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Antimicrobial Resistance of *Campylobacter* spp. and *Arcobacter butzleri* from Pets in Malaysia

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Abstract: The emergence of antimicrobial resistance in pets is not well understood and methods of surveillance are only beginning to be established in a few countries. The consequence of antimicrobial resistant *Campylobacter* and *Arcobacter butzleri* to public health is due to the propensity of the bacteria to swiftly acquire and disseminate resistance gene. Thus, making way for the emergence of new and very pathogenic clones resulting to difficulty in treatment with antimicrobials. The objectives of this study were to determine the antimicrobial resistance patterns and multidrug-resistant (MDR) profiles of *Campylobacter* and *Arcobacter butzleri* isolated from dogs and cats and to evaluate the antimicrobial resistance using the disc diffusion test and Minimum Inhibitory Concentration. Ninety four (94) *Arcobacterbutzleri* and 28 *Campylobacter* isolates were tested against 12 antimicrobials using the disc diffusion method namely Ciprofloxacin (Cip) 5µg; Ampicillin (Amp), 10 µg; Tetracycline (Te), 30 µg; Erythromycin (E), 15 µg; gentamicin (CN), 10 µg; Cefotaxime (CTX), 30 µg; Penicillin G (P), µg; Streptomycin (S), µg; nalidixic acid (NA), µg; Enrofloxacin (Enr), µg; Amoxicillin/Clavulanic acid (AMC), µg and Ceftazidine (CAZ), µg. Using the M.I.C.E. strip, *Campylobacter* and *A. butzleri* isolates showed the exception in the resistance to ciprofloxacin. In comparison, the resistance rates between the disc diffusion and M.I.C. were not significantly different. The resistance patterns showed 18 and 35 antibiotypes for *Campylobacter* and *Arcobacterbutzleri* isolates respectively. *Campylobacter* isolates were found resistant to 9 antimicrobials while *Arcobacter butzleri* showed resistance to 10 antimicrobials. MDR was reported among 50% and 78.9% of *Campylobacter* and *Arcobacterbutzleri* isolates respectively. Antimicrobial resistance in *Campylobacter* and *Arcobacter butzleri* not only increase the risk of treatment failure in both human and animals but also spread antimicrobial resistance genes. Thus, the presence of *Campylobacter* in pets could be a potential source of human infections and environmental contamination.

Keywords: *Arcobacter butzleri,* Cats, *Campylobacter,* Dogs, Multi-Drug Resistance

Introduction

The use of antimicrobial agents has been on the increase in veterinary medicine worldwide. This led to a greater concern about the continued rise of foodborne disease incidence and the resistance of foodborne pathogens to drugs over the last decade (Mc Nulty et al., 2016). The extensive use of antimicrobials lead to its inappropriate usage resulting in increase in bacterial resistance to antimicrobials that directly or indirectly having an impact on both animal and human health (Kroemer et al., 2013.) Though new antimicrobials are being developed, bacteria are reported to be keeping up and adapting defence mechanisms against these antimicrobials, resulting in the development of resistance even to the new antimicrobials (Tillotson and Theriault, 2013).

Campylobacter and *Arcobacter butzleri* are important potential food safety issue due to the increase number of reports published recently. *Campylobacter* are the major cause of gastroenteritis across the globe including some autoimmune conditions such Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (Kaakoush et al., 2015). Pet animals carriers often do not manifest clinical signs of disease, although episodes of diarrhoea have been reported in young animals <1 year of age harbouring *Campylobacter* (Damborg et al., 2016). *Arcobacter butzleri* has been reported as the etiological agent of persistent watery diarrhoea (Arguello et al., 2015). However, the possible effects of antimicrobial use on the emergence of antimicrobial-resistant bacteria in pet animals study are very limited and have been much less studied (Lloyd, 2007). Antimicrobials usage in pets for treatment of infections have been considered to pose little risk, however recent studies suggest that much closer attention needs to be given to this subject because of the close contact between the pets and humans (Weese et al., 2015). To date, small animal welfare is receiving considerable attention resulting in increased expenditure on prevention and treatment of infectious diseases. As a result of these factors, antimicrobial are commonly used in pets especially antimicrobial licensed for human use (Jennings et al., 2016). The world population is increasing and therefore the increased demand for food resources continue to surge. As such, antimicrobials are being used by food animal producers not only for therapy but also to enhance growth and for prophylaxis purpose which results in bacterial resistance (Ballard et al., 2016)

