

Original Research Article

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## Colonization in Piglets by *Campylobacter* Species and its Antimicrobial Profile

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### ABSTRACT

*Campylobacteriosis* is considered an emerging public health problem with a higher morbidity and mortality rate and a significant impediment to socioeconomic development worldwide. Pigs act as a major reservoir host of *Campylobacter* spp. The organism colonizes the intestinal tract and can persist for the entire lifespan in pigs without any disease manifestation and pose a significant threat to farm biosecurity. The emergence of multidrug resistant strains of *Campylobacter* spp. in recent years has been attributed to the indiscriminate use of antimicrobial agents in animal production. In this study, the weekly excretion pattern in piglets was analysed in a government farm in Thrissur. The piglets were found to excrete *Campylobacter jejuni* from the fourth week of age with possible sources of contamination from dams and paddock environment. By the end of tenth week, piglets (66.67 per cent) excreted *C. coli* predominantly. Pigs were also carrying organisms with a regular excretion pattern (45 per cent). Environmental contamination also played a major role in transmission of *Campylobacter* spp. as 20 per cent of feed and soil samples and 30 per cent of wallowing tank water samples were positive for *Campylobacter coli*. Wild birds also acted as a source of paddock contamination with a prevalence rate of 30 per cent. *Campylobacter jejuni* isolates showed higher resistance against azithromycin (97.37 per cent), oxytetracycline (93.42 per cent), co-trimoxazole (88.16 per cent) and ofloxacin (68.42 per cent) and cent per cent resistance against ceftazidime. *Campylobacter coli* isolates showed cent per cent resistance against azithromycin, ceftazidime, co-trimoxazole and higher resistance against chloramphenicol (87.50 per cent), ciprofloxacin (87.50 per cent), doxycycline (87.50 per cent), oxytetracycline (83.33 per cent) and erythromycin (83 per cent).

#### Keywords

Campylobacteriosis,  
Piglets,  
Antimicrobial  
resistance,  
Prevalence,  
Environment

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### Introduction

*Campylobacter* spp. is one of the major causes of foodborne bacterial infection and is among the most frequently reported zoonoses in

humans worldwide with an estimated 400–500 million cases of diarrhoea each year (Ruiz-Palacios, 2007). *Campylobacter* organisms are widespread in the environment and are present in the intestinal tracts of a wide range of birds

and mammals, including domestic animals used for food production (Inglis *et al.*, 2005). They are highly prevalent in food animals such as pigs, poultry, cattle, sheep and ostriches; and also in pet animals like cats and dogs. Pigs are considered as the main reservoir host for *Campylobacter* spp. and are capable of shedding these organisms asymptotically in their faeces which can pose a threat to farm biosecurity. The wide range of reservoirs, contaminated environment and natural water presents an intricate situation (Humphrey *et al.*, 2007). The colonization of the organism in piglets at the farm level occurs at an early age and pose a threat to farm biosecurity. Pigs can also carry a wide range of *Campylobacter* genotypes and piglets can acquire infection from mother and their associated environment which is contaminated (Weijtens *et al.*, 1997). Antimicrobial agents are a leading weapon in the treatment of infectious diseases caused by bacteria and other microbes. The uncontrolled rise in resistant pathogens threatens lives and puts stress on the limited healthcare resources. The development and spread of resistance in zoonotic bacteria including *Campylobacter* with its reservoirs in healthy food producing animals and birds has become a global public health problem.

This study aims to provide an overall picture about colonization of *Campylobacter* spp. in piglets and influence of pigs, wild reservoirs and contaminating environment towards colonization in piglets. This investigation will also throw light on the antimicrobial susceptibility pattern of the isolates obtained during the study and assess the threat by resistant isolates towards farm biosecurity.

### **Materials and Methods**

The present study was undertaken to analyse the colonisation of piglets by *Campylobacter* spp., occurrence of *Campylobacter* spp. in pigs, wild birds and environmental samples

and associated threat towards farm biosecurity. The molecular confirmation of isolates, and antibiotic resistance profile of the positive isolates was also carried out.

### **Collection of samples**

A total of 255 samples was collected from a government licensed farm in Thrissur, Kerala. Fifteen specific piglets from two litters were selected using the unique identification number. Rectal swabs were collected and examined at weekly intervals over the first 10 weeks of life. The sows and associated environment was also sampled on each visit. Samples consisted of piglet rectal swabs (zero to tenth week), sow and boar rectal swabs, faecal samples from pigs, wild bird's droppings, dog rectal swabs, human faecal samples, feed, water, water from wallowing tank, soil, hand swabs and foot swabs. Swab samples from the animals and humans were collected using sterile cotton swabs (HiMedia, India) dipped in Cary Blair medium with charcoal. Faecal samples from pigs and humans were collected in sterile bottles. Feed, drinking water samples, wallowing tank water samples and soil were collected in sterile bottles. The aseptically collected samples were immediately transported at 4°C to the laboratory and were processed within four hours of collection to ensure that the organisms remain viable and culturable.

