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Full Length Research Paper

Prevalence, haemolysis and antibiograms of *Campylobacters* isolated from pigs from three farm settlements in Venda region, Limpopo province, South Africa

P. O. Uaboi-Egbenni[1*], P. O. Bessong[2], A. Samie[3] and C. L. Obi[4]

[1]Department of Microbiology, University of Venda, P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa.
[2]Department of Microbiology, University of Venda, P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa.
[3]Department of Microbiology, University of Venda, P.M.B. 5050, Thohoyandou, Limpopo province, South Africa.
[4]Academic Affairs and Research, Walter Sisulu University, Mthatha, Eastern Cape, South Africa.

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We investigated the prevalence, haemolytic activities and antibiotic susceptibility profiles of *Campylobacter* species isolated from pigs in three farms in the Venda region, Limpopo province, South Africa. During the period of investigation, which spanned over one year, 450 faeces samples from pigs comprising 150 each from the three farms were collected and appropriately transported to the base laboratory at the Department of Microbiology, University of Venda for analysis. In total, the prevalence rate of *Campylobacter* isolates from all three farms was 30.2%, whereas, the rates of 25 - 26% were recorded for farms X, Y and Z, respectively. Out of the isolated *Campylobacter* species 10 (12.5%) were identified as *Campylobacter jejuni* and 70(87.5%) as *Campylobacter coli*. Of the *Campylobacter* isolates from the farms, 23, 22 and 35 were β-haemolytic from farms X, Y and Z, respectively. Susceptibility of the isolates to 12 antibiotics was determined by disc diffusion technique. The overall rate of resistance determined in this study to most of the antibiotics was 84.2%. Most *Campylobacter* isolates from farm X (< 50%) were resistant to ciprofloxacin, cefexime, and erythromycin, while > 50% were resistant to tetracycline, (54.5%) to vancomycin, (100%) to ampicillin and methicillin. The rates of resistance to these antibiotics were not significantly different from farms X and Y ($p < 0.005$). However, most isolates from farm Y were more resistant to ciprofloxacin (57.1%) than isolates from farm X (36.4%) and Z (40.7%). Resistance rates to tetracycline by *C. coli* isolated from farm Y (48%), farm Z (45.5%) and farm X (44.4%) were noted. Similar trend was observed for *C. coli* from farm Y (40%), farm Z (31.8%) and farm X (22.2%) for ciprofloxacin. All *C. coli* from farm Z showed higher level of resistance to ampicillin (100%), Y (64%) and X (55.6%). The *C. jejuni* isolates though few were markedly resistant to fluoroquinolone and the macrolide as well as aminoglycoside antibiotics. Most of the isolates were resistant to at least seven (7) of the antibiotics tested. These observations indicate high levels of resistance to antibiotics commonly used in the swine industry (erythromycin and tetracycline) among the *C. coli* and *C. jejuni* isolates from pigs. Erythromycin and ciprofloxacin are drugs for treatment of human campylobacteriosis. The high prevalence rate of resistance to these drugs among *C. coli* and *C. jejuni* isolates from pig faeces is of public health significance.

Key word: *Campylobacter*, campylobacteriosis, haemolytic, susceptibility, resistance, profiles.

INTRODUCTION

Campylobacter, mainly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as major causes of acute bacterial food-borne gastroenteritis (World Health Organization, 2002). The incidence of human campylobacteriosis in the European Union has increased over the past years and recently exceeded that of *Salmonella* in

*Corresponding author. E-mail: uaboiegbenni@yahoo.com. Tel: +27 15 962 8186.

many countries. Although campylobacteriosis is usually a self-limiting diarrheal disease, severe complications such as septicemia, reactive arthritis, and Guillain-Barre syndrome sometimes occur (Humphrey et al., 2007).

C. jejuni colonize the intestinal tracts of a large number of mammals and birds. Broilers are often carriers of *C. jejuni*. In Thailand, *Campylobacter* species were isolated from 12% of various food samples including pork, chicken and vegetables in Bangkok (Rasrinual et al., 1988; Young, 2005), 40% poultry ceca in India (Das et al., 1996), and 68-100% of poultry samples from retail markets in Taiwan (Shih, 2000).

Reports on *Campylobacter* in pigs revealed higher proportions of *C. coli* than *C. jejuni,* whether they have enteritis or not (Harvey et al., 1999; Steinhauserova et al., 2001). In the U.S., *Campylobacter* species were isolated from 76% gilts, 100% of pregnant sows, 57.8% of newborn piglets, and 100% of weaning pigs (Young et al., 2000). In the Netherlands, fifty-percent of piglets were infected with the same serotypes at seven days of age (Weijtens et al., 1997). The average number of *Campylobacter* colonizing the gut decreased toward the end of the rearing period (Weijtens et al., 1999). A study in Belgium reported the prevalence of *Campylobacter* sp. on pig carcasses at slaughterhouses to be 2% (Korsak et al., 1998), and *Campylobacter* was found in 1.3% of samples from pork from a retail market in U.S. (Duffy et al., 2001). Although the poultry industry has often be considered primarily responsible for human campylobacteriosis, the pork industry has equally been identified as a potential source of human infection (Allos, 2001; Berndtson et al., 1996). Uaboi-Egbenni (2008) also documented the prevalence of campylobacters in dogs and guinea-fowl in Nigeria.