Apart from acquiring antimicrobial resistant bacteria from animals and their products mainly due to the constant influx of the resistance genes into the gut flora through the food chain, human being acquire antimicrobial resistant bacteria due to inappropriate usage of antimicrobials (Teuber et al., 1999). Increasing resistance of bacteria to antimicrobials is becoming a major concern in veterinary medicine, mainly because animals may become carriers of resistant zoonotic agents which may transferred resistance to pathogens affecting humans (Weese, 2008). Reporting of antimicrobial resistance frequently would facilitate better knowledge of antimicrobial resistance and trends in this pattern over time to ensure long-term efficacy of the antimicrobials.

Most cases of *Arcobacter butzleri* enteritis in pets are self-limiting. Several studies have demonstrated that *Arcobacterbutzleri* are found to be resistant to clindamycin, azithromycin, ciprofloxacin, metronidazole, carbenicillin and cefoperazone and sensitive to fluoroquinolones and tetracycline (Fera et al., 2003; Houf et al., 2004). Fluoroquinolones and tetracyclines are the drug of choice if treatment is indicated for the therapy against *Arcobacter butzleri* infection (Son et al., 2007; Vandenberg et al., 2006). Similarly, antimicrobial resistance in 51 isolates of *Campylobacterjejuni* from cats and dogs showed resistance to nalidixic acid, ciprofloxacin, tetracycline, ampicillin, erythromycin and chloramphenicol at 37.3%, 19.6%, 13.7%, 13.7%, 11.8% and 37.3% respectively by E-testing (Acke et al., 2009)

For the past few decades, Multidrug Resistance (MDR) has been found toward all available antimicrobials, presenting one of the biggest threats to public health. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012). Multidrug resistance can be generated by bacteria through accumulation of multiple genes each coding for resistance to a single drug, within a single cell or by the increased expression of genes that code for multidrug efflux pumps (Nikaido, 2009).

Antimicrobial resistance in *Campylobacter* and *Arcobacter butzleri* isolated from poultry and other food animal sources have been reported but very few information in dogs and cats in Malaysia. Thus, the objectives of this study were to determine the antimicrobial resistance patterns of *Campylobacter* and *Arcobacter butzleri* isolates from dogs and cats as well as to determine the Multidrug Resistant (MDR) profiles of *Campylobacter* and *Arcobacter butzleri* isolates.

Materials and Methods

Bacterial Strains and Growth Conditions

For disc diffusion technique, isolates comprising of 15 from *Campylobacter spp.* from dogs and 13 *Campylobacter spp* isolated from cats; and 55 isolates of *Arcobacter butzleri* isolates from dogs and 34 isolates of *Arcobacter butzleri* from cats were obtained from previous studies (Goni et al., 2016; Goni et al., 2017). The isolates were collected and identified as

Campylobacter spp and Arcobacter butzleri using biochemical test and confirmed by Polymerase Chain Reaction (PCR) as reported by Goni *et al.* (2017) before they were stored in cryobeads tubes at -80°C. Similarly, for Minimum Inhibitory Concentration (M.I.C) method, 16 isolates of *Campylobacter* and another 16 isolates of *Arcobacter butzleri* were selected among the same isolates used for disc diffusion method and for the antimicrobial susceptibility testing. The 16 isolates were selectively chosen to represent isolates from dogs and cats in equal proportions. All isolates preserved at -80°C refrigerator were revived on Blood agar (Oxoid) supplemented with 5% defibrinated horse blood. Pure colonies of *Campylobacter* and *Arcobacter butzleri* were then cultured on Mueller-Hinton agar (CM 337, Oxoid and Hampshire, UK) containing 5% defibrinated horse blood. Plates inoculated with *Campylobacter* species were incubated at 42°C for 48 h under microaerobic condition and plates inoculated with *Arcobacter butzleri* were incubated at 30°C for 48 h under aerobic condition.

