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REVIEW ARTICLE

Impact of antibiotic resistant *Campylobacter* isolated from poultry: A concise review

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Abstract

Poultry industry is one of the leading protein sources around the world. In Sri Lanka, it has developed rapidly over the years and it is the main contributor for consumed meat. Campylobacter is one of the microorganisms presents in asymptomatic form in poultry which cause zoonotic disease, Campylobacteriosis to human. Due to improper usage of antibiotics in poultry industry, development of antibiotic resistant Campylobacter strains occurs by causing major public health concern. Hence, it is important to identify the contamination points of Campylobacter during the meat processing and to determine the prevention strategies to control the spread of antibiotic resistant Campylobacter spp. This review focuses on the background of antibiotic resistant Campylobacter species and their distribution by contaminating meat from farm to plate. The impact cause by antibiotic resistant Campylobacter from poultry industry to the public safety is also discussed along with the diagnostic and elimination strategies. Here, the world scenario on antibiotic resistant Campylobacter was reviewed to come up with suggestions to improve the poultry meat produced in Sri Lanka in order to be safe for human consumption and to increase the market value.

Keywords: *Campylobacter, public health, poultry, antibiotic resistance*

INTRODUCTION

Background of the *Campylobacter* species

Campylobacter is a genus of Gram negative, slender spirally curved rod, motile bacteria, consisting 46 species and 16 subspecies (Bacterio.net, 2020; Vandamme *et al*., 2006; Ngulukun, 2017). *Campylobacter* species require low oxygen content in the environment for their survival. It forms into a coccus when expose to atmospheric oxygen. Furthermore, when culture at the room temperature, their survival is poor and it shows optimum culture temperature at 42 °C. The cells of *Campylobacter* species are heat sensitive indicating their gradual death below 0 °C and above 48 °C (Crushell *et al*., 2004). Although, heating is a suitable option for eliminating *Campylobacter,* studies revealed the development of heat resistance by *Campylobacter* strains (De Jong *et al*., 2012; Al-Sakkaf, 2015).

Most of the *Campylobacter* species cause diseases in humans and animals. Poultry has been considered as the natural reservoir of *Campylobacter jejuni,* which is the most common species causing diarrhoea in human (Nyati and Nyati 2013). However, poultry act as asymptomatic careers (Allos, 2001; Kaakoush *et al.,* 2015) marking their higher potential of meat contamination. Consumption of such meat products is the main source of infection to human Campylobacteriosis and by sound understanding of the points at which contamination might occur will be useful in the prevention of such infections by enhancing meat quality.

Among the species which infect humans, *C. jejuni* and *C. coli* are the most abundant organisms causing enteritis and studied widely (Han *et al*., 2016; Yamazaki *et al*., 2017; Gharbi *et al*., 2018; Kulasooriya *et al*., 2019; Pillay *et al*., 2020). Other than humans and poultry, as per World Organization for Animal Health (OIE) report of 2013, *C. jejuni* and *C. coli* also infect non-human primates, cattle, sheep, mink, cats, dogs, ferrets and pigs (www.cfsph.iastate.edu). Although, most of the other *Campylobacter* species are considered as less important, *C. lari* causes recurrent diarrhoea in children and *C. fetus* causes abortions in cattle and sheep; it can affect humans as an opportunistic pathogen (Sauerwein *et al.,* 1993). However, around 550 million annual human diarrhoeal cases reported globally and possibly one fourth due to *Campylobacter*. *C. jejuni* and *C. coli* primarily transmitted by consumption of raw, undercooked or contaminated meat products, of which 33% from poultry (Van Boeckel *et al*., 2015; Mossong *et al*., 2016; Reddy and Zishiri 2017; Sibanda *et al*., 2018). Therefore, it is important to determine ways to improve poultry meat quality by minimizing the food contamination with *Campylobacter* sp.

Significance of antibiotic resistant *Campylobacter*

In humans, majority of the patients infected with *Campylobacter* recover without treatment due to self-limiting gastroenteritis. However, those who are immunocompromised or vulnerable such as children, elderly people and pregnant women may end up in severe, chronic or systemic infections (Gharbi *et al.,* 2018). Therefore, effective treatment is important for control of the disease in humans. From recent studies, it was found out that *Campylobacter* species developed resistance against commonly used antibiotics belonging to groups of macrolides (azithromycin, erythromycin), quinolones (ciprofloxacin) and tetracyclines (Allos, 2001; Blaser *et al*., 2008). There were records of multi-drug resistant *Campylobacter* strains as well raising the alarm to investigate an effective remedy for the treatment of Campylobacteriosis (Chaire *et al*., 2010).

