry products or drinking unboiled water (Kaakoush *et al.,* 2015). In Africa, the prevalence of human campylobacteriosis varies from 7.7–18.5%, and *Campylobacter* is persistently found in stools of both diarrheic and non-diarrheic children (Gahamanyi *et al.,* 2020). This is often linked to poor hygiene and sanitation (Osbjer *et al.,* 2016). However, there is a limited number of reports on the prevalence of *Campylobacter* both in humans and animals probably because *Campylobacter* is not among the pathogens screened for by medical and veterinary hospitals in most of the developing countries. The occurrence of *Campylobacter* in Tanzania may be higher than what is reported due to the lack of enough reporting and antibiotic prescription is done before laboratory confirmation (Rukambile *et al.,* 2021). In some rural areas, people share the same house with livestock and the likelihood of contaminating kitchen utensils, environment, and playground with feces is high, and thus increasing the risk of contracting zoonotic infections (Rukambile *et al.,* 2021).

Apart from human gastroenteritis, *Campylobacter* infections have been associated with several complications such as the reactive arthritis (RA), Reiter's Syndrome (RS), irritable bowel syndrome (IBS), Guillain-Barré Syndrome (GBS), Inflammatory Bowel Disease (IBD), Crohn's disease (CD), and Ulcerative Colitis (UC) (Esan *et al.,* 2017).

It is known that the majority of human cases of campylobacteriosis are self-limiting and do not require antibiotic treatment (Guévremont *et al.,* 2006). However, treatment is advised for severe cases of *Campylobacter* infections especially among children and immune-compromised individuals (Kaakoush *et al.,* 2015). Drugs of choice include erythromycin (macrolide) and ciprofloxacin (quinolone) but *Campylobacter* strains that are resistant to these drugs are increasingly being reported (Sproston *et al.,* 2018). The increasing resistance is partly attributed to misuse of antimicrobials in both human and veterinary medicine (Guévremont *et al.,* 2006).

Campylobacter jejuni and *C. coli* account for more than 90% of human campylobacteriosis cases (Moore *et al.,* 2005). However, atypical *Campylobacter* species are gaining considerable attention as important human and animal pathogens (Zhang *et al.,* 2009; Man, 2011). Infections caused by *Campylobacter* are usually under-reported due to difficulties in isolation procedures (Lastovica, 2016). For instance, it has been estimated that 40% of the bacteria from human faeces diagnosed through microscopy cannot be cultured in the laboratory (Berg, 1996). In contrast to other gastrointestinal pathogens, the culturing of *Campylobacter* species is laborious due to their microaerophilic nature and vulnerability to temperature fluctuations (Park, 2002). Conventional procedures for the isolation

and identification of *Campylobacter* from various sources require bacterial enrichment for two days and subculturing to selective agar, followed by phenotypic identification (Jacob *et al.,* 2011). Furthermore, commonly used selective media and added antimicrobials may inhibit the growth of certain *Campylobacter* species (Bessede *et al.,* 2011). Species like *C. concisus*, *C. sputorum*, *C. curvus*, *C. rectus*, and some strains of *C. hyointestinalis* grow in a microaerobic atmosphere that is supplemented with hydrogen to facilitate their recovery (Jacob *et al.,* 2011). Moreover, *Campylobacter* species may become dormant as viable but non-culturable (VBNC) forms difficult to grow on commonly used media (Singh *et al.,* 2011) unless a primary enrichment step is included in the isolation protocol (Thornval *and* Hoorfar, 2021). Consequently, the epidemiology and role of non-*C. jejuni/C. coli* species in human gastroenteritis are not fully understood (Lastovica, 2006).

The emerging *Campylobacter* species have been neglected but the integration of molecular techniques and suitable culture media in current diagnostic tests has helped in promoting the awareness of atypical species as relevant human and animal pathogens (Lastovica, 2016). *Campylobacter concisus* has been associated with gastroenteritis, inflammatory bowel disease, gingivitis, and periodontitis (Man, 2011; Liu *et al.,* 2018). *Campylobacter hominis* has been isolated in a blood sample of a septicemic patient (Linscott *et al.,* 2005), while *C. gracilis* has been associated with bacteremia, head infections, periodontitis and empyema (Gorkiewicz *et al.,* 2003; Liu *et al.,* 2018). In cattle, the most commonly reported species are *C. fetus*, *C. lanienae*, *C. sputorum*, *C. jejuni*, and *C. hyointestinalis* (Linton *et al.,* 1997; Inglis and Kalischuk, 2003; Mshelia *et al.,* 2010).