Disc Diffusion Method

Campylobacter and *Arcobacter butzleri* isolates were tested for susceptibility to 12 antibiotics based on the Clinical Laboratory Standard Institute (CLSI) (2010) protocol. The following antibiotic discs (Oxoid, Hampshire, UK) were used: Ciprofloxacin (Cip), 5 µg; ampicillin (Amp), 10 µg; Tetracycline (Te), 30 µg; erythromycin (E), 15 µg; gentamicin (CN), 10 µg; Cefotaxime (CTX), 30 µg; Penicillin G (P), 10 µg; Streptomycin (S), 10 µg; Nalidixic Acid (NA), 30 µg; Enrofloxacin (Enr), 5 µg; Amoxicillin/clavulanic acid (AMC), 10 µg and Ceftazidine (CAZ), 10 µg. Each inoculum was prepared in 0.9% NaCl suspension by mixing the pure bacterial colonies obtained from the fresh culture plates. The inoculum turbidity was adjusted to 0.5 McFarland standard ($\sim 1.5 \times 10^8$ cfu/mL). One microliter of the suspension of each inoculum was transferred onto Mueller-Hinton agar (Oxoid) plate supplemented with 5% defibrinated horse blood to make a confluent lawn of bacterial growth. All plates were allowed to dry for 5 min before placing the antimicrobial disc onto the agar. All plates inoculated with *Campylobacter* were incubated at 42°C for 24 h under microaerobic conditions generated by gas pack (BD CampyPak[TM], Becton, Dickson and Company, Polymouth, England, UK) and plates inoculated with *Arcobacter butzleri* were incubated under aerobic condition at 30°C for 48 h. The diameters of the zones of inhibition were measured after incubation. For erythromycin, the zone of diameter breakpoint for bacteria isolated from animals were recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (2002) and for ciprofloxacin, ampicillin,

tetracycline, erythromycin, gentamicin, cefotaxime, penicillin G, streptomycin, nalidixic acid, enrofloxacin, amoxicillin/clavulanic acid and ceftazidime, the diameter of breakpoint for Enterobacteriaceae were recommended by the CLSI (2010). For the quality control, reference strains for *C. jejuni* (ATCC 33560) and *Arcobacter butzleri* (CCUG 17812) were used. The isolates were classified as sensitive, intermediate and resistant using zone diameter breakpoints of the CLSI (2013; 2012).

Minimum Inhibitory Concentration (MIC) method

For this method, Minimum Inhibitory Concentration Evaluator (M.I.C.E) strips (Oxoid, Hampshire, UK) were used for the determination of the MIC based on the recommendation of the manufacturer. Erythromycin, ampicillin, ciprofloxacin and tetracycline were the antimicrobials used. Plates were prepared as in disc diffusion method. The surface of the agar plate is left to dry for 5 min after applying the suspension of each inoculum. An M.I.C.E strip was applied on each plate. The surface of the strip with the MIC scale is placed on the surface of the plate in such a way that is facing upward and the top end of the strip containing the antimicrobial positioned at the end of the plate. A sterile forcep was used to facilitate the complete contact of the strip with the surface of the agar. All plates inoculated with *Campylobacter* isolates were incubated under microaerobic condition as above and plates streaked with *Arcobacter* were incubated aerobically at 30°C for 48 h. The inhibitory concentration was read at the point where the elliptical zone of inhibition intersected the test strip and that concentration of the antimicrobial was considered as MIC for the organism. For quality control, reference strains of *C. jejuni* (ATCC 33560) species and *Arcobacter butzleri* (CCUG 17812) were used. The reading for each isolate was recorded and classified as being resistant or sensitive based on the MIC breakpoint according to the manufacturer's instructions (CLSI, 2013; 2012). For each test, *C. jejuni* ATCC 33560 and *Arcobacter butzleri* (CCUG 17812) was used as quality control strain.

