

# Do the Current *Campylobacter* Detection Methods in Poultry Carcass Fail To Include Viable But Non-Culturable (VNBC) Cells?

*Campylobacter*, a microaerophilic, spiral-shaped, Gram-negative bacterium, is a major cause of bacterial gastroenteritis worldwide. *Campylobacter* genus includes 12 species and *C. jejuni* and *C. coli* are the most common isolates and involved in human gastrointestinal infection [1]. Centers for Disease Control and Prevention estimated that *C. jejuni* causes 2.4 million cases in the United States each year and is the causative agent for 5-14% of overall diarrheal diseases worldwide. Campylobacteriosis with *C. jejuni*, is characterized by the rapid onset of fever, abdominal cramps, and bloody diarrhea. Sporadic cases are most common and are often associated with handling and consumption of undercooked poultry and poultry products as *C. jejuni* is part of the normal intestinal flora of chicken. The presence of *C. jejuni* in processed chicken carcasses offered for retail sale was determined by both conventional bacteriological cultural techniques and polymerase chain reaction (PCR). The PCR base identification methods were able to detect and identify higher percentage of *Campylobacter* spp. in various type of the specimens including foods, fecal and clinical samples [2-5] but the current PCR base methods used to detect *Campylobacter* failed to differentiate the vegetative and viable but non-culturable (VBNC). On the other hand, the bacteriological culture method represents only vegetative cells those grow on conventional culture agar plates.

It has been reported that effects of temperature, aeration and presence of chemicals as well as storage duration can cause the transition of *C. jejuni* cells from a vegetative state to a VBNC state [6]. Alternatively, it has been found that dormant state of *C. jejuni* cells can be resuscitated in *in vivo* culture condition [7-9]. A recent study has shown that quorum-sensing autoinducers play vital role in reviving VBNC cells in *Vibrio cholera* [10]. Similarly, resuscitation-promoting factors were reported to be responsible for the growth of non-culturable *Mycobacterium tuberculosis* [11,12]. However, the exact molecular mechanisms behind the activation of VBNC cells or resuscitation into planktonic condition in *Campylobacter* are still unknown. Current methodologies used in surveillance and microbiological quality control only focus on vegetative *C. jejuni* cells. Morphology transition from spiral cells in logarithmic phase to predominantly coccoid cells and its role in human infections have been reported [13,14]. That information indicates that there is a gap between the colony count techniques used in quality control/surveillance assay and real number of *C. jejuni* cells present in the poultry and poultry products.

Recently, U.S. food and drug administration (FDA) has introduced this bacterial pathogen in addition to *Salmonella* for routine analysis in retail poultry and poultry products. Currently, very little is known about the survival and recontamination to other



Serajus Salaheen, Nityananda Chowdhury and  
Debabrata Biswas\*

Department of Animal and Avian Sciences and Center for Food Safety and Security Systems, University of Maryland, College Park, MD 20742, USA

**\*Address for Correspondence**

Dr. Debabrata Biswas, Assistant Professor, Department of Animal and Avian Sciences and Center for Food Safety and Security Systems, University of Maryland, College Park, MD 20742, USA; E-mail: dbiswas@umd.edu

**Copyright:** © 2014 Salaheen S, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Submission:** 03 June 2014

**Accepted:** 05 June 2014

**Published:** 07 June 2014

product and processing environment that are required to make safer products in the processing plant. It is essential to determine potential threat of vegetative and VBNC *C. jejuni* contamination and their survival ability in various conditions in poultry carcass and treatment system, and develop the precise processing and molecular methods to improve their detection. In recent years, several molecular techniques including PCR (polymerase chain reaction) and sequencing have been tested and recommended for routine use for surveillance of environmental samples and microbiological quality control. However, methods capable of detecting VBNC *Campylobacter* are scarce. Josefsen et al. [15] proposed a technique for detecting both viable and VBNC *Campylobacter* cells using real time PCR and propidium monoazide, but further validation of the technique is required. More research is also required to evaluate the survivability of *Campylobacter* across the poultry carcass and treatment system. Some possible ways to improve the system are as follows:

- i) Develop technology for rapid identification of vegetative and VBNC *C. jejuni* cells;
- ii) Develop a statistical framework necessary to evaluate the potential health risks with this bacterial pathogen in both vegetative and VBNC forms;
- iii) Determine the most effective intervention points to control the vegetative and VBNC *C. jejuni* in chicken carcass and processing environment;
- iv) Develop risk monitoring techniques to detect potential hazards of vegetative and VBNC *C. jejuni* cells in the distribution chain;
- v) Develop, complement and maintain an aggressive technology transfer system that effectively communicates the work of the processing industry.