The polymerase chain reaction (PCR) and other molecular diagnostic tests based on nucleic acids are attractive due to their benefits including their higher sensitivity, ease-of-use, improved turnaround time, relatively low cost, and potential to be fully automated (Ghosh *et al.,* 2014). The breakthrough in technology and easy access to commercial kits has led to shifting from traditional laboratory diagnostic techniques to the newer molecular ones (Amjad, 2020). The analysis of the 16S rRNA gene by PCR and sequencing techniques has assisted in the phylogenetic identification of *Campylobacter* species including those unidentified by conventional techniques (Al-Nasrawi, 2016). Some of the disadvantages of molecular-based techniques include expensive initial setups, lack of discrimination between living and dead bacteria, and not contributing to public health surveillance and outbreak investigations due to lack of isolates (Amjad, 2020).

Despite being time-consuming (up to five days to get pure colonies), *Campylobacter* isolation by culture is still useful as it allows to get pure colonies and testing of antimicrobial susceptibility (Ghosh *et al.,* 2014). Bacterial isolates are crucial for understanding and evaluating phenotypic and genotypic characteristics of individual isolates but they can also be used for surveillance studies by national or international organizations (McLain *et al.,* 2016).

In Tanzania and most of the low and middle-income countries (LMICs), the reports on the role of *Campylobacter* spp. in gastroenteritis are scanty due to limited capacity in laboratory diagnosis and the absence of national surveillance programmes (Coker *et al.,* 2002; Gahamanyi *et al.,* 2020). Thus, the information available for both human and animal campylobacteriosis is limited (Komba *et al.,* 2013) which undermines its importance as a public health concern. The current study aimed at molecular detection of *Campylobacter* species in human and cattle faecal samples in Kilosa District, Tanzania using PCR amplification of the 16S rRNA gene and Sanger sequencing.

Materials and methods

Study design and sample collection

This cross-sectional study was conducted in Kilosa district of east-central Tanzania (6° S and 8°S, and between 36° 30′ and 38°E) from July 2019 to October 2019. Human stool samples were randomly obtained from patients with abdominal discomfort seeking medical care at Kilosa District Hospital during the time of the study. We selected the 1st, 3rd, 5th, etc. patients reporting to the Microbiology Laboratory for stool examination. Cattle faecal samples were

randomly collected using sterile gloves from healthy lactating cows belonging to Mbumi ward. The ward was purposively selected based on the number of lactating cows and accessibility. Farms and cows were randomly selected by lottery. Identified farms were given numbers and selection was done by the lottery through picking the corresponding numbers. Upon reaching each farm, animals were also given numbers and the same procedure was repeated. The criteria to include the animals were being a lactating cow and apparently being healthy.

A total of 70 human stool samples and 30 cattle rectal grab faecal samples were collected in sterile dry screw-top containers containing Dimethyl Sulfoxide (DMSO), packed in a cool box, and transported to the Microbiology Laboratory at Sokoine University of Agriculture for DNA extraction within 8 hours of collection. In case DNA extraction could not be done within 8 hours of sample collection, the stool samples were refrigerated at 4^{o}C and processed the following day.

DNA extraction and Campylobacter species identification

Approximately, 1g faecal sample in DMSO was diluted (10% wt/vol) in buffered peptone water (BPW) (9 ml) and vortexed until the sample was thoroughly homogenized. Two hundred (200) µL of the homogenized faecal sample was used for genomic DNA extraction using Quick-DNA™ Faecal/Soil Microbe Microprep Kit (Zymo Research Corp, Irvine, California, USA) based on the manufacturer's instructions. Eluted DNA concentration and purity were checked using a NanoDrop™ spectrophotometer (Biochrom, Cambridge, England) before storage at -20^{o}C.

Detection of *Campylobacter* was done by multiplex PCR using specific primers *cj0414* for *C. jejuni* and *ask* for *C. coli* as previously described (Yamazaki-Matsune *et al.,* 2007). Then, PCR of the 16S rRNA gene was performed on DNA samples negative for *C. jejuni* and *C. coli* using genus-specific primers including a 19bp-forward primer (C412F) and an 18bp-reverse primer with complementary sequence (C1228R) as previously described (Linton *et al.,* 1996). Positive control DNA was extracted from *Campylobacter jejuni* (ATCC® 33560™) while deionized water was included as the negative control. The PCR final volume was 25 µL, including 12.5 µL of 2X Master Mix (Thermo Fisher Scientific, Seoul, South Korea), 1 µL (10 µM) of C412F primer, 1 µL (10 µM) of C1228R primer, 1 µL of template DNA, and 9.5 µL of sterile deionized water. All primers were made by Integrated DNA Technologies, Inc. (Singapore Science Park, Singapore).

