Rapid Detection of *Campylobacter jejuni* in Poultry Products Using Quantum Dots and Nanobeads Based Fluorescent Immunoassay

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Abstract: *Campylobacter jejuni* causes 2.1 to 2.4 million cases of foodborne illnesses in the United States each year with some of the cases linked to eating undercooked poultry or handling raw poultry and poultry products. Thus, a rapid, specific method is needed to detect *C. jejuni* on poultry and poultry products. The objective of this research was to develop a sensitive immunoassay method for rapid detection of *C. jejuni* by using both magnetic nanobeads to separate and concentrate the target bacteria and quantum dots (QDs) as fluorescent markers. In this research, both streptavidin conjugated QDs 620 (8 nm diameter) and magnetic nanobeads (150 nm diameter) were separately coated with the specific biotin conjugated anti-*C. jejuni* antibody. The conjugated magnetic nanobeads then were mixed with a sample containing *C. jejuni*. After immunomagnetic separation, the magnetic nanobeads-*C. jejuni* conjugates were mixed with the conjugated QDs. Then, unattached conjugated QDs were removed using immunomagnetic separation. A spectrometer was used to measure the fluorescence of the complexes of magnetic beads-*C. jejuni*-QDs. The results showed that this method could detect *C. jejuni* in pure culture, ground turkey, chicken juice or chicken carcass wash solution at concentrations down to 2-3 cells/0.1 mL sample (20-30 cfu/mL). The total detection time was less than 2 h. This study would provide the poultry industry a more effective rapid method for detection of major foodborne pathogens in poultry products to ensure food safety.

Key words: *Campylobacter* detection, magnetic nanobeads, quantum dots, Immunoassay

INTRODUCTION

*Campylobacter jejuni* is one of the most important bacterial pathogens associated with foodborne diseases reported worldwide. *C. jejuni* is estimated to cause 2.4 million cases of foodborne illness in the United States each year (CDC, 2010, 2012). Many of the previous cases have been linked to eating undercooked poultry or handling raw poultry and poultry products. Studies have found *Campylobacter* contamination of up to 88% of chicken carcasses (Clark, 2013; FDA, 2013). The development of rapid, sensitive and specific methods for detection of food-associated bacterial pathogens remains challenging for ensuring food safety. Immunoassays are one category of rapid methods that have been developed for detection of pathogenic bacteria in food, environmental and clinical samples. Although conventional culture methods hypothetically allow the detection of a single cell of specific pathogens, they are extremely time-consuming, typically requiring at least 24 h and complicated multi-steps to confirm the analysis. Even current well studied rapid methods such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) can take 10 to 24 h and 4 to 6 h, respectively, with detection limits varying from 10\(^2\) to 10\(^3\) cfu/mL.

Quantum dots (QDs) as colloidal nanocrystalline semiconductor were first studied and reported in 1998 (Bruchez et al., 1998; Chan and Nie, 1998). QDs are composed of CdSe/ZnS core/shell nanocrystals. The inorganic fluorophores QDs have advantages over traditional organic dyes and fluorescent proteins due to their high fluorescence quantum yields, their excellent stability against photobleaching and chemical degradation, and their properties of broad absorption spectra, narrow-emission spectra (Jaiswal et al., 2003; Koeper et al., 2005; Shao et al., 2011). Hahn et al. (2005) reported that under continuous excitation, QDs retain high fluorescence intensities for hours whereas the typical organic dye bleaches within seconds. Water soluble QDs are suitable for biological detection and biomolecular conjugation. Desirable biological molecules can be coupled to QDs and retain their biological activities, which leads to the development of molecular conjugates of QDs that are biocompatible and suitable for use in cell biology and immunoassays. Researchers have investigated the applications of QDs for biological detection of cellular imaging (Chalmers et al., 2007; Jaiswal and Simon, 2004) and bacteria (Su and Li, 2004; Yang and Li, 2005, 2006; Xue et al., 2009; Wang et al., 2011).

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Magnetic nanobeads (MNBs) are well-established core-shell nanoparticles with controlled size. The magnetic core consisted of Fe₃O₄ has an external magnetic force for the immunomagnetic separation. Nanobeads exhibit properties of ferrofluids and remain stable colloids (Lekowska-Kochaniak et al., 2002; Liu et al., 2003). Currently, researchers conjugated MNBs with antibodies, aptamers, antibiotics, probes, proteins and dyes for bacterial separation and detection, protein purification and toxin decapsulation (Gu et al., 2006; Varshney and Li, 2007; Gao et al., 2009; Goransson et al., 2010).