The antibiotic resistant *Campylobacter* species cause significant loss to the public health sector due to unresponsiveness of the treatment (CDC, 2019). In order to find solutions, it is necessary to dig out the roots of developing antibiotic resistance in *Campylobacter*. Antibiotics prevent the growth and replication process by binding to the target for its function. Iovine (2013) had mentioned four mechanisms of antibiotic resistance: 1) alter antibiotic target 2) inactivate the drug 3) decrease membrane permeability and 4) express antimicrobial efflux pumps. Currently, rise of multidrug resistance turned out as an eye opener in *Campylobacter* resistance studies (Hao *et al*., 2016). *C. jejuni* 1655 strain with multiple drug resistance showed several mutations in *gyrA,* 23S *rRNA*, *aphA*, *tetO* and *aadE* genes. It is also consisted with *pTet* plasmid, which resulted in multidrug resistance (Yang *et al*., 2019). Therefore, performing antibiotic susceptibility testing prior to treatment is recommended in clinical practice.

The origin of this multidrug resistance directs toward animal husbandry practices, mainly poultry production (Wieczorek *et al.*, 2018). A genetic study on multidrug resistant *Campylobacter* spp. isolated from both diarrhoeic humans and poultry meat in China revealed the presence of identical genotypes (Du *et al*., 2018). However, the findings from Silva and colleagues, mentioned that there was no relationship between *Campylobacter* isolates from human and poultry (Silva *et al*., 2016). Hence it arouses necessity of more research to determine the correlation between antibiotic resistance in *Campylobacter* from poultry origin.

In Sri Lankan context, antibiotic resistant *Campylobacter* was isolated in few studies. Kottawatta and co-researchers reported high resistance (80%) of *C. jejuni* against ciprofloxacin and nalidixic acid while low resistance (11%) against erythromycin (Kottawatta *et al*., 2017). However, a recent study by Perera and colleagues showed that *Campylobacter* isolated from poultry faecal samples are 100% resistant to ciprofloxacin, nalidixic acid and trimethoprim-sulfamethoxazole antibiotics, around 70% for amoxicillin and tetracycline followed by lower resistance to streptomycin (Perera *et al.*, 2018). This shows the change of antibiotic resistant profiles over the years. Hence, it indicates the potential of transferring resistant genes in *Campylobacter* species implicating its need of recurrent investigations.

***Campylobacter* contamination during poultry meat processing**

C. jejuni and *C. coli* are the two main food-borne pathogens in human Campylobacteriosis of which both are prevalent in poultry (Friedman *et al.*, 2004). Interestingly, newly hatched chicks do not have *Campylobacter* and showed colonization of the species after one or two weeks of birth in broilers (Evans and Sayers, 2000). This could be due to the presence of maternal immunity at birth and the formation of gut flora with maturity (Sahin *et al.,* 2001; Newel and Fearnley, 2003). *C. jejuni* and *C. coli* are present in poultry gastrointestinal tract as commensals which mask the disease and such asymptomatic careers lead to meat contamination during processing (Beery *et al*., 1988; Byrd *et al*., 1998; Keener *et al*., 2004).

In Sri Lanka, poultry is the most leading livestock industry and main protein source for humans (Department of Census and Statistics, 2019). The deep litter open house with half wall system is commonly used in Sri Lanka and spilling waterers were located within the pen leading to moist humid environment (Line, 2006) which favours the spread of *Campylobacter* within the flock. In most farms, rest of the half walls are covered using a mesh and due to improper maintenance, there are holes which insects, pests such as house flies, house mice and other wild animals can enter. This is another fact which makes the whole farm contaminate with *Campylobacter* spp. from faecal droppings (Kalupahana *et al.*, 2013; Nichols, 2005). Once the first bird becomes colonised by *Campylobacter* in their gut, faecal shedding is an important feature which can result in transmission to all coprophagic birds. In few days, majority of birds in a positive flock get colonised extremely rapidly by bird-to-bird transmission within the flock (Newell and Fearnley, 2003).

During poultry processing, evisceration is performed manually after opening the vent (DAPH 2015), which becomes the first point of meat contamination. In large scale poultry meat processing line, from the point of bird arrival to packing, there are few main steps such as hanging and stunning, throat cutting, bleeding, scalding, defeathering, evisceration, chilling, grading, portioning and boning. Once *Campylobacter* from asymptomatic career contaminate the meat at one step of the processing line, it passes through all the remaining steps and leads to cross contamination (Bashor *et al.*, 2004; Wagenaar *et al.*, 2013). In most of the developed countries, poultry meat processing is fully automated and easy to minimize contamination. However, in most of the Asian countries including Sri Lanka, semi-automated or completely manual processing is practiced (FAO, 2016). Therefore, great care must be taken to avoid *Campylobacter* contamination during meat processing.

DISCUSSION

Campylobacter elimination strategies in poultry farms

Antibiotic resistant *Campylobacter* spp. is on the rise in poultry around the world due to malpractices and misuse of antibiotics in livestock sector. Such strains can cause severe consequences in vulnerable and immunocompromised patients being unresponsive to antibiotic treatment. Therefore, knowledge on identification and determination of susceptibility towards antibiotics is important as per public health concerns.