The DNA amplification was performed using the model MiniAmp™ plus Thermal Cycler (Applied Biosystems, Massachusetts, USA). The cycling conditions used were initial denaturation at 95^{o}C for 5 minutes, 35 cycles each of 94^{o}C for 30 seconds, 55^{o}C for 45 seconds and 72^{o}C for 45 seconds, and a final extension at 72^{o}C for 7 minutes. The PCR products were held at 4^{o}C before analysis. PCR products (5µl) were mixed with 2µl Loading STAR (Dyne bio, Seongnam-si, Korea) diluted with 5µl of nuclease-free water and analyzed by gel electrophoresis where 10µl of the mixture was loaded onto 1.5% SeaKem® LE Agarose gel (Lonza Inc.-Rockland, Maine, USA) in 0.5X TAE buffer. After electrophoresis, PCR product bands were visualized using a Dual UV Transilluminator (Core Bio System, Huntington Beach, California, USA) under ultraviolet (UV) light. Then, the image was photographed with iBright™ CL1000 Imaging System (Thermo Fisher Scientific, Seoul, South Korea). The size of the amplification products (816bp) obtained was compared to the Dyne 100 bp DNA ladder (Dyne bio, Seongnam-si, Korea). The PCR products (816 bp) were purified using Pure Link ™ Quick PCR purification Kit (Invitrogen, Vilnius, Lithuania) and sequenced at SolGent (Solutions for Genetic technologies, Daejeon, South Korea) using the *Campylobacter* genus-specific primers by Sanger method. However, during purification of PCR products, nine samples from humans and one from cattle did not yield enough DNA required by the sequencing company.

Data analysis

The data were analyzed with GraphPad Prism 8.4.0 (GraphPad Software, La Jolla, California, USA; 2020). Descriptive statistics (frequencies and percentages) were computed to determine proportions for different attributes. The GenBank sequences with the best and the high scoring matches with sequences of this study were selected using the NCBI BLASTN search.

Sequences were edited, aligned, and analyzed using BioEdit sequence alignment software (version 7.2.6.1) (Hall, 1999). Multiple sequence alignment by Muscle (Edgar, 2004), computation of evolutionary distances by the Jukes-Cantor method (Jukes and Cantor, 1969), and the phylogenetic tree building by the Neighbor-Joining (NJ) method (Saitou and Nei, 1987) were done with the Molecular Evolutionary Genetics Analysis (MEGA X) software (MEGA Inc, Englewood, New Jersey, USA) (Kumar *et al.*, 2018). The phylogenetic analysis was carried out by comparing the sequences of this study to 16S rRNA genes of reference strains downloaded from LPSN (Parte *et al.,* 2020). To confirm the reliability of our analysis, bootstrap analysis was performed with 1,000 resampled datasets and it was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). All the 16S rRNA gene sequences derived from sequencing were submitted to GenBank for obtaining accession numbers.

Results

A total of 70 human stool samples (male=35; female=35) were collected. The age of patients ranged from 2 to 89 years with 10 patients (14.3%) being children ≤ 15 years of age. Overall, the detection rate of *Campylobacter* spp. in human samples was 65.7%. The PCR products with predicted size (816bp) were obtained in some of the screened samples (Figure 1). Of the *Campylobacter* spp. positive samples (n=46), 24 (52.2%) were from females and 22 (47.8%) were from males. *Campylobacter* species were detected in nine of the 10 (90%) children ≤ 15 years of age. Of the five diarrheic cases, four were positive for *Campylobacter*. Of the diarrheic patients, two were children ≤15 years. *Campylobacter* species were detected in six (20%) of the 30 faecal samples collected from cattle.