In this research, we successfully developed a sensitive immunoassay method using magnetic nanobeads and quantum dots for rapid detection of C. jejuni in pure culture, chicken juice, ground turkey or chicken carcass wash solution.

MATERIALS AND METHODS
Pathogenic bacterial cultures and surface plating methods: Stock cultures of C. jejuni (ATCC 29428) purchased from American Type Culture Collection (Manassas, VA) were grown for 18-20 h at 42°C in Campylobacter enrichment broth (Remel, Lenexa, KS). Serial 10-fold dilutions were made in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) and the viable cell numbers of C. jejuni were determined by surface plating on Campylobacter enrichment selective agar (Remel, Lenexa, KS). Stock cultures of S. Typhimurium (ATCC 14028), L. monocytogenes (ATCC 43251) and E. coli O157:H7 (ATCC 43888) purchased from American Type Culture Collection (Manassas, VA) were grown for 18-20 h at 37°C in brain heart infusion (BHI) broth (Remel, Lenexa, KS). After serial 10-fold dilutions in PBS, the viable cell numbers of S. Typhimurium, E. coli O157:H7 and L. monocytogenes were determined by surface plating on selective agars.

Preparation of immunomagnetic nanobeads and QDs: Biotinylated rabbit anti-C. jejuni polyclone antibody (4.5 mg/ml) obtained from Thermo Scientific (Rockford, IL). A 1:10 dilution of anti-Campylobacter antibody was prepared with PBS (0.01 M, pH 7.4) for further use. Streptavidin capture ferrofluid conjugated magnetic nanobeads (MNBs 150 nm diameter) purchased from R&D Systems (Minneapolis, MN) were coated with the anti-C. jejuni antibodies. To avoid using extra reagents, the ratio of the streptavidin conjugated magnetic nanobeads and biotinylated antibody were calculated according their binding sets. In this step, 20 μL of immunomagnetic nanobeads were mixed with 20 μL biotin-conjugated rabbit anti-Campylobacter antibody (0.5 mg/mL). Streptavidin conjugated quantum dots (QDs) 620 (1 μM, 8 nm diameter) purchased from Ocean Nanotech (San Diego, CA) were coated with the biotin labeled anti-C. jejuni by biotin-streptavidin complex. The ratio of the streptavidin conjugated QDs and biotinylated antibodies were tested to minimize the materials used. 20 μL of 1:3 diluted QDs 620 were mixed with 20 μL biotin-conjugated rabbit anti-Campylobacter antibody (0.5 mg/ml). Both MNBs-antibody and QDs-antibody mixtures were shaken on a RKVSD 10101 mixer (ART, Inc., Laurel, MD) at a speed of 10 rpm at room temperature for 30 min.

Inoculation of chicken juice, chicken carcass wash and ground turkey wash solutions: For preparation of chicken juice, commercially frozen chickens without giblets were placed in a container and thawed over night at room temperature. Chicken juice was obtained from each chicken and mixed together (Birk et al., 2004). The mixed chicken juice was subsequently cleared by centrifugation to eliminate large particles, by sterilized filtration and was then stored at -20°C. Before use, the sterilized chicken juice was thawed at 4°C overnight. Post-chilled chicken carcasses were individually washed with 100 mL of 0.1% buffered peptone water (BPW) by hand shaking for 1 min and the wash solutions were collected. Each of 25 g samples of ground turkey (purchased from local grocery store) was mixed with 225 mL of 0.1% PBW and stomacher washed for 1 min. The wash solutions were collected. All chicken juice, chicken carcass wash and ground turkey wash solutions were divided into two portions. One portion was inoculated with various dilutions of C. jejuni broth cultures to obtain final inoculation concentrations of 10⁷ to 10⁹ cfu/mL wash solution during the testing day. The second portion was not inoculated with the bacterial culture and served as controls. The final inoculation concentrations from 10⁷ to 10⁹ cfu/mL and controls were confirmed with the tests using bacterial plating methods.

