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Mini Review

Campylobacter Infection and its Sensitivity in Retail Pork

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Abstract

Food safety and security are serious concerns to the world. The growing population, depleting resources demand that judicious production is key and ensuring that the product is safe from the farm is a collective responsibility. Pork is the source of most consumed animal protein on the planet. Concerns of various type from chemical residues, environment pollution and source of pandemic diseases have challenged the growth of the pork industry. There is strong correlation between contamination of pork by pathogens like, *Campylobacter, Salmonella, Toxoplasma gondii, Listeria monocytogens, Staphylococcus aureus, Trichinella spiralis* and human health hazard. The purpose of this study is to outline the possible risk factors, antibiotic susceptibility pattern, prevalence, possible reason behind high prevalence and developed resistance and possible control measures for *Campylobacter* spp. (*C. coli and C. jejuni*). The risk factor analysis-based research clearly indicated that possible contamination is due to unhygienic slaughtering, evisceration and processing practices. Prevalence is more in the retail meat of Nepal than other countries. The commonly used antibiotics in Nepal are not fully sensitive. The developed resistance might be due to overuse or misuse of antibiotics which may lead to post antibiotics era. Strict slaughtering procedure, HACCP (hazard analysis and critical point) and GMP (good manufacturing practices) during slaughtering are prerequisities for *Campylobacter* control in carcass. We need to focus through individual, policymaker, health professional level for enhancement of pig industry with strict biosecurity measures at farm level of Nepal.

Keywords: antibiotic resistance; Campylobacter; pork; Nepal

Introduction

Foodborne pathogens are the contributor to human illnesses, public health problems and deaths each year. The outbreak of foodborne disease takes place through meat (Nsoesie *et al.*, 2014). About 76 million illnesses yearly in the United States is due to foodborne pathogens; however the related cases are declined in recent year due to advancement in food processing practices (CDC, 2010). It has been estimated that around two millions of *Campylobacter* related human foodborne illness in US in 1997 (Tauxe, 2002). The infection rate of *Campylobacter* is much greater in young <5 years of age, and *C. jejuni* was predominant over *C. coli* (Gallay *et al.*, 2007). The burden due to various foodborne organisms are increasing day by day. Campylobacteriosis is a significant cause of mortality in infants and children's (WHO, 2001). Foodborne pathogens have the public health impact and around $14 billion annual cost of illness and a loss of 61000 quality adjusted life year in USA (Hoffmann *et al.*, 2012). In human clinical manifestation of *Campylobacter* infection includes enteritis ranging from loose feces to severe dysentery, post sequelae infection includes Guillain-Barre syndrome (GBS) characterized by flaccid paralysis and Reiter's Syndrome (RS) characterized by relative arthritis (Altekruse and

Tollefson, 2003). Outbreak of disease provides causes behind the illness, types of foodborne illness, and further strategies should be adopted to overcome the foodborne illness (CDC, 2010).

In developing countries pork is taken as the highest consumed animal protein in the World (Delgado *et al.,* 2001). It is considered as the major meat in China hence consumption increasing day by day with increasing economic development (Guo *et al.,* 2005). But the social thought, social perception towards pork meat concerned as the mainstreaming problem in the world. The religious taboos are present in commercial production of pork meat and it is explicitly forbidden for the upper castes of Nepal (Gurung *et al.,* 2014). An additional concern in research is due to increasing in number of newly found *Campylobacter* species as well as increase in antibiotic resistant *Campylobacter* species like *C. jejuni* (WHO, 2001). Nepal has about 1.16 million heads pig, among them 53% concentrated in hill region, 33% in terai region and 11% in mountain region (MOAD, 2013). The research of various meat sellers, meat suppliers, processing industry, hotels, restaurants in Pokhara, Dharan, Kathmandu, Jhapa and Rasuwa of Nepal show that the total daily sales of pork in major pig/pork hub is 23.84 metric tons with a total value of Rs.7,175,000 (Gurung *et al.*, 2014). Pork is contaminated with various pathogens like *Campylobacter, Salmonella, Toxoplasma gondii, Listeria monocytogens, Staphylococcus aureus* and *Trichinella spiralis.* Nepalese data shows about sixty five percent pork handlers adopting control measures for pork borne disease but none of them had even heard about campylobacteriosis (Ghimire *et al.*, 2013). Research has shown that the heavy contamination of pork meat in Nepal is by antimicrobial resistant pathogen (Ghimire *et al.*, 2014).