### **Processing of samples**

All the samples collected were subjected to isolation and identification of *Campylobacter* spp as per the procedure described by OIE terrestrial manual, 2017 with modifications. The selective enrichment of the samples was carried out in Blood Free *Campylobacter* (modified Charcoal Cefoperazone Deoxycholate, mCCD) broth (HiMedia, India) under microaerophilic conditions at 42°C for 48 h. All the samples except rectal swabs were

subjected to enrichment at the ratio of 1:9 in mCCD broth. Rectal swab samples were swabbed directly onto P-mCCDA (Polymyxin B supplemented modified Charcoal Cefoperazone Deoxycholate Agar) plates and incubated under microaerophilic conditions at 42°C (Thermo CO<sub>2</sub> incubator) for 48 h.

### **Molecular detection**

All the positive isolates were subjected to multiplex polymerase chain reaction (mPCR) for the confirmation of *Campylobacter* spp. All the isolates were analysed for the presence of *Campylobacter* genus specific *16SrRNA* gene (816 bp). Species level identification was done by the detection of *C. jejuni* specific *mapA* gene (589 bp) and *C. coli* specific *ceuE* gene (489 bp). All the *Campylobacter* isolates were analysed for the presence of *cadF*, a conserved virulence gene (400 bp) (Fig. 1 and 2).

### **Antibiogram**

All *Campylobacter* isolates were examined for their antimicrobial drug susceptibility/resistance pattern by disc diffusion method, as per the procedure described by Baserisalehi *et al.*, (2007) against 33 different antibiotics.

### **Results and Discussion**

On analysing 255 samples collected from various sources which included rectal swabs from piglets, pigs and dogs, faecal samples from pigs and humans, wild birds droppings, environmental and feed samples and worker's hand swabs and foot swabs 84 samples were positive for *Campylobacter* spp.

Sampling of piglets was initiated for piglets three days after birth where sows were found to carry *Campylobacter* spp. but none of the piglets were positive for *Campylobacter* spp. Out of 15 piglets screened regularly at weekly

interval, five piglets started excreting *Campylobacter* spp. from fourth week onwards with a regular pattern of shedding among piglets. By the end of tenth week, ten piglets were positive for *Campylobacter* spp. with a prevalence rate of 66.66 per cent. Till six weeks, piglets were excreting *Campylobacter jejuni* and some had mixed infection of *C. coli* and *C. jejuni* which was confirmed by molecular techniques and piglets were weaned and kept in boar pen. Boar rectal swabs were also positive for *Campylobacter* spp. From seventh week *C. coli* was predominantly isolated from piglets. By the end of tenth week all piglets started excreting *C. coli*. Overall *Campylobacter coli* (37/54) was the predominant species isolated followed *C. jejuni* (17/54).

On screening of pig rectal swabs and faecal samples, prevalence rate of 45 per cent and 30 per cent was obtained with a higher prevalence among sows. Thirty per cent prevalence was recorded from wild birds mainly from *Corvus splendens*. Two feed samples were positive for *Campylobacter* spp. which was obtained from pens of pigs fed with chicken waste. Three wallowing water samples collected from pregnant sow and adult pens were positive for *Campylobacter* spp. Two soil samples obtained from the vicinity of sheds were contaminated with *Campylobacter* spp. and 40 per cent of worker's foot swab samples collected after operations were positive for *Campylobacter* spp. Of three dog rectal swab samples collected from the farm, one sample was found to be positive for *Campylobacter* spp. (Table 1).