Data Analysis

Statistically, kappa (κ) test was used to examine the strength of observed agreement between disc diffusion method and M.I.C using M.I.C.E strips for each antimicrobial tested on both methods performed using SPSS version 20.0 (SPSS Chicago, USA) with the following interpretation criteria: κ<0.00- poor; $0.00 \leq$ κ<0.20- slight; $0.20 \leq$ κ0.40- fair; $0.40 \leq$ κ<0.60- moderate; $0.60 \leq$ κ<0.80- substantial; κ>0.80-almost perfect as suggested by McHugh (2012).

Results

The 12 antimicrobials were chosen from OIE Recommendation for Veterinary Critically Important Antimicrobial (2012) list and WHO Recommendation for the Critically Important Antimicrobials for Human (WHO, 2011). Five (17.8%) and 2 (2.1%) of the isolates from dogs and cats respectively showed resistance to more than one antimicrobial using the disc diffusion method. The highest prevalence of resistance among all the *Campylobacter* isolates was to penicillin G (53.2%) followed by ceftazidine and erythromycin (50%) as shown in Fig. 1. Similarly, the highest prevalence of resistance among all the *Arcobacter butzleri* isolates was 98.9% to both penicillin G and ampicillin followed by streptomycin (85.2%) as shown in Fig. 1. Results from this study showed that multidrug resistance among the isolates of *Campylobacter* and *Arcobacter butzleri* were 78.9% and 50% respectively for both dogs and cats using the disc diffusion test.

Of the 16 *Campylobacter* and *Arcobacter butzleri* isolates tested for resistance using the M.I.C.E strips, 60% of *Campylobacter* isolates were resistant to ampicillin,

66.6% to and tetracycline 80% to erythromycin, while 73.3% of *Arcobacter butzleri* isolates were resistant to ampicillin, 80% resistant to tetracyclines and 60% resistant to erythromycin. However, none of the isolates were resistant to ciprofloxacin as shown in Fig. 2.

In comparison of the resistance rates between disc diffusion and M.I.C methods for *Campylobacter* isolates, the kappa value (κ) showed a fair agreement for ampicillin (κ = 0.211), poor agreement for tetracycline (κ = -0.80) and slight agreement for erythromycin (κ = 0.17). For *Arcobacter butzleri* isolates, the kappa value showed agreement for ampicillin (κ = 0.72) and erythromycin (κ = 0.72) and poor agreement for tetracycline (κ = -0.647). The susceptibility of the *Campylobacter* and *Arcobacter* isolates evaluated using M.I.C. The susceptibility of 16 *Campylobacter* and *Arcobacter butzleri* isolates evaluated using M.I.C. Evaluator strips and disc diffusion test for four antimicrobials were compared as shown in Fig. 3 and 4 respectively. The resistant rates and distribution of the *Campylobacter* and *Arcobacter butzleri* in different concentrations of antimicrobials are indicated and shown in Table 1 and 2 respectively.
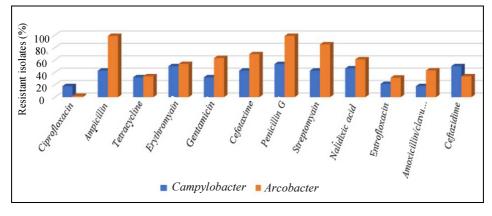