***Campylobacter* Genus**

Campylobacter is a microaerophilic, Gram-negative rod exhibiting motility and the causes serious foodborne bacterial disease worldwide (Silva *et al.,* 2011). The bacteria cause serious diarrhea (Ghimire *et al.,* 2014). *Campylobacter* can grow in meat at pH 5.8 but some strain of *Campylobacter* can also grow in meat at pH 6.4. Thermal temperature of about 50°C can inactivate most of the strain (Gill and Harris, 1982). *Campylobacter* was first time identified in 1906 by two British veterinarians analyzing the presence of "large numbers of a peculiar organism" in the uterine mucus of a pregnant female (Silva *et al.,* 2011; Skirrow, 2006; Zilbauer *et al.,* 2008). Smears of uterine mucus when stained in Loffler's blue shows large numbers of organism, most of them are comma shaped and spirillar form formed by joining end to end (Skirrow, 2006). *Campylobacter* requires 5% to 10% oxygen and 1% to 10% carbon dioxide environment for suitable growth and its multiplication (Bolton and Coates, 1983). Ingestion in small concertation around 500 in number results in gastroenteritis (Black *et al.*, 1988). Campylobacteriosis is a collective term used to designate any disease caused by *Campylobacter* biotype. The form of *Campylobacter* causing enteritis are mainly due to *C. jejuni* and *C. coli*. Pigs are the major inhabitant of *C. jejuni*, *C. coli* and other *Campylobacter* species (Oosterom *et al.,* 1985) . Pigs are reservoir of *Campylobacter* whatever they show enteritis or not but the proportion of *C. coli* than *C. jejuni* is more in pigs (Steinhauserova *et al.*, 2001).

Global Epidemiology

There is an evidence to show high increase in incidence of campylobacteriosis globally in the past decade. Europe, North America and Australia are more prevalent for campylobacteriosis. Epidemiological data from Asia, Africa and the Middle East shows the regions are endemic for campylobacteriosis (Kaakoush *et al.,* 2015). The incidence and number of cases reported in various region of country may vary (Sarjit and Dykes, 2017). The variations are more likely due to different sensitivity pattern of detection, area, population of case profile, bio control protocols, availability of resources, different food and feeding practices in these regions (Kaakoush *et al.*, 2015). The reported cases of non *jejuni/coli Campylobacter* like *C. lari*, *C. upsaliensis, C. fetus* and campylobacteriosis in human by *C. coli and C. jejuni* are minimum as tip of iceberg though it is worldwide (Wagenaar *et al.,* 2013). The diversity of *Campylobacter* is not restricted within the pig group as a whole but also within segregated individual (Weijtens *et al.,* 1999).

Risk Factors

Campylobacter transmission between animals include consumption of contaminated food and water, animals contact and international travel (Kaakoush *et al.,* 2015). Acquired immunity difference between individual to individual is considered as an important factor governing transmission of campylobacteriosis in developing countries (Havelaar *et al.,* 2009). Female pigs are more prone to campylobacteriosis due to slight decrease in immunity of female during estrous period and pregnancy (Ghimire *et al.*, 2014). Contamination is high during evisceration by fecal content and use of contaminated knife during processing and cutting (Chaichin *et al.*, 2011). Poor sanitary practice in pork shop and processing unit is the most possible risk factor in Nepalese context *(Ghimire et al.,* 2014). Fecal, pharyngeal, environmental factors during swine slaughtering are the major contamination factors for pork meat (Borch *et al.*, 1996). Normal enteric flora of animals (pig, cattle, poultry) contains *Campylobacter* pathogen (Gallay *et al.*, 2007; Ghimire *et al.*, 2014; Stern *et al.*, 2003). So contamination of carcass with intestinal content is the important risk factor for its high prevalence in retail carcass (Payot *et al.*, 2004; Young *et al.,* 2000). Chilling and blast freezing of meat to reduce microorganisms growth and deterioration showed significant reduction in

Campylobacter (Nesbakken *et al.*, 2008; Pearce *et al.*, 2003). Ghimire *et al.* (2013) reported the main risk factors for contaminations in Nepal are lack of safety tools adopted by pork handlers and processor and lack of eduction among them (Table 1). Possible reason behind contaminations of carcass are unhygienic slaughtering, processing and evisceration practices (Table 1).