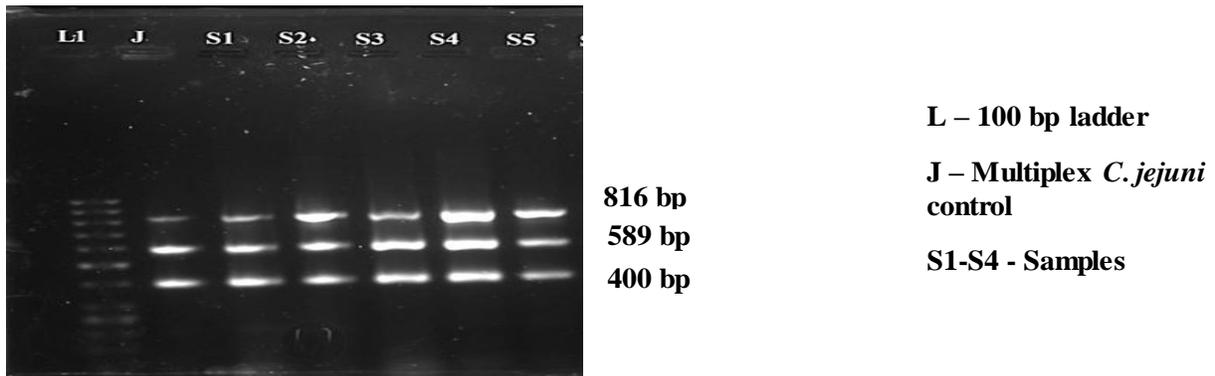
### **Antibiotic resistance profiling**

All the positive isolates of *Campylobacter* spp. obtained from samples were subjected to antibiotic sensitivity test by standard disc diffusion method.

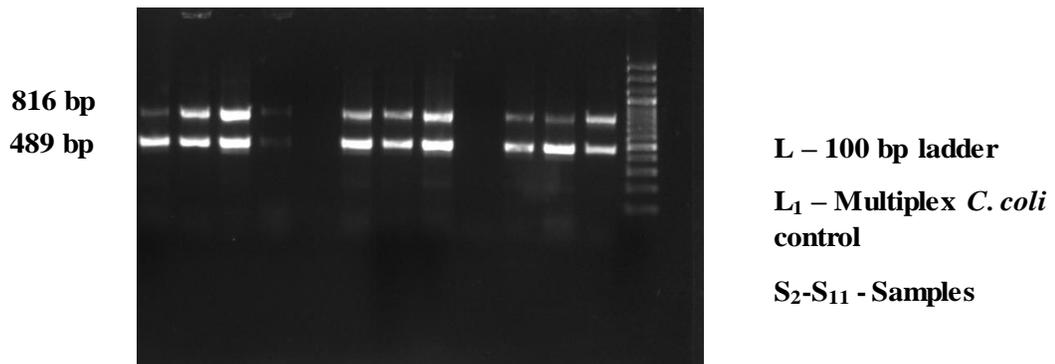
**Table.1** Occurrence of *Campylobacter* spp. in government licenced farm in Thrissur

Sl.no	Source	Total no. of samples	Positive samples	Occurrence %
1	Rectal swabs – Piglets (0-10 weeks)	150	54	36
2	Pigs- Rectal swabs	20	9	45
3	Pigs – Faecal samples	20	6	30
4	Wild birds- droppings	10	3	30
5	Feed	10	2	20
6	Drinking water	10	-	-
7	Wallowing tank samples	10	3	30
8	Soil	10	2	20
9	Worker’s hand swabs and foot swabs	10	4	40
10	Faecal sample – Workers	2	-	-
11	Dog rectal swabs	3	1	33.33
	<b>Total</b>	<b>255</b>	<b>84</b>	<b>32.9</b>

**Fig. 1** Multiplex PCR for *Campylobacter jejuni*



**Fig.2** Multiplex PCR for *Campylobacter coli*



The prevalence of Campylobacteriosis has been reported worldwide and it is one of the most widespread zoonotic gastro enteric infectious diseases of the last century. Pathogenicity of *Campylobacter* spp. is mainly attributed by its low infectious dose, toxin production, carrier status in poultry and coccid dormant stage. These organisms have become the major pathogens of foodborne illnesses because of its severe consequences of infection like Guillain-Barre syndrome, reactive arthritis and irritable bowel syndrome. In this study, carriage rate among piglets was about 33.33 per cent from fourth week of age, which was lower than that reported by Weijtens *et al.*, (1997), who found 85 per cent of piglets positive after four weeks. Steinhäuserová *et al.*, (2001) found 41 per cent of healthy piglets up to 8-week-old positive which was mostly similar to results in this study. Piglets started excreting *Campylobacter* spp. from the fourth week onwards with a regular pattern of shedding. By the end of tenth week, ten piglets were positive for *Campylobacter* spp. with a prevalence rate of 66.66 per cent. Regular increase in incidence among piglets was mainly from sows which carried the infection which points to be a likely source of infection. Comparable results were obtained from the study conducted by Weijtens *et al.*, (1997), who found out that 9 out of 10 sows can carry *Campylobacter* spp. and could infect 85 per cent of piglets with during initial four weeks of life. Alter *et al.*, (2005) also found similar results where they found incidence of 56.6 per cent at fourth week and genotypic similarities was found between sow and piglets. Other probable sources can be from the boar which was positive and piglets were shifted into its pen after weaning as well as from piglets which was positive initially. However, Thakur and Gebreyes (2005) found that 77 per cent of nursery pigs carried *C. coli* on antibiotic free farms but on farms using antibiotics the prevalence was only 28 per cent. Hence farming practices can directly affect *C. coli* prevalence among piglets.