Fig. 1: Percentage of *Campylobacter* and *Arcobacter butzleri* isolates resistant to antimicrobials using disc diffusion method
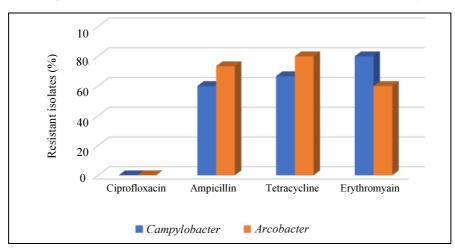

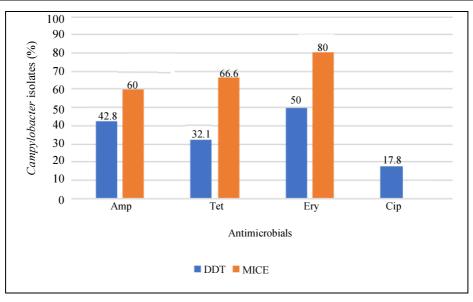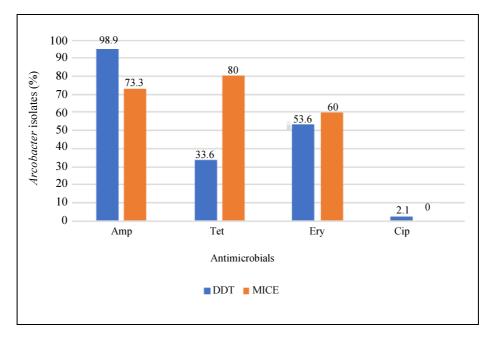**Fig. 2:** Antimicrobial resistance patterns of *Campylobacter* and *Arcobacter butzleri* isolates using M.I.C.E strip

Fig. 3: *Campylobacter* isolates resistant to type of antimicrobials using Disc Diffusion Test (DDT) and M.I.C. Evaluator strips (M.I.C.E)

Fig. 4: *Arcobacter butzleri* resistant to type of antimicrobials using Disc Diffusion Test (DDT) and M.I.C.E strips

Table 1: Distribution of MICs for *Campylobacter* isolated from dogs and cats

Antimicrobials	Concentration (µg/mL)													
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Ampicillin	-	-	-	-	1	-	2	1	-	-	2	-	-	1
Tetracycline	-	-	-	-	1	1	1	1	1	-	-	-	3	1
Erythromycin	-	-	-	-	-	2	-	1	-	-	2	2	1	-
Ciprofloxacin	-	-	-	1	4	8	1	1	-	-	-	-	-	-

Resistant isolates are indicated by bold and underlined

Table 2: Distribution of MICs for *Arcobacter butzleri* isolated from dogs and cats

Antimicrobials	Concentration (µg/mL)													
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Ampicillin	-	-	-	-	-	-	-	-	-	2	2	-	-	-
Tetracycline	-	-	-	-	-	-	-	-	-	1	3	5	-	-
Erythromycin	-	-	-	-	-	-	-	-	-	3	3	4	-	2
Ciprofloxacin	-	-	1	2	1	6	-	2	3	-	-	-	-	-

Resistant isolates are indicated by bold and underlined

Table 3: Antibiogram of *Campylobacter spp.* isolates from dogs and cats

Antibiotypes	No of isolates	% of isolates	Antibiogroups	Common Antimicrobial(s) resistance
CipCtxPTeAmpENaAmcCaz	1	3.6	1A	CipCtxPTeAmpENaAmcCaz
CipTeESNaCazEnr	1	3.6	2A	NaEnr
CnCtxTeSNaAmcEnr	1	3.6	2B	
CnPAmpENaCazEnr	1	3.6	2C	
CnTeSNaCazE	2	7.1	3A	Caz
CnCtxSNaCazEnr	2	7.1	3B	
CtxPAmpESCaz	2	7.1	3C	
PTeESNaCaz	1	3.6	3D	
PESAmpAmcCaz	1	3.6	3E	
CnPAmpSAmcCaz	1	3.6	3F	
CipPENaCazEnr	1	3.6	3G	
CtxPTeAmpNa	2	7.1	4A	P
CtxPAmpES	1	3.6	4B	
CipCtxPAmpNa	1	3.6	4C	
CnCtxPEAmc	1	3.6	4D	
CipCnTeAmc	1	3.6	5A	
PAmpECaz	1	3.6	5B	
CtxPAmpE	1	3.6	5C	