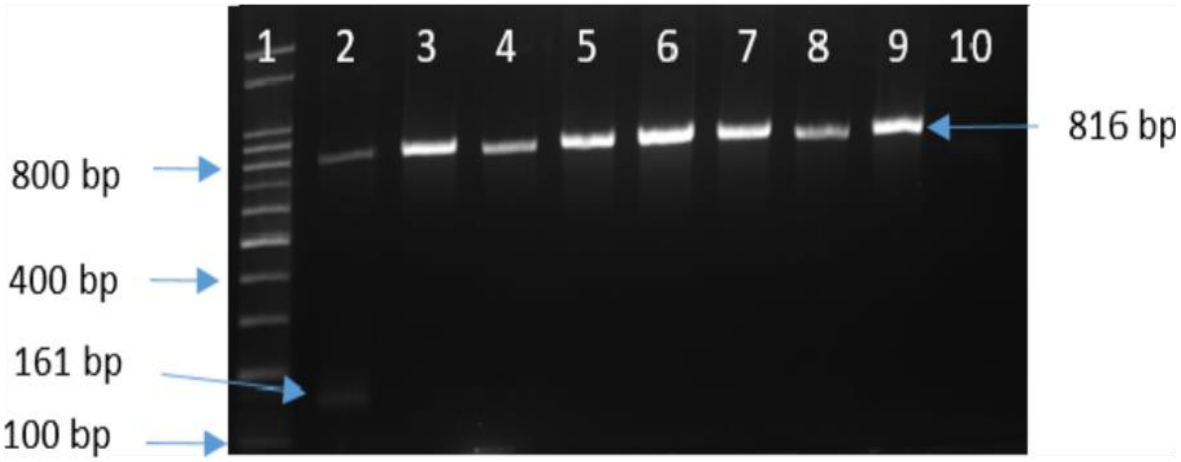

Figure 1. PCR products showing bands for Campylobacter genus (816 bp) and Campylobacter jejuni (161 bp). Lanes: 1: 100bp molecular weight marker; 2-4: bands from human samples; 5: positive control; 6-9: bands from cattle samples; 10: negative control.

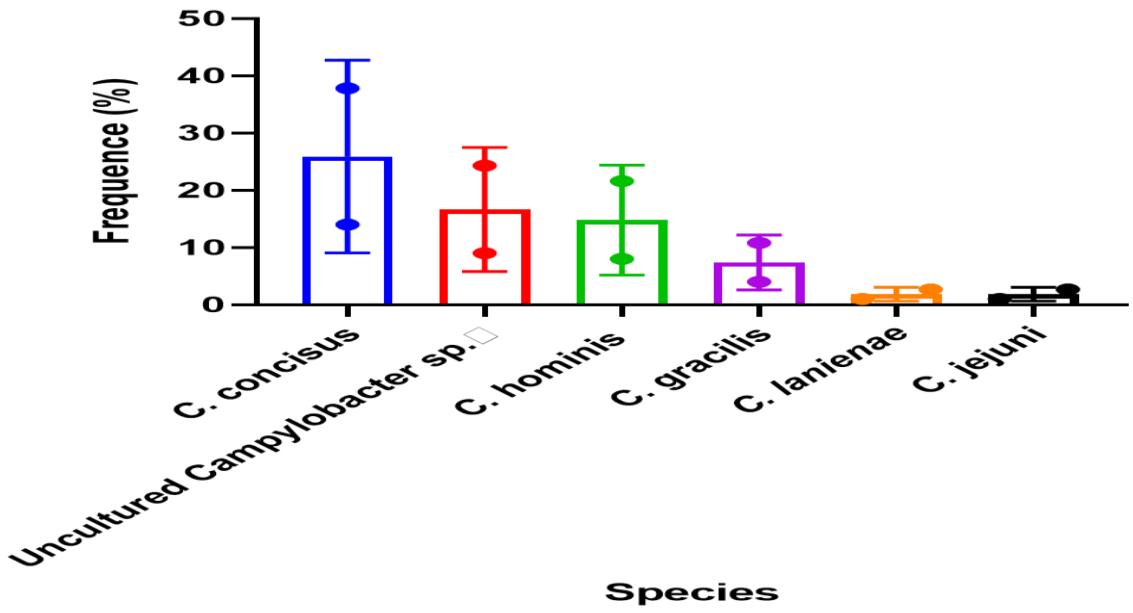

Figure 2. Distribution of Campylobacter species from human samples identified by sequencing. The dots on the whiskers of the box plot (outside the box) refer to the detection rates of each species in percentage while the dots inside the box refer to the frequency of each species