The gastrointestinal tracts of other food animal species have also been shown to be frequently colonized with campylobacters, particularly, *C. jejuni* and *C. coli* (Minihan et al., 2004). Reported rates of *Campylobacter* carriage in food animals have varied widely between studies (Busato et al., 1999). The high prevalence of campylobacters in pigs has been reported previously in numerous studies and dressed pig carcasses have been shown to be more frequently contaminated than either beef or sheep (Nesbakken et al., 2003). This is most likely attributable to the fact that pig carcasses undergo a communal scalding process combined with the fact that the skin remains on the carcass following all of the dressing procedures (Moore et al., 2005).

Thermotolerant campylobacters (*C. jejuni/C. coli*) constitute the most frequent cause of intestinal infections worldwide. The clinical spectrum of *Campylobacter* enteritis ranges from a watery, nonbloody, noninflammatory diarrhea to a severe inflammatory diarrhoea with abdominal pain and fever. Disease is less severe in developing countries than in developed countries. The disease is characterized by bloody stool, fever, and abdominal pain that is often more severe than that observed in *Shigella* and *Salmonella* infections. In developing countries the features reported are watery stool, fever, abdominal pain, vomiting, dehydration, asthenia, anorexia and presence of faecal leukocyte; patients are also often underweight and malnourished (Rao et al., 2001; Coker et al., 2002). Stools remain positive for several weeks.

Treatment appears to be beneficial if it is administered early enough in the course of the disease (Salazar-Lindo et al., 1986). The recommended drugs are erythromycin, or amoxicillin or a fluoroquinolone (ciprofloxacin, norfloxacin) or tetracycline, provided the bacterium has not acquired a resistance. However, the emergence of antibiotic resistant strains has further opened a new dimension as to how to combat the disease together with the emerging resistant strains. In 1988, the emergence of fluoroquinolone resistance in *Campylobacter* strains was first reported in Spain, and since then the emergence of fluoroquinolone resistance has been identified in many countries, including Finland, the Netherlands, England, and Canada (Berndston et al., 1996), but not in South Africa. In Spain, 100% of *Campylobacter* strains isolated from broilers and pigs were found to be resistant to fluoroquinolones (Berndtson et al., 1996).

There is a dearth of information and research on the prevalence, epidemiology and antibiograms of campylobacters in pigs in South Africa in spite of the reports in Europe and other parts of the world on pigs as potential sources of infections for humans. This study therefore ascertained the prevalence, antibiotic susceptibility profiles and haemolytic activities of campylobacters isolated from pigs in three farms in the Venda region, Limpopo province of South Africa in order to provide updated information and data on the research subject.

MATERIALS AND METHODS

Collection of faeces

A total of four hundred and fifty (450) freshly voided faeces comprising one hundred and fifty from each of the three farms were collected at random from pigs in sterile 50 ml plastic containers and were adequately kept in coolers packed with crushed ice, stored at 4°C and immediately transported to the laboratory for analysis. The faeces were immediately processed. About 2 gm of the samples was transferred to 6 ml of sterile phosphate-buffered saline and left to emulsify at room temperature for 10 - 20 min to release the bacteria. The suspension and bacteria were used directly for the identification of *Campylobacter*. The three (3) sampling sites are separated from each other by about 200 km but are located in the same province. The three farms were code named X, Y, and Z for ethical reasons.

Isolation and identification of *Campylobacter* sp. by conventional culture methods

Ten microlitres of the faecal suspension was spread on the surface of a charcoal cefoperazone deoxycholate agar plates (CM 739 [Oxoid] with cefoperazone supplement SR 155E). The plates were incubated under microaerophilic conditions employing the Campygen gas generating kit (Oxoid CM025) at 42°C for 48 h.

Colonies suspected to be *Campylobacter* were further purified on blood agar plates (Blood Agar Base No.2 (Oxoid) supplemented with 5% sterile laked horse blood). All the isolates were characterized by their catalase, oxidase reactions, hydrogen sulphide production, indoxyl acetate, hippurate and susceptibility to nalidixic acid by standard procedures (On and Holmes, 1991, 1992). The resulting isolates were subsequently stored at -80°C in brain heart infusion broth with 15% glycerol until further investigation.