Prevalence

Pork is highly contaminated with pathogen like *Campylobacter, Salmonella, Toxoplasma gondii, Listeria monocytogens, Staphylococcus aureus* and *Trichinella spiralis.* These pathogens cause serious human health hazard. *Campylobacter* is normally inhabitant in intestine of pig. *Campylobacter* prevalence is high at slaughter, farm and retailed meat and it is contaminated mainly by *C. jejuni and C. coli*. However *C. coli* infection is more common in pig than *C. jejuni* (Uddin *et al.*, 2013). Ghimire *et al.* (2014). reported *C. coli* prevalence is more than *C. jejuni* in retail meat and slaughter house of Nepal (Table 2). Tap water sources used in slaughter houses are highly contaminated with *Campylobacter* followed by tube well and water jar in Nepal (Bhattarai *et al.*, 2019). The various countrywise prevalence pattern shows , retail carcss is full of threat against *Campylobacter* infection (*C. coli and C. jejuni*) (Table 2). *Campylobacter* infection in pork meat in Nepal was found to be (38.85%.) This finding is more than the research done in other countries like New Zealand, US, UK, Ireland, Italy (Table 2). But comparable to the research done in 2003 in US study (33%) (Pearce *et al.*, 2003). The finding in Nepal is less than the research done in Tanzania (66.7%) (Mdegela et al., 2011) and dressed rib meat in US at 2011, (49%) (Abley *et al.*, 2012).

Table 1: Response pattern of pork meat shop and pork handlers in Chitwan, Nepal to analyze possible risk factors

Response pattern of pork handlers		
No response	**Intermediate response**	**Low response**
Butchers response about use of anal plugs during slaughtering	Butchers response about contamination of carcass with intestinal content during slaughter	Slaughter house practicing chilling of carcass immediately after slaughter
Slaughter slabs having provision of separate dirty section	Nuisance of flies in pig meat shops	Workers wearing apron daily
Workers wearing gloves and masks	Workers washing hands regularly before and after pork handling	
	Workers washing hands regularly with soap and	

Poor knowledge of pork handlers and butcher is principle reason behind high contamination in pig carcass.
Source: (Ghimire et al., 2013)

Table 2: Countrywise prevalence pattern of *Campylobacter*

Country	No. of Sample	Sample location	Prevalent species	Positive sample	% of positive sample	Reference
Nepal	139	Retail and slaughter house	*C. coli* *C. jejuni*	42 12	30.21 8.63	(Ghimire *et al.*, 2014)
	200 (water used)	Slaughter house	*Campylobacter* spp.	12	6	Bhattarai *et al.*,2019)
New Zealand	230	Uncooked retail	*C. coli* *C. jejuni*	3 18	1.3 7.8	(Wong *et al.*, 2016)
US	384	Retail	*Campylobacter* spp.	5	1.3	(Duffy *et al.*, 2016)
	282	Composite carcass, rectal, colon, equipment's	*Campylobacter* spp.	93	33	(Pearce *et al.*, 2003)
UK	1309 (muscle tissue) 131 (offal's)	Retail	*Campylobacter* spp.	66 24	5 18.3	(Little *et al.*, 2008)
Ireland	197	Retail	*Campylobacter* spp.	10	5.07	(Whyte *et al.*, 2004)
Italy	106	Retail	*C. coli* *C. jejuni*	3 3	2.8 2.8	(Samma *et al.*, 2016)

Pig carcass in Nepal is highly contaminated with foodborne *Campylobacter* spp.

Bacterial Sensitivity

Antimicrobial resistance in many countries around the world are mainly due to lack prediction of specific antimicrobial agents, lack of susceptibility testing and use of large amount of antimicrobial agents (Aarestrup *et al.*, 2008). Antibiotics resistance is due to use of antibiotics in improper dose (above therapeutic dose) without concerning environment and health hazards (Yang *et al.*, 2019). The large uses of various antimicrobial agents develop resistance in host animals. The research in Denmark shown that the use of animal daily dosages (ADDs) for treatment of gastrointestinal disease in weaners and slaughter pigs are more followed by ADDs for respiratory problems of weaners and general problems of slaughter pigs (Aarestrup *et al.*, 2008). *Campylobacter* strains of Nepal are not fully sensitive to antibiotics but resistivity pattern lies in middle range (Table 3). Various research reported from various countries are less resistant to antibiotics as compared to Nepal (Table 3). But the susceptibility pattern of bacteria depends on the origin site and different methods and media used for the culture and subculture of bacteria during sensitivity analysis (Jones *et al.*, 1986).