The results of sequencing confirmed the presence of *Campylobacter* species in all submitted sequences (37 from humans and 5 from cattle). The remaining PCR products did not give enough quantity of DNA required by the sequencing company after the purification step. The species were confirmed based on percent identity (above 99%), the query cover, and the E-value. In humans, C. *concisus* was the most prevalent (37.8%), followed by uncultured *Campylobacter* spp. (24.3%), *C. hominis* (21.6%), and *C. gracilis* (10.8%). *Campylobacter lanienae* and *C. jejuni* occurred at a frequency of 2.7% each (Figure 2). For cattle, all the five (100%) 16S rRNA sequences matched with *C. lanienae*. The percent identity between species was: *C. concisus* and *C. hominis* (92.91%), *C. concisus* and uncultured *Campylobacter* spp. (91.78%), *C. concisus* and *C. jejuni* (93.49%), and *C. jejuni* and *C. hominis* (91.14%). The pairwise distances between species were: *C. concisus* and *C. hominis* (0.07), *C. concisus* and uncultured *Campylobacter* spp. (0.08), *C. concisus* and *C. jejuni* (0.07), and *C. jejuni* and *C. hominis* (0.1). The mean genetic distance was 0.07.

The 16S rRNA genes of *Campylobacter* spp. from this study were compared with 16S rRNA sequences of different strains of *Campylobacter* spp. by BLASTN search. Following submission to the GenBank, gene sequences were allocated with the following accession numbers: MT126449 to MT126453; MT130973 to MT130991; and MT131150 to MT131167.

The phylogenetic analysis was carried out by comparing the 16S rRNA genes of this study to 16S rRNA genes of reference strains (*C. concisus*, *C. hominis*, *C. gracilis*, and *C. lanienae*) and uncultured *Campylobacter*. The analysis of sequence data from *Campylobacter* species in this study revealed a high nucleotide sequence similarity to different reference strains. *Campylobacter hominis* clustered closer to *C. gracilis* than it was with *C. concisus*. It was also noted that *C. lanienae* formed a separate cluster at the bottom of the tree. Uncultured *Campylobacter* and *Campylobacter* spp. RM 12175 were also found among the sequences of this study (Figure 3). The tree was rooted using *Helicobacter aurati* and *Arcobacter molluscorum*.

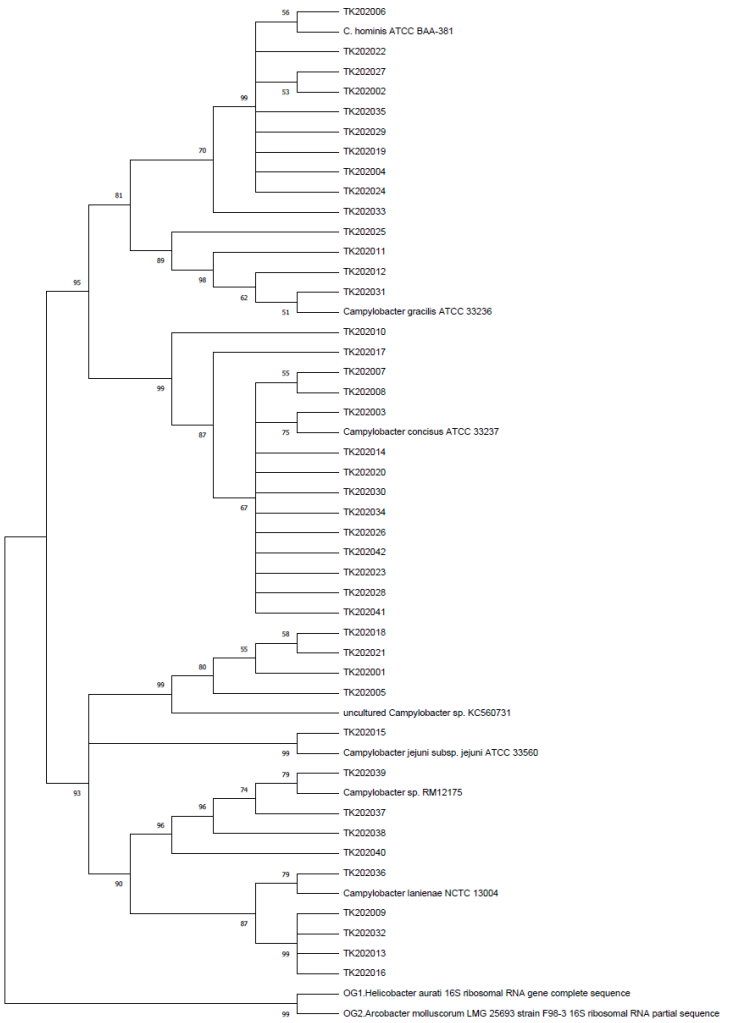

Figure 3. Molecular phylogenetic analysis of Campylobacter species using 16S rRNA sequences by the neighbor-joining method. Bootstrap values (%) based on 1000 replicates are indicated at nodes. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA X. Reference strains were included in the analysis. The tree was rooted using H. aurati and A. molluscorum