Confirmation of presumptive positive *Campylobacter* strains

Presumptive identification of *Campylobacter* isolates was done using the Dryspot *Campylobacter* test kit (Oxoid Basingstoke, Hampshire England). The test is specific for pathogenic *Campylobacter* strains belonging to *C. jejuni, C. coli, Campylobacter upsaliensis*, and *Campylobacter lari*. However, the other *Campylobacter* species are not included in this identification scheme. The manufacturer's instructions were strictly adhered to in this procedure. Briefly, 48 h cultures of suspected *Campylobacter* showing the usual cultural characteristics were selected. The reagents were removed from the refrigerator and allowed to attain room temperature according to the manufacturer's instructions. The extraction tubes were placed in test tube rack. Thereafter, one drop of extraction reagent 1 was added into the extraction tube. Sufficient growth of the suspected *Campylobacter* culture was removed to fill the inner diameter of a sterile loop. These cells were thoroughly suspended in a drop of reagent 1. The loop was allowed to stay in this reagent for 3 min. Two drops of extraction reagent 2 was added to the previous mixture and mixed thoroughly using the retained loop. With the aid of a paddle pastette provided with the kit, 1 drop (50 µl) of the neutralized extract was placed onto the test circle and a drop onto the control circle. With the aid of the flat end of the paddle pastette, the extract was mixed into the dry control reagent spot until completely suspended to cover the reaction area. The same pastette was used to repeat this procedure for the test reagent. The test cards were rocked for about 3 min. This process was repeated for all suspected *Campylobacter* isolates. Agglutination under normal lighting condition indicated that the organism was *Campylobacter*.

The presumptive positive campylobacters were further subjected to Mast diagnostic *Campylobacter* kits consisting of urease, indoxyl acetate and hippurate test solution. Briefly, 24 h cultures of the *Campylobacter* were inoculated into the urease and hippurate test solutions according to the manufacturer's instructions. These were then incubated for 4 h for colour development. For urease, development of pink colour was indicative of urease enzyme production (*C. lari*), while development of pink colour in hippurate solution indicated production of hippuricase enzyme (*C. jejuni*). In the case of indoxyl acetate solution, change of colour from colourless to blue/purple was indicative of the presence of *C. jejuni* or *C. coli*. The indoxyl acetate strips were impregnated with wet cultures and allowed to stay for 3 - 5 min. Development of purple colour within this period was indicative of positive reaction (*C. jejuni* and *C. coli*) (Popovic-Uroic et al., 1990; On and Holmes, 1992; Elviss et al., 2008).

Blood haemolysis test

To ascertain pathogenicity of isolates, the identified *Campylobacter* sp. were subjected to haemolytic test according to the procedure of Samie et al. (2007). Briefly, a 24 h broth culture of *Campylobacter* sp. were cultured by spread plate method on Columbia agar supplemented with defibrinated sheep red blood cells. Plates were incubated at 37 ± 2°C for 24 h. Thereafter, plates were observed for complete, partial and no haemolysis.

Antimicrobial agents

The antibiotics tested in this study were: Trimethroprim (2.5 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tetracycline (30 µg), ampicillin (10 µg), erythromycin (15 µg), streptomycin (10 µg), methicillin (µg), cefexime (30 µg), imipenem (µg), kanamycin (30 µg) and vancomycin (30 µg) (Oxoid, Unipath Ltd, Basingstoke, England).

Antimicrobial sensitivity testing

The method of Gaudreau and Gilbert (1997) was used. Briefly, the confirmed *Campylobacter* isolates were inoculated into plates of Mueller-Hinton agar supplemented with 5% sheep red blood cells in plates carrying a maximum of five (5) antimicrobial discs. All plates were incubated at 35°C under a microaerophilic atmosphere obtained with a gas generator envelope (Oxoid, Unipath Ltd, Basingstoke, England) for 24 h. The resulting zone diameters were measured with a graduated metre rule.

Preparation of bacterial genome

Genomic DNA was obtained by the whole-cell lysate method as described by Marshall et al. (1999). Briefly, cells from a 24 - 48 h culture grown on Columbia blood agar were resuspended in sterile distilled water to an optical density of 2.5 at 540 nm. The suspensions were boiled 100°C for 20 min in Eppendorf tube. The resulting templates were either used immediately for polymerase chain reaction (PCR) or were kept at 4°C for up to 1 month.