Sensitivity analysis of *Campylobacter* for retail pork shows Nepal is intermediate resistant to Chloramphenicol, Gentamycin, Ciprofloxacin, Nalidixic acid, Tetracycline, Cotrimoxazole, Ampicillin, Erythromycin and Colistin. Ampicillin, Amoxicillin/ clavulanic acid and Chloramphenicol are found sensitive in Brazil. Gentamicin, Streptomycin, Erythromycin and Chloramphenicol are found sensitive in Poland. Ciprofloxacin, Tetracycline and Nalidixic acid are sensitive in New Zealand. Ciprofloxacin, Erythromycin and Gentamycin are found sensitive in West Indies (Table 3). Among these antibiotics, Chloramphenicol is found sensitive in both Brazil and Poland whereas slight resistant in USA, West Indies and Nepal. Ciprofloxacin is found sensitive in New Zealand and West Indies but slight high resistant in Brazil. Gentamycin and Erythromycin found sensitive in both Poland and West Indies but slight resistance in Nepal (Table 3).

Table 3: Antibiotic Sensitivity pattern of *Campylobacter*

Country	Species	Bacterial sensitivity				References
		Full Sensitive	Intermediate resistant		Full Resistant	
			Low resistant	High resistant		
Nepal	(*C. coli*)	_	Chloramphenicol, Gentamycin Ciprofloxacin Nalidixic acid, Tetracycline, Cotrimoxazole	Ampicillin, Erythromycin, Colistin.	_	(Ghimire *et al.*, 2014)
Brazil	*(C. jejuni, C. coli)*	Ampicillin, Amoxicillin/ clavulanic acid, Chloramphenicol	Streptomycin, Gentamycin	Ciprofloxacin, Clindamycin	Cephalothin, Nalidixic acid, Norfloxacin, Tetracycline, Trimethoprim	(Biasi *et al.*, 2011)
Poland	*(C. jejuni)*	Gentamicin, Streptomycin, Erythromycin, Chloramphenicol	Ciprofloxacin, Nalidixic acid, Tetracycline	_	_	(Wieczorek and Osek, 2013)
New Zealand	*(C. jejuni)*	Ciprofloxacin, Tetracycline, Nalidixic acid	Erythromycin	_	_	(Harrow *et al.*, 2004)
USA	(*C. coli*) in finishing farm	_	Chloramphenicol, Ciprofloxacin, Erythromycin, Nalidixic acid,	Tetracycline	_	(Gebreyes *et al.*, 2005)
West indies	(*C. coli*)	Ciprofloxacin, Erythromycin, Gentamycin	Ampicillin, Chloramphenicol, Metronidazole	Tetracycline	_	(Matthew-Belmar *et al.*, 2015)

The *Campylobacter* species are generating resistivity day by day with most of commercially available antibiotics worldwide.

Reason Behind High Prevalence and High Antibiotics Resistivity

The main possible attributes for high prevalence in Nepal are the poor management practices, unhygienic slaughtering, evisceration and processing practices. It may transmit during lairage when non infected animal gets contact with infected animals. The scalding water gets contaminated when it passes through mouth and pharynx and subsequently fill inside lungs with harmful pathogen present in pharyngeal region and contamination also takes place during dehairing, polishing using brushes and scrapes (Borch *et al.,* 1996). Transboundary diseases like swine fever, inadequate slaughter facility, breeding stock of inferior quality are also the reason behind high prevalence in Nepal. Chilling decreases bacterial load in carcass (Oosterom *et al.,* 1985). Prevalence is high in unchilled carcass of Nepal (Ghimire *et al.,* 2014). Significantly higher prevalence of *Campylobacter* spp. is found in slaughter slab and retail shop where wooden chopping board (Achano) and weighing machines are not cleaned daily (Ghimire *et al.,* 2014). Research confirmed the epidemiological role of poultry meat, beef meat in *Campylobacter* transmission is high (SAMMARCO *et al.*, 2010). So high contamination of all level of meat suggests potential threat of cross contamination in slaughter house, shop and market. Resistance in *Campylobacter* is relatively common in research might be due to misuse and overuse of unnecessary antibiotics, haphazard use of such antibiotics (Little *et al.*, 2008) . Which may lead to post antibiotics era where minor injuries once may kill. In countries without standard treatment guidelines, antibiotics are prescribed by health workers, professionals, veterinarians and overuse by public themselves (WHO, 2018). The main reason in Nepal is high use of antibiotics for therapeutic purpose and as a growth promoter (Ghimire *et al.,* 2014). So Nepalese consumers are consuming multiple antibiotics resistant *Campylobacter* in feed knowingly or unknowingly.