Assay procedure: The assay procedure is outlined in Fig. 1. First, 100 μL of the each diluted 10⁻¹⁰⁻¹⁵ cfu/mL cultures was mixed with 40 μL antibody coated magnetic nanobeads (MNBs-Ab) for 30 min. The MNBs-Ab-cell conjugates were separated from the solution by putting the tube on a magnetic separator for 2 min and then removing the liquid with a syringe. After washing twice with PBS, the captured cells were mixed with 40 μL anti-C. jejuni antibody coated QDs 620 (QDs-Ab) for another 30 min. After removing excess QDs solution by immunoseparation, the final conjugates MNBs-C. jejuni-QDs were ready to be measured by a spectrometer. The fluorescence measurement was performed using a laptop-controlled portable system which included a USB2000 miniature fiber-optic spectrometer, a LS-450 LED light source module, a R400-7 UV-vis optical probe (Ocean Optics, Inc., Dunedin, FL) and a probe/cuvette holder housed in a dark box. Fluorescence intensity produced by QDs 620 gave a fluorescent peak at 620
Streptavidin conjugated nanobead

Biotin-labeled antibody

C. jejuni

Streptavidin conjugated QD

Other bacteria

\( \lambda_{ex} = 395 \text{ nm} \)

\( \lambda_{ex} = 620 \text{ nm} \)

Fluorescence Wavelength

Fig. 1(a-b): Procedure of the immunoassay for detection of Campylobacter jejuni: (a) Schematic diagram of the detection procedure. Biotinylated rabbit anti-C. jejuni antibody-coated streptavidin-capture ferrofluid conjugated MNBs were used for capturing C. jejuni cells from the samples. The biotinylated rabbit anti-C. jejuni antibody-coated streptavidin conjugated QDs were used as fluorescence labels. (B) The C. jejuni were detected through the spectrometer measurement of fluorescence intensity produced by bound QDs.

nm when QDs were excited at 395 nm wavelength. The fluorescent peaks of interference reaction signals increased with the increasing cell number of target cells. Separation of target bacteria from multiple bacteria in a pure culture was tested for the recovery rate, as well as detection limit. All tests were duplicated.

Test for possible interfering with multiple bacteria: The immunoassay was tested for possible cross-reaction with other foodborne bacteria including S. Typhimurium, L. monocytogenes and E. coli O157:H7. Those bacteria were tested individually and in combination with the target C. jejuni.

Statistical analysis: The results of experiments on the detection of C. jejuni at different concentrations in pure culture and three different poultry samples were compared individually to those of negative controls. The means and standard deviations of measured fluorescent intensity in each test were calculated and statistically evaluated by means of one-way analysis of variance and Student's t-test for multiple comparisons among all concentrations of C. jejuni using JMP®10 statistical software (SAS Institute, Inc., Cary, NC). In comparison, the means were considered to be significantly different when \( p < 0.1 \). Coefficient of determination \( R^2 \) represent the linear relationships of the change in fluorescent intensity vs. C. jejuni at concentrations ranging from 0 to \( 10^2 \) cells/0.1 mL in all samples were calculated using Excel.

RESULTS AND DISCUSSION

Tests for possible interfering with mixed multiple bacteria: The results of the bacterial interference test are shown in Fig. 2. All S. Typhimurium, L. monocytogenes and E. coli O157:H7 were detected in the presence of other bacteria.

Fig. 2: Representative fluorescence spectra obtained for the mixed bacterial cultures: samples of negative control (NC), S. Typhimurium (S) at level of 6x10^2 cfu/0.1 mL, L. monocytogenes (L) at levels of 2x10^2 cfu/0.1 mL, E. coli O157:H7 (E) at levels of 3x10^2 cells/0.1 mL, mixture of S. Typhimurium, L. monocytogenes and E. coli O157:H7 (SLE) at levels of 2x10^2 cfu/0.1 mL and the mixture of SLE cultures with C. jejuni (CSLE) at levels 1x10^3 cfu/0.1 mL.
Fig. 3(a-d): (a) Representative fluorescence spectra obtained for samples negative control and different C. jejuni in PBS at levels of $2 \times 10^0$, $2 \times 10^1$, $2 \times 10^2$ and $2 \times 10^3$ cells/0.1 mL in pure culture; (b) Representative fluorescence spectra obtained for samples containing C. jejuni at concentrations of 0 (or negative control), $3 \times 10^0$, $3 \times 10^1$, $3 \times 10^2$ and $3 \times 10^3$ cells/0.1 mL in ground turkey solutions; (c) Representative fluorescence spectra obtained for samples containing C. jejuni at concentrations of 0 (or negative control), $3 \times 10^0$, $3 \times 10^1$ and $3 \times 10^2$ cells/0.1 mL in wash chicken carcass solutions; and (d) Representative fluorescence spectra obtained for samples containing C. jejuni at concentrations of 0 (or negative control), $2 \times 10^0$, $2 \times 10^1$ and $2 \times 10^2$ cells/0.1 mL in chicken juice solutions.
monocytogenes and E. coli individual or mixed cultures show low fluorescent signal similar to the negative control. Only the mixtures with the target C. jejuni culture show above 2000 counts fluorescent intensities.