PCR identification of *Campylobacter* strains

In order to ascertain if the dryspot positive *Campylobacter* isolates were genuinely campylobacters, they were subjected to PCR identification using the general primers for the identification of *Campylobacter*. These primers are also specific for other members of the campylobacteriaceae (*Helicobacter* and *Arcobacter*). However, *Arcobacter* and *Helicobacter* sp. show negative reaction to the *Campylobacter* dryspot kit. Hence, any amplification of the primer sequences at the 1,004-bp fragment within the coding region of 16S rRNA confirmed such isolates as *Campylobacter* sp. and not *Helicobacter* or *Arcobacter* sp. The PCR-restriction fragment length polymorphism (RFLP) method used in this study was as previously described by Marshall et al. (1999). Briefly, amplification was done in 50-µl reaction volume containing 5 µl of whole-cell lysate, 1 µl each primer, 10x buffer (Roche), 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide (Roche) and 5U Taq DNA polymerase (Roche). The PCR amplification was performed with a – thermocycler (ESCO Swift Mini Thermal Cycler Version 1.0, ESCO Technologies, Philadelphia U.SA). The samples were subjected to an initial denaturation for 2 min at 95°C, followed by 30 amplification cycles, each consisting of 94°C for 30 s, 52°C for 30 s, and 72°C for 90 s. A final primer extension at 72°C for 10 min was included. Oligonucleotides primers employed in this study were CAH16S 1a (5' – AAT ACA TCA AAG TCG AAC GA – 3') and CAH16S 1b (TTA ACC CAA CAT CTG ACG AC – 3'), respectively. The oligonucleotides used in this study were synthesized by Roche Applied Science (Manheim, Germany).

RESULTS

Cultural studies

A total of four hundred and fifty (450) freshly voided

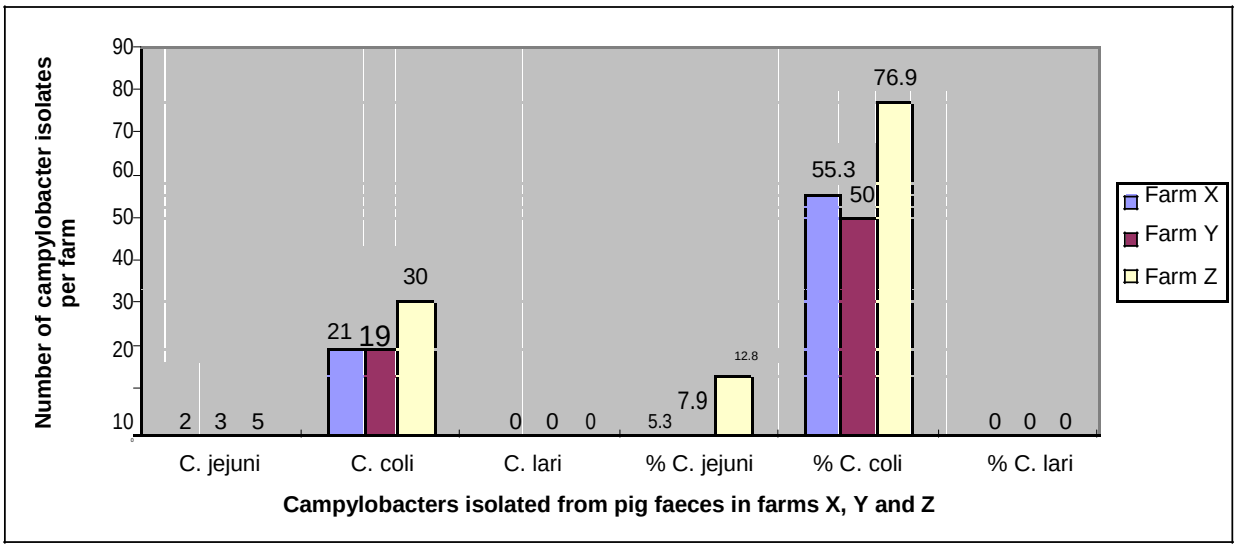

Fig.1 : Shows the number of *C. jejuni , C. coli, C. lari* and their percentages isolated from three pig farms in South Africa