Amp: ampicillin; Cn: gentamicin; Cip; ciprofloxacin; Enr: enrofloxacin; Na; nalidixic acid; Te: tetracycline; E: erythromycin; S: streptomycin; Ctx: cefotaxime; P: penicillin G; Amc: amoxicillin/clavulanic acid; Caz: ceftazidime

Table 4: Antibiogram of *Arcobacter butzleri* isolates from dogs and cats

Antibiotypes	No of isolates	% of isolates	Antibiogroups	Common Antimicrobials resistance
CtxPAmpAmcNaESCnEnrTe	2	2.1	1A	CtxPAmpNaESCnEnrTe
CtxPAmpAmcCazNaESCip	1	1.1	2A	CtxPAmpNaESte
CtxPAmpAmcCazNaESCnEnrTe	25	26.1	2B	
CtxPAmpAmcCazNaESTe	1	1.1	2C	
CtxPAmpAmcCazNaES	2	2.1	3A	CtxPAmpAmc
CtxPAmpAmcNaESCn	1	1.1	3B	
CtxPAmpAmcCazNaSCn	1	1.1	3C	
CtxPAmpAmcESCnTe	1	1.1	3D	
CtxPAmpAmcCazESCn	1	1.1	3E	
PAmpAmcCazNaSCn	5	5.2	4A	CtxPAmpAmc
CtxPAmpAmcENaS	3	3.1	4B	
CtxPAmpAmcCazNaS	2	2.1	4C	
CtxPAmpAmcCazSE	2	2.1	4D	
CtxPAmpSTeECn	2	2.1	4E	
CtxPAmpAmcCazNaTe	2	2.1	4F	
CtxPAmpAmcCazCnS	1	1.1	4G	
CtxAmpAmcESNa	1	1.1	4H	
CtxPAmpNaSTeE	1	1.1	4I	
CtxPAmpAmcCazTeE	1	1.1	4J	
PAmpSAmcCnNa	6	6.2	5A	CtxPAmp
PAmpSAmcETe	2	2.1	5B	
PAmpSCazNaCip	2	2.1	5C	
CtxPAmpSAmcCaz	2	2.1	5D	
CtxPAmpAmcCazE	2	2.1	5E	
CtxPAmpAmcCazTe	2	2.1	5F	
CtxPAmpSAmcCn	1	1.1	5G	

Table 4: Continue

CtxPAmpAmcCazNa	1	1.1	5H	
CtxPAmpSCnE	1	1.1	5I	
PAmpSAmcENa	1	1.1	5J	
CtxPAmpAmcCaz	6	6.2	6A	PAmp
CnPAmpAmcSNa	2	2.1	6B	
CnPAmpAmcS	1	1.1	6C	
CnPAmpES	1	1.1	6D	
CtxPAmpS	1	1.1	7A	
PAmpAmcE	1	1.1	7B	Amp

Amp: ampicillin; Cn: gentamicin; Cip; ciprofloxacin; Enr: enrofloxacin; Na; nalidixic acid; Te: tetracycline; E: erythromycin; S: streptomycin; Ctx: cefotaxime; P: penicillin G; Amc: amoxicillin/clavulanic acid; Caz: ceftazidine

Antiobiogram of *Campylobacter* and *Arcobacter butzleri* isolated from dogs and cats produced different numbers of antibiotypes using the disc diffusion method. For *Campylobacter,* eighteen antibiotypes were detected in five antibiogroups according to the number of antimicrobials that each isolate was resistant and 35 antibiotypes were detected in 7 antibiogroups for *Arcobacter butzleri*. The most common antimicrobial resistant patterns for *Campylobacter* isolates were CnTeSNaCazE, CnCtxSNaCazEnr, CtxPAmpESCaz and CtxPTeAmpNa (7.1%) (Table 3) and for *Arcobacterbutzleri,* it was CtxPAmpAmcCazNaESCnEnrTe (26.1%) (Table 4).