All the piglets excreted *C. jejuni* till seven weeks which can be mainly be attributed to

feeding of chicken waste which was positive for *Campylobacter* spp. and presence of wild birds which may have contributed to *C. jejuni* contamination. Similar results were obtained from the work done by Jensen *et al.*, (2006). They observed that 35 per cent of environmental samples (soil and water) were positive for *Campylobacter* spp. and wildlife reservoirs like rats, crow-birds also contributed predominantly to paddock contamination predominantly with *C. jejuni*. Ramonaite *et al.*, (2015) also observed higher prevalence of *C. jejuni* among crows (39.2 per cent) which contribute to paddock contamination. From seventh week, *C. coli* was predominantly isolated from piglets and by the end of tenth week all piglets started excreting *C. coli* which can be mainly be attributed to alteration of intestinal colonisation by *C. coli* and contributed by carrier sows and boar which contaminate paddock environment and also from contaminated soil and water. Trimble *et al.*, (2013) analyzed soil, compost and processing waste water and found a prevalence rate of 64.3 per cent in soil and 45 per cent in waste water and these contributed to paddock contamination. Workers also contributed to contamination of associated pens as 40 per cent of samples analyzed after operations were positive for *Campylobacter coli*.

All the 20 *C. jejuni* isolates obtained from different sources were subjected to antibiotic sensitivity test by standard disk diffusion method. *Campylobacter jejuni* isolates showed more than 90 per cent sensitivity to clindamycin and nitrofurantoin which is in tune with the findings of Joby (2016) from Thrissur. Amikacin, kanamycin, streptomycin, chloramphenicol, ceftazidime and levofloxacin sensitivity patterns is in agreement with the findings of Joby (2016). In this resistance exhibited by the isolates against azithromycin and erythromycin was 97.37 and 92.10 per cent respectively which was in tune with results obtained by Karikari *et al.*, (2017) from Africa. All the 64 *C. coli* isolates obtained from samples were sensitive to nitrofurantoin which is in accordance with the findings of Joby

(2016). The sensitivity patterns of amoxyclav (62.50 per cent) and levofloxacin (20.83 per cent) are in accordance with Dadi and Asrat (2008). Parkar *et al.*, (2013) showed similar results for ampicillin (62.50 per cent) and gentamycin (sensitivity patterns as found in the present study. Hundred per cent of the isolates were resistant to azithromycin, ceftazidime, cotrimoxazole and ofloxacin which are in agreement with the reports of Baserisalehi *et al.*, (2007). The resistance of fluoroquinolone group (ciprofloxacin and levofloxacin) and the resistance patterns for clindamycin, erythromycin and gentamicin of the present study are in agreement with the findings of Han *et al.*, (2016).

Pigs and piglets have been reported as carriers of several genotypes of *Campylobacter* spp. The present study conforms to this finding and shows that piglets are probably infected from their mother and the associated environment. Feeding of chicken waste and wild birds contaminating the paddock environment with *C. jejuni* can act as a source of contamination and pigs can readily acquire *C. coli* from their surroundings, in turn leading to permanent colonization in the intestines of piglets. The dominant species observed in the piglets studied changed constantly over the 10-week sampling period, but the trend was for *C. coli* to replace the *C. jejuni* which showed predominance in the beginning. In addition, the sow can acquire new species from her piglets and surroundings, and also display a constant change in the dominant genotypes detected.

In conclusion, *Campylobacter* spp. are most widely distributed and found mostly in warm blooded animals. The study revealed weekly prevalence of *Campylobacter* spp. in piglets and sources which contributed to colonisation of organism in piglets. As food safety and its association with biosecurity are highly significant, the potential threat to farm biosecurity due to colonisation in piglets is significantly higher. Contamination of paddock environment can contribute to transmission of *Campylobacter* spp. among animals and

occupational group. In order to reduce the risk of contamination of food and infections caused by *Campylobacter* spp., it is important to adopt appropriate practices for reducing *Campylobacter* colonization in pigs and hygienic practices throughout the swine production chain. The pathogenic potential of the organism is evident due to the presence of the virulence gene of *Campylobacter* spp. The development of resistance to more antibiotics can be of public health significance. Development of strict biosecurity measures should be adopted in the farm in order to reduce environmental contamination and transmission. An antimicrobial intervention is necessary to reduce *Campylobacter* spp. in colonized pigs and piglets.

### Acknowledgement

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