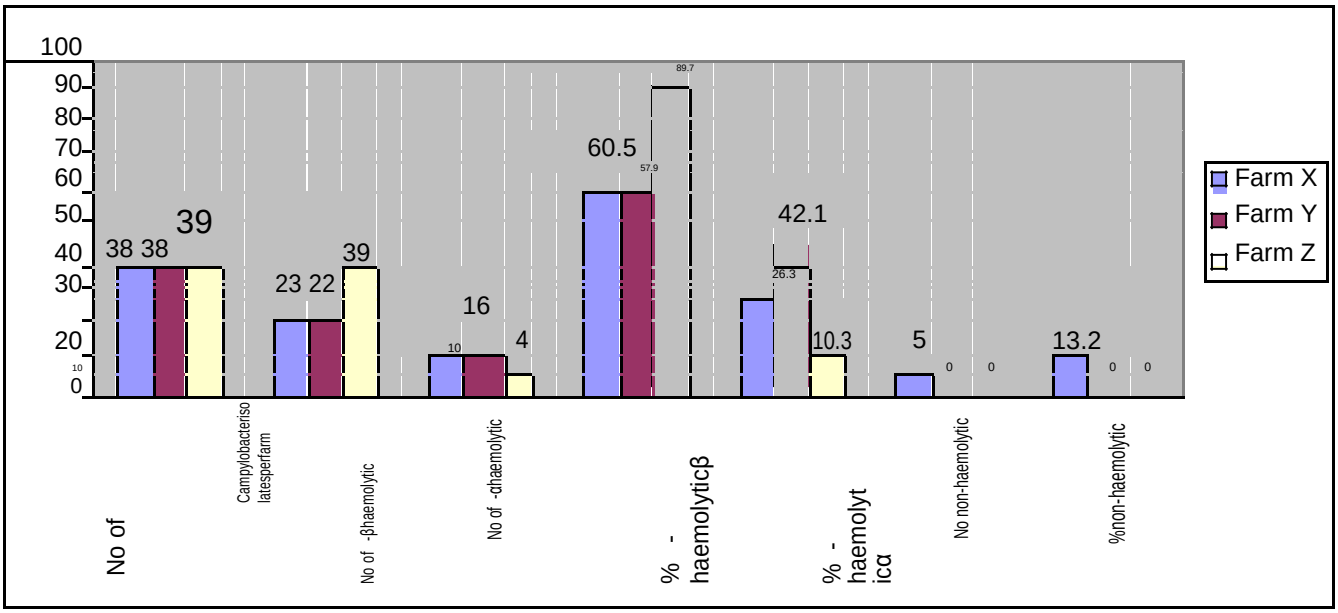

Fig. 2: Results of haemolysis of sheep red blood cells by *Campylobacter* isolates

faecal samples were processed for the detection of campylobacters over the course of the study. Of the 138 presumptive isolates from pigs on mCCDA from the farms, 25.6% (115 out of 450) were confirmed as campylobacters. Figures 1 and 2 show the percentage incidence of *Campylobacter* strains from pig faeces in the three (3) farms. From, there was an equal percentage of occurrence of campylobacters in farms X and Y with a value of 25.3%. However, there was a higher incidence of campylobacters among pigs in farm Z (26%) than in farms X and Y. It must be noted here that some strains that could not be resuscitated did not form part of the analysis and subsequent studies. With the aid of the *Campylobacter* dryspot and Mast diagnostic kits, 2(5.3%) of the isolates from farm X were identified as *C. jejuni*, while 21 (55.3%) were identified as *C coli*. In farm Y, 3 (7.9%) were *C. jejuni*, while 19 (50%) were *C. coli*. In farm Z, 5 (12.3%) were *C. jejuni*, while 30 (76.9%) were *C. coli*. Overall, the prevalence of *C. jejuni* among pigs in the three farms was 10 (12.5%), while it was 70 (87.5%) for *C. coli* (Figure 2). From this finding, there was a preponderance of *C. coli* compared with *C. jejuni* in all