Discussion

The rapid, accurate isolation and characterization of food-borne pathogens can contribute to the prevention of infectious diseases and save lives by allowing early initiation of effective treatment (Vizzini *et al.,* 2019; Thornval *and* Hoorfar, 2021). Furthermore, molecular characterization of *Campylobacter* strains isolated from the same or different samples is very important in tracing human infections to potential sources. Molecular detection techniques based on PCR are fast, but require the extraction of genetic materials (DNA or RNA), specific instrumentation, highly trained laboratory personnel, and hence not suitable for rapid and point-of-care analysis (Vizzini *et al.,* 2019). These methods are also criticized for not being able to differentiate between leaving and dead cells (Thornval *and* Hoorfar, 2021).

For many years *C. jejuni* and *C. coli* have been the most commonly reported species of *Campylobacter* associated with gastroenteritis in humans (Moore *et al.,* 2005). Nonetheless, advances in molecular diagnostic techniques have proven that isolation by culture contributes to increased detection rates of *C. jejuni* and *C. coli* over other species which biases both the outcome of the diagnosis and the relative contribution of other species to *Campylobacter* infections (Bullman *et al.,* 2012). The routine culture-based methods fail to detect

over a third of *Campylobacter* positive samples (Bullman *et al.,* 2012). The scarcity of atypical *Campylobacter* species reported so far is attributed to the limitations and bias of culture-based methods (Bullman *et al.,* 2012). The low detection of other *Campylobacter* species has been associated with their fastidious nature like incubation temperatures, atmospheric conditions, length of incubation, nutrient requirements, and differing susceptibilities to antimicrobial agents required for optimal growth (Bojanić *et al.,* 2019). Also, some injured *Campylobacter* strains are capable of transforming into a viable but non-culturable (VBNC) state upon exposure to adverse conditions such as suboptimal nutrients, oxygen, osmotic pressure, temperature, and light which complicates their detection (Bullman *et al.,* 2012). This would require the use of enrichment media to enable the growth of desired ones over the contaminants which may overgrow *Campylobacter*. Therefore, this study reports the detection of *Campylobacter* spp. in both humans and cattle by molecular methods. To the best of our knowledge, there are limited reports in Tanzania detecting the presence of *Campylobacter* species directly from faeces without a culture-based approach. Previous authors adopted cultural methods (Komba *et al.,* 2015; Chuma *et al.,* 2016), which, probably, did not give a true picture of *Campylobacter*-related infections because some of the fastidious species may have failed to grow on selective media.

The reported detection rate of *Campylobacter* spp. (65.7%) in humans was similar to that reported in Nigeria (Gwimi *et al.,* 2015) but higher than those reported previously in Tanzania (Jacob *et al.,* 2011; Chuma *et al.,* 2016), Fiji (Devi *et al.,* 2014), India (Salim *et al.,* 2014) and Cambodia (Osbjer *et al.,* 2016). These observations, probably indicate that the true incidence of non-*C. jejuni*/*C. coli* species is probably underestimated as one of the major contributors to human gastroenteritis where routine detection of *Campylobacter* by culture methods is still used for diagnosis (Bullman *et al.,* 2012). The difference in detection rates could be attributed to the sampling strategy, isolation methods, PCR conditions, level of sanitation, and geographically related variations. *Campylobacter concisus* and *C. hominis* were the most predominant species occurring at 37.8% and 21.6%, respectively. This concurs with the findings reported in Denmark (Aabenhus *et al.,* 2002) and Australia (Mahendran *et al.,* 2011). In South Africa, It was previously reported that *C. concisus* was the second species with a higher prevalence after *C. jejuni* (Lastovica, 2016). However, *C. concisus* has been isolated from diarrheic patients without other pathogenic microorganisms suggesting that it could be an emerging cause of human gastroenteritis (Mahendran *et al.,* 2011; Lastovica, 2016). *Campylobacter concisus* has been reported to be an oral bacterium causing gingivitis and periodontitis but also playing a role in the development of Crohn's disease (CD), a special form of inflammatory bowel disease (IBD) in humans (Liu *et al*., 2018).