Based on the sensitivity to oxygen and oxygenic radicals several selective media are available to culture *Campylobacter* spp. such as Charchoal Cefoperazone (CCDA), Preston and Butzler agars (Baylis *et al.*, 2000). Other than standard culture methods, fluorescent *in-situ* hybridization method (FISH), latex agglutination and polymerase chain reaction methods are currently in use for the detection of *Campylobacter* spp. (Wilma *et al.*, 1992; Lehtola *et al.,* 2006; Debretsion *et al.*, 2007). A study done by Portner reported the presence of viable but non-culturable (VBNC) *Campylobacter* strains (Portner *et al.*, 2007). Hence, culture-based conventional methods may mislead as negative for *Campylobacter* spp. Therefore, proper diagnosis of the *Campylobacter* species and resistant strains from poultry is necessary for the decision making on control measures.

Campylobacter spp. is susceptible for oxygen, freezing and thawing stresses, low humidity and drying. Therefore, to eliminate *Campylobacter*, considerations have to be made based on those factors along the meat processing line (Silva *et al.*, 2011). In order to prevent human *Campylobacter* and spread of the disease, poultry meat contamination has to be prevented as the main protein source is poultry in the world as well as in Sri Lanka. Poultry farms act as the primary source of contamination in poultry meat which is considered as the origin of human Campylobacteriosis. At the point of slaughtering, risk of contaminating *Campylobacter* negative carcasses from positive ones can occur. Therefore, it is important to make strategies to process *Campylobacter* negative flocks and positive flocks separately in the poultry processing plants. Although, it is difficult to eliminate *Campylobacter* completely, reduction of contamination and the affected animals can lead to reduce cases of human Campylobacteriosis (Wagenaar *et al.,* 2006; Havelaar *et al.*, 2007; Silva *et al.*, 2011).

Suggestions to improve food safety

Most of the developed countries have established measures to track antibiotic resistant *Campylobacter* and to conduct awareness and control programs. However, in developing countries, antibiotic resistant *Campylobacter* gets very little attention or even unnoticed because of the asymptomatic nature in poultry (Kottawatta *et al.*, 2017). But, it may seriously affects the consumer safety and if the meat is free of *Campylobacter*, it can get higher consumer demand and increase value.

As the primary source of *Campylobacter*, proper farm management plays a critical role. In wild and domestic birds, *Campylobacter* is common inhabitant in their gut and they can shed it to the chicks in poultry pens. Therefore, establishing hygienic barriers such as, proper fencing and biosecurity system need to be installed. Farm personnel can practice routines to minimize spread of *Campylobacter* by sanitizing hands and utensils, changing boots and attending younger flocks first or assigning separate staff to different age groups of chicken (Humphrey *et al.,* 2007). In Sri Lanka, most of the farmers practice all-in-all-out system which allows farmers to disinfect the premises after one round of poultry flock (Kottawatta *et al.,* 2017). Due to poor hygiene of most farms, the use of antibiotics is immense although it is not recommended. In order to avoid such malpractices while improving the poultry health, use of prebiotics and probiotics such as lactic acid bacteria or polysaccharides can be introduced to farmers (Hariharan *et al.,* 2004). Furthermore, application of bacteriocins from bacteria such as *Paenibacillus polymyxa* (Stern *et al.*, 2005) or bacteriophages against *C. jejuni* showed promising results of reducing the faecal content of *Campylobacter* in poultry (Carrillo *et al.*, 2005).

Poultry processing line is the other critical hard point of contaminating meat by *Campylobacter*. Therefore, proper cleaning and disinfection is important to make the premises and thereby meat free of food-borne pathogens. Denmark has been producing "*Campylobacter-free*" certified poultry meat (Krause *et al.*, 2006). This is an adding value to the meat produced while promoting public safety.

Considering all the above facts, it shows the necessity of developing effective mitigation strategy to control *Campylobacter* in poultry. Minimizing antibiotic resistance and spread of antibiotic resistant strains by the control of antimicrobial treatment has to be implemented using strict regulations on poultry industry. The epidemiological status of *Campylobacter* in poultry farms needs to be recorded continuously and share the knowledge among stakeholders to decrease the prevalence and antimicrobial resistance. Furthermore, frequent screening of asymptomatic poultry and apparently healthy flocks need to be carried out to determine novel antibiotic resistant or multi-drug resistant strains of *Campylobacter* spp.

CONCLUSION

Presence of antibiotic resistant *Campylobacter* spp. in poultry is an underlying issue which needs to be paid more attention in Sri Lanka. Improper management of poultry industry particularly by excessive use of antibiotics as a prophylactic measure is the main cause of the development of antibiotic resistant *Campylobacter* spp. It leads to human Campylobacteriosis unresponsive to treatment which can be prevented by biosecurity in poultry farm and meat hygiene. Furthermore, producing food-borne pathogen free certified meat will add value to meat and gain higher income to improve poultry industry in Sri Lanka.

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