Discussion

In this study, *Campylobacter* species and *Arcobacter butzleri* isolates from dogs and cats were evaluated for antimicrobial resistance. Antimicrobials are used in humans and pet animals for treatment of diseases. However, lack of prudent use and overuse of the antimicrobials facilitate the spread of the resistance genes from resistant bacteria to susceptible bacteria. The overall percentage of *Campylobacter* isolates resistant to erythromycin, nalidixic acid, ampicillin and penicillin G was higher than to ciprofloxacin, gentamycin, cefotaxime and enrofloxacin. This result is similar to the findings of Vandenberg *et al.* (2006). Presently, antimicrobial treatment of *C. upsaliensis* associated diarrhea is limited due to lack of available information. As a result of this, antimicrobial therapy of the case of infection caused by *C. upsaliensis* is still not clear (Bourke *et al.*, 1998).

The level of resistance of 16 *Campylobacter* isolates tested using the disc diffusion and M.I.C.E strip were compared statistically for the four antimicrobials used (ampicillin, erythromycin, tetracycline and ciprofloxacin). Both *Campylobacter* and *Arcobacter butzleri* isolated from dogs and cats were 100% susceptible to ciprofloxacin using the M.I.C. method. However, there is a fair agreement for ampicillin and poor agreement for tetracycline but M.I.C. detected higher rate of resistance than disc diffusion method between the two antimicrobials. There was a slight

agreement for erythromycin among the *Campylobacter* isolates between the two methods. Similarly, *Arcobacter butzleri* showed agreement for ampicillin and erythromycin and poor agreement for tetracycline. Previous studies showed a high-level agreement between the two methods particularly for aminoglycosides and fluoroquinolones, while a low-level agreement was observed for other antimicrobials (Luangtongkum *et al.*, 2007). Disc diffusion technique is often preferred than M.I.C method because is often reliable, easy and inexpensive method of testing antimicrobial susceptibility (Yildirim and Ersin, 2005).

The low agreement between MIC and disc diffusion technique could be due to the few numbers of isolates used for M.I.C compared to the disc diffusion method due to the laborious work involved and its expensive cost (Luangtongkum *et al.*, 2007). However, the overall levels of resistance were not significantly different between the disc diffusion method and MIC determination. The sensitivity of MIC for susceptibility testing in routine practice is limited compared to the disc diffusion method due to the high cost of the strips (Miflin *et al.*, 2007; Luangtongkum *et al.*, 2007; Moore *et al.*, 2006). Evaluator strips and disc diffusion test for four antimicrobials were compared. Higher sensitivity is seen in disc diffusion technique than M.I.C methods as seen in other studies (Miflin *et al.*, 2007; Moore *et al.*, 2006). However, lack of a standardized protocol and resistance break points for the isolates are the main issues that contribute to the variability in antimicrobial susceptibility (Houf *et al.*, 2004).

Antimicrobial resistance may arise through different mechanisms (intrinsic or acquired) that vary depending on the organisms and the class of antimicrobial agent involved. Intrinsic resistance are caused by natural genes occurring in the DNA of the host animal while the acquired resistance mechanisms involve the acquisition of genes that encode antimicrobial resistance (Umber and Bender, 2009). However this increase in the application of antimicrobial agent have resulted in disruption of the balance of the ecosystem thereby creating the enrichment of Multi-Drug Resistance (MDR) bacteria (Levy, 1997). Multi-drug resistance is a great threat to human that effect the choice of

antimicrobial for therapy (Kurincic *et al.*, 2005). From the present study, 78.9% of *Arcobacter butzleri* isolates and 50% of *Campylobacter* isolates were resistant to three or more classes of antimicrobials using the disc diffusion test. Cases of multidrug resistance have been reported in several studies to occur in *Arcobacter butzleri* and *Campylobacter*. Similar study by Son *et al.* (2007) tested *Arcobacter butzleri* from chicken carcass and reported 71.8% of the isolates were resistant to two or more antimicrobials. Another study in *Campylobacter* isolates showed cases of multidrug resistance (Campana *et al.*, 2010; Han *et al.*, 2007; Jalo *et al.*, 2018).