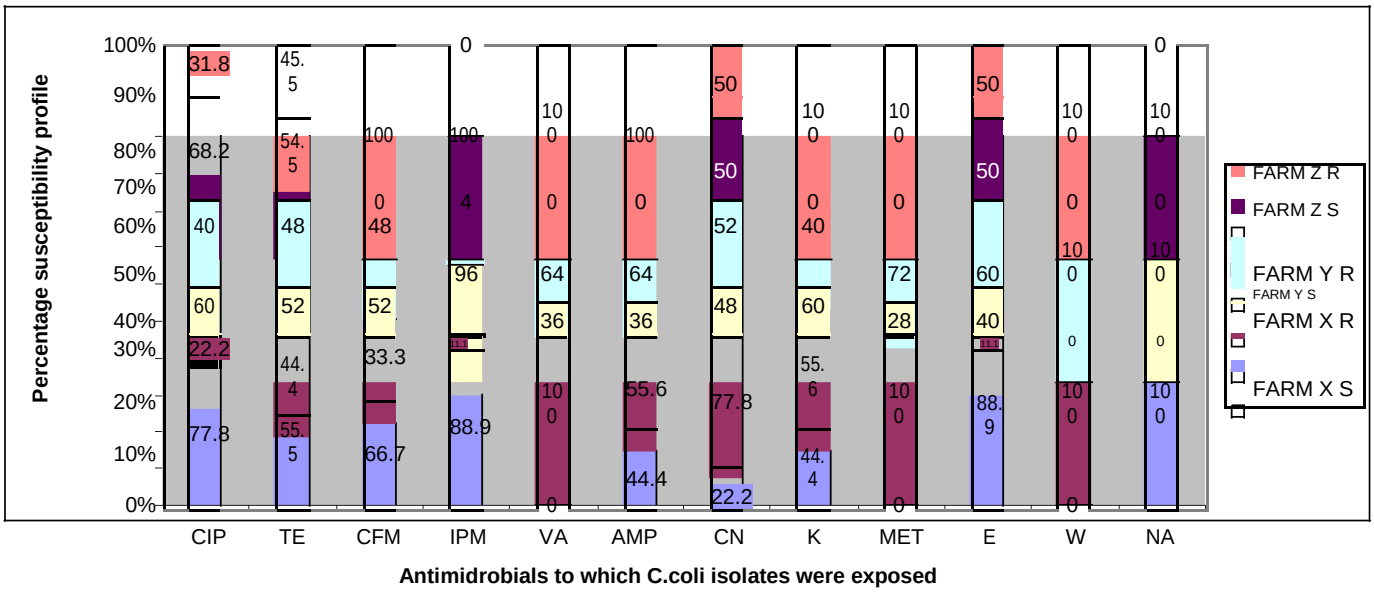

Fig. 3: Percentage susceptibility profile of *Campylobacter coli* exposed to 12 antibiotics (Key: S = Susceptibility; R = Resistance; CIP= Ciprofloxacin; TE= Tetracycline; CFM = Cefexime; IPM = Imipenem; VA= Vancomycin; AMP = Ampicillin; CN= Gentamycin; K= Kanamycin; MET= Methicillin; E=Erythromycin; W= Trimethoprim; NA= Nalidixic acid).

farms.

Blood haemolysis

Of the 115 *Campylobacter* strains from the pigs tested for sheep blood haemolysis, 23 were β-haemolytic, 10 were α-haemolytic and 5 non-haemolytic in farm X, 22 were β-haemolytic, 16 were α-haemolytic for farm Y, 35 were β-haemolytic and 4 were α-haemolytic for farm Z (Figure 3).

Antimicrobial resistance

Sixty-six isolates were tested for their antibiotic susceptibility profiles. Most *Campylobacter* isolates from farm X (< 50%) were resistant to ciprofloxacin, cefexime, and erythromycin, while > 50% were resistant to tetra-cycline (54.5%), vancomycin (100%), ampicillin (54.5%), kanamycin (63.6%), and methicillin (100%). In farm Y, ≥ 50% were resistant to ciprofloxacin (57.1%), tetracycline (50%), cefexime (53.6%), vancomycin (67.9%), gentamycin (53.6%), methicillin (75%), and erythromycin (53.6%), except imipenem and kanamycin which were 10.7 and 46.4% resistant, respectively. In farm Z, > 50% but < 100% were resistant to tetracycline, gentamycin and erythromycin, while 40.7% were resistant to ciprofloxacin. Approximately 100% of *Campylobacter* species in this farm were resistant to cefexime, vancomycin, kanamycin, and methicillin. Resistance to most of the antibiotics tested was more pronounced among isolates from farm Z. Majority of the isolates from farm Y were more resistant to ciprofloxacin (57.1%) than isolates from farm X (36.4%) and farm Z (40.7%) (Figure 4 and 5). There were no significant differences in resistance profiles amongst *Campylobacter* isolates in the farms ($p < 0.005$). Study of individual species resistance showed that in farm X, all *C. jejuni* isolates were resistant to Ciprofloxacin, tetracycline, cefexime, imipenem, vancomycin, gentamycin, kanamycin and methicillin. Of the three *C. jejuni* isolates from farm Y, ≤ 100% were resistant to all the antimicrobials except tetracycline (33.3%) and nalidixic acid in which all were susceptible. In farm Z, ≤ 100% were resistant to 10 of the antimicrobials except ciprofloxacin to which 80% were resistant and nalidixic acid to which 100% were susceptible. For *C. coli*, in farm X < 50% were resistant to ciprofloxacin, tetracycline, cefexime, imipenem and erythromycin except vancomycin, ampicillin, gentamycin, kanamycin, methicillin and trimethoprim to which ≤ 100% were resistant. In farm Y, < 50% of *C. coli* were resistant to ciprofloxacin, tetracycline, cefexime, imipenem and kanamycin, while ≤ 100% were resistant to vancomycin, ampicillin, gentamycin, methicillin, erythromycin and trimethoprim. In farm Z, ≤ 50% were resistant to ciprofloxacin, tetracycline, gentamycin and erythromycin, while all *C. coli* isolates (100%) were resistant to cefexime, vancomycin, ampicillin, kanamycin, methicillin and trimethoprim. Analysis of the zone diameters was done using CLSI/NCCLS (2002) for enterobacteriaceae.

The PCR micrographs of the DNA from *Campylobacter* strains from sheep are as indicated in. The purified DNA from the *Campylobacter* strains amplified at the 1004 bp, which is the specific region for the conserved 16S rRNA for members of the genus *Campylobacter*. The bands formed were confirmed as those for *Campylobacter*. Specific identification by the Mast diagnostic kits