### References

1. Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, et al. (2011) *Campylobacter* spp. as a Foodborne Pathogen: A Review. *Front Microbiol* 2: 200.
2. Lawson AJ, Shafi' MS, Pathak K, Stanley J (1998) Detection of *Campylobacter* in gastroenteritis: comparison of direct PCR assay of faecal samples with selective culture. *Epidemiol Infect* 121: 547-553.

3. Kulkarni SP, Lever S, Logan MJ, Lawson AJ, Stanley J, et al. (2002) Detection of Campylobacter species: a comparison of culture and polymerase chain reaction based methods. *J Clin Pathol* 55:749–753.
4. Keramas G, Bang DD, Lund M, Madsen M, Bunkenborg , et al. (2004) Use of Culture, PCR Analysis, and DNA Microarrays for Detection of Campylobacter jejuni and Campylobacter coli from Chicken Feces. *J Clin Microbiol* 42: 3985–3991.
5. Singh H, Rathore RS, Singh S, Cheema PS (2011) Comparative analysis of cultural isolation and PCR based assay for detection of Campylobacter jejuni in food and fecal samples. *Braz J Microbiol* 42: 181-186.
6. Rollins DM, Colwell RR (1986) Viable but nonculturable stage of Campylobacter jejuni and its role in survival in the natural aquatic environment. *Appl Environ Microbiol* 52(3), 531-538.
7. Jones DM, Sutcliffe EM, Curry a (1991) Recovery of viable but non-culturable Campylobacter jejuni. *J Gen Microbiol* 137: 2477-2282.
8. Cappelier J, Magras C, Jouve J, Federighi M (1999) Recovery of viable but non- culturable Campylobacter jejuni cells in two animal models. *Food Microbiol* 16: 375-383.
9. Baffone W, Casaroli A, Citterio B, Pierfelici L, Campana R, et al. (2006) Campylobacter jejuni loss of culturability in aqueous microcosms and ability to resuscitate in a mouse model. *Int J Food Microbiol* 107: 83-91.
10. Bari SMN, Roky MK, Mohiuddin M, Kamruzzaman M, Mekalanos JJ, (2013) Quorum-sensing autoinducers resuscitate dormant *Vibrio cholerae* in environmental water samples. *Proc Natl Acad Sci U S A* 110: 9926-9931.
11. Wivagg CN, Hung DT (2012) Resuscitation-promoting factors are required for  $\beta$ -lactam tolerance and the permeability barrier in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 56: 1591-1594.
12. Kana BD, Gordhan BG, Downing KJ, Sung N, Vostroktunova G, et al. (2008) The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro. *Mol Microbiol* 67: 672-684.
13. Chaisowwong W, Kusumoto A, Hashimoto M, Harada T, Maklon K, et al. (2012) Physiological Characterization of Campylobacter jejuni under Cold Stresses Conditions: Its Potential for Public Threat. *J Vet Med Sci* 74: 43-50.
14. Tholozan JL, Cappelier JM, Tissier JP, Delattre G, Federighi M (1999) Physiological Characterization of Viable-but-Nonculturable Campylobacter Physiological Characterization of Viable-but-Nonculturable Campylobacter jejuni Cells. *Appl Environ Microbiol* 65:1110–1116.
15. Josefsen MH, Löfström C, Hansen TB, Christensen LS, Olsen JE, et al. (2010) Rapid quantification of viable Campylobacter bacteria on chicken carcasses, using real-time PCR and propidium monoazide treatment, as a tool for quantitative risk assessment. *Appl Environ Microbiol* 76: 5097-5104.