Campylobacter lanienae was detected in both human stool and cattle faecal samples. It has previously been recovered from healthy pigs and cattle (Guévremont *et al.,* 2008). This species has been reported as a probable aetiological agent of human gastroenteritis (Lévesque *et al.,* 2016). However, other workers have suggested that *C. lanienae* has restricted pathogenicity or is a non-pathogenic *Campylobacter* (Costa and Iraola, 2019). *Campylobacter lanienae* was reported to be closely related to the *C. fetus* group but with two special features : (i) additional four to 10 flagellin genes and (ii) the lack of genes necessary for selenium metabolism (Miller *et al.,* 2017). The detection rate of this species was higher than the one previously reported (Inglis and Kalischuk, 2003). Further characterization of *C. lanienae* could shed more light on its genetic diversity and source (Guévremont *et al.,* 2008). The limited literature on *C. lanienae* does not allow us to critically assess its epidemiology and the reasons behind its infrequent detection.

Data on concurrent isolation of *Campylobacter* species in both humans and cattle are limited. In this study, the detection rates were 65.7% and 20%, in humans and cattle, respectively. Our findings showed higher detection rates when compared to the rates reported earlier in Tanzania (Kusiluka *et al.,* 2005), and Cambodia (Osbjer *et al.,* 2016). However, previous studies started by culturing the faeces which may justify the low detection rates. Further comparative studies on *Campylobacter* species isolated from humans and cattle are necessary to understand

their epidemiology and be able to explain the source of infection. However, specific molecular techniques like the whole-genome sequencing (WGS) and multilocus sequence typing (MLST) would be appropriate for source attribution studies.

Since the development of the polymerase chain reaction (PCR) and DNA sequencing, comparison of the gene sequences of various bacterial species has shown that the 16S ribosomal RNA gene is highly conserved within a species and among species of the same genus and thus, it can be used as the gold standard for speciation of bacteria (Woo *et al.,* 2002). Phylogenetic analysis based on the 16S rRNA gene is of paramount importance for bacterial taxonomy (Dewhirst *et al.,* 2005) and it has been applied to *Brucella* (Ntirandekura *et al.,* 2020) and *Campylobacter* identification (Gorkiewicz *et al.,* 2003). Our findings concur with the reported species of non-*C. jejuni*/*C. coli* group with *C. concisus* being the predominant species (Mukhopadhya *et al.,* 2011). In cattle, our results are in agreement with previously reported occurrences where *C. lanienae* had higher proportions compared to *C. jejuni* and C. *hyointestinalis* (Guévremont *et al.,* 2008). Figure 3 highlights the taxonomic position of the strains obtained in this study compared to reference strains and supported the sequencing results.

The current study had some limitations including the sample size and lack of culture-based species identification. The sample size used could not allow us to estimate the prevalence or generalize the findings at national or regional levels. This could affect also the diversity of identified *Campylobacter* strains. Considering that we did not culture the stool samples, the comparison is made based on previous studies carried in sometimes different conditions or settings. Another challenge was the inability to check antimicrobial resistance genes in extracted DNA due to the presence of various DNA from faecal microbiota. However, this study highlights the advantages of molecular methods over culture-based ones in the detection of *Campylobacter* spp. in clinical samples. We recommend further studies on the burden of disease due to emerging *Campylobacter* species and associated sequelae necessary for informing policymakers and guide in designing appropriate public health interventions.

Conclusion

The findings of this study highlight the higher detection rates of the less-frequently isolated *Campylobacter* species (*C. concisus* and *C. hominis*) in patients attending Kilosa District Hospital. *Campylobacter lanienae* was mainly detected in cattle faecal samples suggesting cattle as another possible reservoir. These *Campylobacter* species are often neglected due to their cultural behavior and fastidious nature but, they have proven to be zoonotic with a public health concern. It is therefore, important that health practitioners and public health authorities recognize the possibilities of occurrence of *Campylobacter* species other than *C. jejuni* and *C. coli* which are not screened on a routine basis in many countries and hence, go unreported. Molecular-based techniques offer an alternative to culture-based methods especially when it comes to the atypical *Campylobacter* species as they provide results in a short time and up to species level.

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