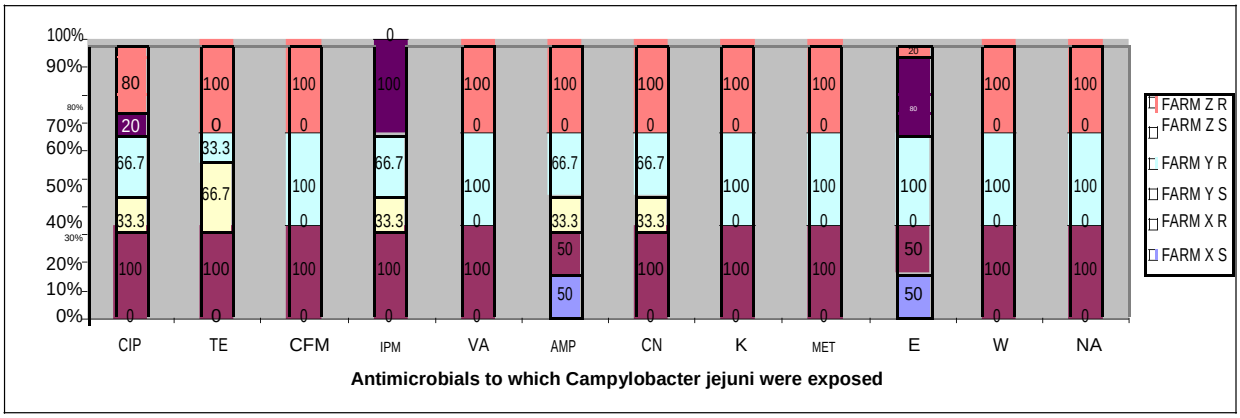

Fig.4: Percentage susceptibility profile of *Campylobacter coli* exposed to 12 antibiotics (Key: S = Susceptibility; R = Resistance; CIP= Ciprofloxacin; TE= Tetracycline; CFM = Cefexime; IPM = Imipenem; VA= Vancomycin; AMP = Ampicillin; CN= Gentamycin; K= Kanamycin; MET= Methicillin; E=Erythromycin; W= Trimethoprim; NA= Nalidixic acid).

Fig.5: PCR products of amplified DNA from pig *Campylobacter* isolates aligning at the 1004bp of a 1.9kb ladder (a) pig and (b). (a) Lane 1= 1.9kb ladder; lane 2,3,4,5,6,7, amplified bands of DNA from pig *Campylobacter* strains; (b) lane 1= 1.9kb DNA ladder; lanes 2,3,4,5,6,7 are amplified bands of DNA from pig (farm Y) *Campylobacter* strains.

differentiated the isolates into *C. jejuni* and *C. coli.*

DISCUSSION

Elucidating the shedding patterns and prevalence of *Campylobacter* strains in the faeces of farm animals is a prerequisite for effective healthcare strategy against zoonotic infections. In the current study, the prevalence of campylobacters from pigs and their antimicrobial susceptibility pattern was determined. Of the 450 fresh faecal samples processed for all farms, 84.6% were identified as campylobacters. There was an equal prevalence (25.3%) among pigs' faeces in farm X and Y, but a slightly higher predominance in faeces from farm Z. In an epidemiological study carried out on 95 pigs originating from eight farms, Weijtens et al. (1993) found 11% of the stomach samples of pigs to be positive for *Campylobacter*, whereas, 85% of faeces were positive. A prevalence of campylobacters in pigs ranging from 63 - 100% has been reported in other studies (Weijtens et al., 1993; Saenz et al., 2000; Young et al., 2000; Pezzotti et al., 2003; Payot et al., 2004). In our findings, none of the farms was free from *Campylobacter.* There were no significant differences in the prevalence of *Campylobacter* sp. in the three farms (X, Y, Z) with the average prevalence value at 25.3, 25.3 and 26% respectively ($p < 0.005$). Weijtens et al. (1993, 1999) reported high counts of shedding of *Campylobacter* genotypes in faeces of pigs.

In terms of prevalence, *C. coli* were more predominant than *C. jejuni* in all farms. Farm Z had a higher incidence of *C. jejuni* and *C. coli* than the rest farms. This report is in line with previous reports which revealed that swine

were predominantly contaminated by *C. coli*, whereas, *C. jejuni* were more frequently isolated from poultry (Aarestrup et al., 1997; Weijens et al., 1999; Van Looveren et al., 2001; Pezzotti et al., 2003). The reason for this discrepancy in the distribution pattern of *C. jejuni* and *C. coli* in swine has not been elucidated. However, it is not implausible that host adaptation or differences in rearing practices may be significant factors.

Quite a large population of *Campylobacter* isolates from pig faeces where β-haemolytic on 5% sheep redblood cells. This observation is in line with the report of Samie et al. (2007). This is a confirmation that the *Campylobacter* strains could be pathogens of medical significance. The predominance of *C. jejuni* in human infections has led to the disregard of the impact that less prevalence species like *C. coli* can play in human infections. Nevertheless, a recent survey by Tam et al. (2003) showed that the morbidity resulting from *C. coli* has actually been underestimated.

The PCR micrographs of the DNA from *Campylobacter* strains from all animals were as shown in Figure 1. The purified DNA from the *Campylobacter* strains amplified at the 1004 bp, which is the specific region for the conserved 16S rRNA for members of the genus *Campylobacter*. The bands formed are in line with those of Marshall et al. (1999).