Control Strategies for Campylobacter and Developed Resistance

There is no doubt various identified and no identified factors hampers pig industry causing human health hazard. Franco (1988) reported *Campylobacte*r is major reservoir in pork meat. Incidence for food borne pathogen on pork during handling of pork and pork product is also reported (Mcmullen, 2000). Strict slaughtering procedure, HACCP (hazard analysis and critical point) and GMP (good manufacturing practices) during slaughtering restrict its spread and check microbial growth (Borch *et al.,* 1996). The CCP (critical control point) made the specific steps to limit microbial contamination during slaughtering and dressing includes; I) lairage, (II) killing, (III) scalding, (IV) dehairing, (V) singeing/flaming, (VI) polish, (VII) circumanal incision and removal of the intestines, (VIII) excision of the tongue, pharynx and tonsils, (IX) splitting, (X) post mortem inspection procedures and deboning of the head. Adequate cooling, aeration, and proper cooking maintains food safety and preclude contaminations (Franco, 1988). Lowering moisture content by evaporation, increasing acidity by lactic acid fermentation, salting, curing and thermal treatment controls microbial growth and spoilage of pork meat (Gurung *et al.,* 2014). Chilling procedure is effective to check *Campylobacter* growth (Ghimire *et al.,* 2014). Antibiotic resistance is rising dangerously in all parts of the world. Emergence of new resistance is threatening and spreading rapidly hence we need to focus on induvidual, policy maker and health professional level. In individual level, we need to focus on safe use of antibiotics. We must prefer antimicrobial drugs which are prescribed by veterinarians and never go for haphazard use of antibiotics. We use antibiotics for growth promotion or for disease control in healthy animals should be restricted. In policy maker level we need to ensure national action plan to tackle antibiotics resistance through improve surveillance, strengthen policies, programs and implementation of effective control measures. Health professionals need to focus on current guideline of prescription and report the antibiotics resistant infection to surveillance team.

Conclusion and Recommendation

No doubt pork is highly consumed animal protein throughout the world and pig is reservoir for *Campylobacter*. Prevalence of *Campylobacter* is more in pig carcass of Nepal. This research addressed various risk factors, microbial sensitivity pattern, prevalence, reason behind high prevalence and developed antibiotics resistance and their corresponding control strategies comparing with research based articles. This research highlights high prevalence and high antibiotic resistance in retail meat of Nepal than other countries. Unhygienic slaughtering, evisceration and processing practices limit the pig industry in Nepal. Higher prevalence of *Campylobacter* spp. in slaughter slab and retail shop where wooden chopping stump (Achano) and weighing machines are not cleaning daily of Nepal. Overuse and haphazard use of antibiotics may lead to post antibiotics era where minor injuries may kill. We need to focus through induvidual, policy maker and health professional level. We need to focus on the use for safe antibiotics prescribed by registered veterinarians. We need to prepares various policies and action plan to tackle antibiotics resistance through policy making level. Good manufacturing practices, hazard analysis and critical control practices and strict biosecurity are recommended for all entrepreneur involving in pig industry rather than use of unnecessary antibiotics. The governmental and non governmental agencies are recommended to consider various policy issue to tackle antibiotics resistance. Strict biosecurity measures must to be adopted in farm level focusing on segregation of diseased animals, developing resistance to disease through use of various vaccine, cleaning and disinfection in individual and farm level. Training the butchers about hygienic slaughtering practices are necessary. Pork transporters and suppliers are recommended to adopt preservation methods like evaporation, lactic acid formentation, salting, curing or smoking, boiling, cooking, roasting, pasteurization and sterilization.

Author's Contribution

Aasish Gautam & Sushil Neupane jointly designed the research plan; performed experimental works, collected required data & analysed the data. Aasish Gautam prepared the manuscript. Krishna Kaphle critical revised and finalized the manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of paper.

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