Detection of C. jejuni from pure cultures and different poultry samples: Figure 3(a) shows the results of the experiments to enumerate cells of C. jejuni in pure culture. The difference of fluorescent intensities for the lowest detection concentrations, 1-2 cells/0.1 mL of C. jejuni, cells, above their background fluorescent signal at 620 nm are over 1000 counts. The results of the experiments to enumerate cells of C. jejuni in ground turkey wash solution are shown in Fig. 3(b). The difference of fluorescent intensities for the lowest concentration, 1-3 cells/0.1 mL of C. jejuni cells, above their background fluorescent signal at 620 nm are close to 1000 counts. Figure 3(c) shows the results of the experiments to enumerate cells of C. jejuni in chicken carcasses wash solution. The difference of fluorescent intensities for the lowest detection concentrations, 1-3 cells/0.1 mL of C. jejuni cells, above their background fluorescent signal at 620 nm are 800 counts. The results of the experiments to enumerate cells of C. jejuni in chicken juice are shown in Fig. 3(d). The difference of fluorescent intensities for the lowest detection concentrations, 1-2 cells/0.1 mL of C. jejuni cells, above their background fluorescent signal at 00620 nm are 900 counts.

Figure 4 shows the means of fluorescent detection data at different concentrations of C. jejuni for each sample. It can be seen that the fluorescence intensity increased with the increasing cell number of Campylobacter from 2.3x10^2 to 10^3 cells/0.1 mL. These results demonstrated that the more bacterial cells in the sample, the greater amount of the QDs they could bind and, thus, the stronger fluorescence signal they produced. Linear relationships are found for all of different poultry samples with R^2 = 0.90 to 0.99. The use of magnetic nanobeads for bacterial separation and concentration in combination with QDs as fluorescent labels provides a method that gives the quantitative detection of C. jejuni. Conjugating nanobeads and QDs with desirable antibodies that are specific to target bacteria resulted in a detection method that is both sensitive and specific for C. jejuni in pure culture, chicken juice, chicken wash and ground turkey wash solutions at a concentration of 2-3 cells/0.1 mL sample (20-30 cfu/mL). The difference of fluorescent intensities for the lowest detection concentrations, 1-3 cells/0.1 mL of Campylobacter cells, above their background fluorescent signal at 620 nm are from 800 to 1000 counts. Possible cross-reactivity against S. Typhimurium, L. monocytogenes and E. coli O157:H7 was tested and no interference spectrum showed up. Fluorescence intensity increased with the increasing cell number of C. jejuni and linear relationships were determined with R^2>0.90. Results demonstrated that the more bacterial cells in the sample, the greater amount of the QDs they could bind and, thus, the stronger fluorescent signal they could produce. The detection time from sampling through to result report was within 2 h. The fluorescence intensities for different concentrations of C. jejuni in each type of poultry samples are significantly different (p<0.1).

Campylobacter jejuni is the most common Campylobacter species associated with foodborne illness. Conventional microbiological methods to identify C. jejuni are not only tedious and time-consuming, but also prone to misdiagnoses due to the viable but
nonculturable (VBNC) state (Tholozan et al., 1999; Ziprin et al., 2003; Lin et al., 2008; Meleo et al., 2011). Molecular method based PCR and real-time PCR assays may provide an alternative to culture methods for the detection of C. jejuni. However, those assays either need 24 to 48 h enrichment (Sails et al., 2003; Oliveira et al., 2005; Mayr et al., 2010) or only work at high detection limits (LaGier et al., 2004; Debretson et al., 2007; Leblanc-Maridor et al., 2011). Immunoassays using magnetic nanobeads to capture bacteria from food samples in combination with quantum dots as a fluorescent label have been studied for quantitative detection of E. coli O157:H7 (Wang et al., 2011b). Listeria monocytogenes (Wang et al., 2007; 2011a, b), Salmonella (Joo et al., 2012) and Staphylococcus aureus (Chen and Zhang, 2012). The development of a quantum dots and magnetic nanobeads based immunoassay for rapid and sensitive detection of C. jejuni described in this study is the first quantum dots immunoassay used to detect C. jejuni from poultry samples.

Conclusion: Our research demonstrates a rapid, specific method to detect C. jejuni in poultry samples using the magnetic nanobeads to separate and concentrate the target bacteria and the quantum dots as fluorescent labels. The detection limit of 2-3 cells/0.1 mL achieved within 2 h in this study is comparable with most rapid methods that have been investigated in recent years.

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REFERENCES