After exposure to twelve (12) antibiotics, several strains showed multiple resistance to most of the antibiotics used. The resistance patterns displayed by *Campylobacter* isolates from all farms to fluoroquinolone (ciprofloxacin) and macrolides (erythromycin) classified as second line and first line antimicrobials are of particular importance, since patients suffering from campylobacteriosis are usually treated with these antimicrobials agents.

In the study, 22.2, 40 and 31.1% of *C. coli* were resistant to ciprofloxacin, while 11.1, 60 and 50% of *C. coli* were resistant to erythromycin in farm X, Y and Z, respectively. For ampicillin, 55.6, 40 and 100% of *C. coli* were resistant to this antimicrobial in farm X, Y and Z, respectively. Norma et al. (2007) in their study in Canada observed a high incidence of *C. coli* resistant to ciprofloxacin and erythromycin. The resistance of *C. coli* to ampicillin as exemplified in this study was higher than those reported from France and Denmark (20 and 17%), respectively but lower than that reported from Spain (65.9%) for farm X, Y but not for farm Z (100%). Resistance to β-lactam antimicrobials in pathogenic bacteria develops through bacterial conjugation. This resistance-transfer mechanism is very crucial because it permits genetic exchange of information between species of bacteria (Davis and Conner, 1994; EFSA, 2007; Norma et al., 2007). In South Africa, data on anti-microbial susceptibility of *Campylobacter* isolated from pigs are scanty. Due to high prevalence of resistance shown by *Campylobacter* sp. to β-lactam antimicrobials (ampicillin) it is no longer recommended for use in farms (Davis and Conner, 1994).

In this study, we observed resistance frequently to antimicrobials most commonly used in swine industry, tetracycline and erythromycin. Different resistance patterns were observed for one aminoglycoside antibiotic for all isolates tested for *C. coli*. 77.8, 52 and 50% were resistant to gentamycin in farms X, Y and Z, respectively. However, the report of Norma et al. (2007) in Canada showed a low level of resistance (0.2%) of *Campylobacter coli* to gentamycin. The recommendation of gentamycin as an alternative therapeutic antimicrobial against human campylobacteriosis by Fernandez et al. (1994) in Chile should be considered with caution judging from the high prevalence of resistance to gentamycin observed in this study. Sato et al. (2004) and Inglis et al. (2005) observed high levels of resistance to tetracycline (45%), which is in line with our observation of ≥ 50%.

Saenz et al. (2000) in their study reported no erythromycin resistance among *C. jejuni* isolates from broilers and high levels of resistance *C. coli* isolates from pigs (81.1%) compared to those from humans (34.5%). However, in our study, one *C. jejuni* isolate out of two was resistant to erythromycin in farm X; all three *C. jejuni* isolates in farm Y were resistant to erythromycin, while one of the five *C. jejuni* isolate in farm Z was resistant to erythromycin. In addition, more resistance was associated with *C. coli* than with *C. jejuni*. A study in Quebec found that 61% of *C. coli* isolated from pigs but none of *C. coli* isolates from chickens were resistant to erythromycin (Guevrement et al., 2006). Resistance of *Campylobacter* species to antibiotics may suggest the widespread use of antibiotics as growth promoters in farm animals (Piddock et al., 2003; Pezzotti et al., 2003; Moore et al., 2001).

Resistance profiles to tetracycline and fluoroquinolones were very high in our study and this is consistent with other reports (Saenz et al., 2000; Norma et al., 2007). This may have stemmed from the fact that these antibiotics have been used in the swine industry for several years.

All the *Campylobacter* isolates were susceptible to nalidixic acid in this study, so that cross-resistance between nalidixic acid and ciprofloxacin was not found in all the quinolone susceptible *Campylobacter* strains. This finding is however in contrast to the observation of Saenz et al. (2000) who found cross-resistance between ciprofloxacin and nalidixic acid resistant strains in Spain.

Moore et al. (2005) mentioned the use of mobile genetic elements in campylobacters as mechanism for the extrusion of antibiotics out of the bacterial cell. Considering the feeding habits of pigs, it is plausible that they can ingest commensals carrying resistant genes from the feeds, facilitating transfer to pathogenic bacteria in the lumen of farm animals. Such pathogenic acquisition of resistant genes from exogenous source can result in global prevalence of resistant pathogens. Angulo et al. (2004) and Blake et al. (2003) made similar arguments regarding acquisition of exogenous genes by lumen pathogens in farm animals.

Surveillance and monitoring of antimicrobial use must be done to ensure the controlled use of antibiotics. The use of antibiotics as growth promoters and prophylaxis for animals should be carefully evaluated and monitored because acquisition of antibiotic resistant strains of campylobacters by man through ingestion of animal's food have serious health implications. .

Finally, the high prevalence of antibiotic-resistance among isolates of campylobacters in pig in the Venda region of South Africa needs further investigation. This study will be expanded to include the restriction analysis, antibiotic resistant genes and phylogeny of the *Campylobacter* isolates.

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