The fastidious nature and the growth requirement in microaerophilic atmosphere, the quality of the break point are usually not adequate; therefore both disc diffusion and MIC methods can be used for the susceptibility resting of the *Campylobacter* and *Arcobacter* (Hakanen *et al.*, 2002). Routine susceptibility testing using MIC method is not practically possible when compared to the disc diffusion method due to the financial cost of the strips or if using conventional way, it is laborious (Miflin *et al.*, 2007).

Similarly, higher level of resistance of *Arcobacterbutzleri* isolates to ampicillin, penicillin G and streptomycin were observed. These results were comparable to the findings of Otth *et al.* (2004) who showed that 90% of the *Arcobacter* isolates showed ampicillin resistance. The findings of Aydin *et al.* (2001) reported resistance to ampicillin from broiler chicken at 64.1%.

Arcobacter butzleri from this study were shown to be highly susceptible to ciprofloxacin using disc diffusion (71.5%) and M.I.C.E strip (80%). This is followed by enrofloxacin (55.7%), amoxycillin/clavulanic acid (52.6%) and tetracycline (50.5%). These results were in accordance with Son *et al.* (2007) which reported high susceptibility to tetracyclines. However, *Campylobacter* isolates have been shown to have low resistance to ciprofloxacin (5/28) and amoxycillin/clavulanic acid (5/28) followed by enrofloxacin (6/28). Therefore, ciprofloxacin is the antimicrobial of choice. Similarly, lack of standardized protocol and resistance break points for the isolates are the main issues that contribute to the variability in antimicrobial susceptibility (Houf *et al.*, 2004).

Antimicrobial-resistant human pathogens are unrelated to animal sources in the great majority of cases. A study suggested that less than 4% of antimicrobial-resistance problems in humans could be associated with animal sources and that this resistance is largely related to zoonotic organisms (Bywater, 2004). Transmission of bacteria by direct contact between household pets and humans is favoured through close contact such as petting, licking and physical injury or through the domestic environment such as contamination of food, furnishings. It is clear that pets can pose a risk in the transmission of antimicrobial resistant bacteria to humans. However, the risk of transmission is higher in children than adult human beings because of their closer physical contact with pets and as well as household environments contaminated by the pets. However, propagation of acquired resistant gene is also facilitated through faecal shedding therefore promoting their spread in the human population and environment (Guardabassi *et al.*, 2004).

Conclusion

The increasing rates of antimicrobial resistance among isolates of *Campylobacter* and *A. butzleri* in dogs and cats and also increase in multidrug resistance is of prime public health concern as seen in this study. However, pets could be disseminating the resistant strains through faeces in the environment. Similarly, close contact with pet represents a health risk to humans and therefore posed a challenge to the public health. This study asserts the importance of resistance and dissemination of pathogens and the demand to further explore the mechanism of antimicrobial resistance acquisition and the role of virulent genes in disease pathogenesis with a view to ensuring effective prevention and control of the spread of resistant strains.

Compliance with Ethical Standards

Acknowledgement

Author's Contributions

MDG and SA designed and carried out the experiment and drafted the manuscript. ZZ, GKD and AYO provided the fund and critically analysed the manuscript, MIJ, AAB. SMJ and MAA critically analysed the manuscript and provided guidance. Additionally, the authors have read and approved the final version of this manuscript.

Conflict of Interest

There is no conflict of interest in this paper to declare.

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