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Development of rapid immuno-based nanosensors for the detection of pathogenic bacteria in poultry processing plants

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Abstract

Foodborne diseases is a major public health concern and costs billions of dollars losses every year. For example, 93.8 million cases of gastroenteritis reported in Europe, United States, South America and Asia. Different methods depending on various scientific principles are used for detection of pathogenic bacteria relating foodborne diseases, conventional methods including culture-depending methods, microscopic, PCR, serological and biochemical methods are still used but majority of this methods suffers from a number of disadvantages includes long time-analysis, high cost, limited sensitivity and lab-based. Therefore, there is an urgent need for development of rapid screening assays for field applications with high sensitivity. In this study, we developed colorimetric immuno-sensor was developed and evaluated as a novel rapid detection nanosensing platform for the detection of foodborne pathogens such as Salmonella Typhimurium, Salmonella Enteritidis, Staphylococcus Aureus and Campylobacter Jejuni. The sensing platform composed of cotton swab and nanoparticles. Cotton swaps were functionalized with general or specific recognition receptors for collecting and pre-concentration of bacteria from the surfaces. Then the cotton swab was dipped in a developing solution to indicate the presence of specific bacteria. The developing solution has a cocktail of different coloured nanospheres conjugated with different recognition receptors for the various bacteria analytes was used to generate the different colours. The nanosensor was used for testing the bacteria on different surfaces mimicking the poultry processing units such as glass slides, stainless steel and chicken meat.

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Keywords: Foodborne Pathogens; immunobiosensors; Salmonella Typhimurium: Salmonella Enteritidis: Staphylococcus Aureus: Campylobacter Jejuni.

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Bacteria, virus, parasites and fungi are playing a major role for causing human foodborne illness. Though there are hundreds of bacterial stain found in the bodies, mostly they are involved in the essential activities. The pathogenic bacteria are present in the guts and intestinal tract of animals[1,2] therefore, the food chain is the one of the main source for carrying the pathogens to the human body. *Salmonella Typhimurium, Salmonella Enteritidis, Staphylococcus Aureus* and *Campylobacter Jejuni* are the frequently reported as foodborne pathogens[3,4]. It is important to detect the contaminated pathogens in the human consuming foods. There are several methods applied for the detection, however they are time consuming and more expensive. Therefore there is a need for the rapid and sensitive method for the detection of food contamination. We have developed a low cost use and through cotton swab immune-bead based biosensor for foodborne pathogen detection.

Salmonella typhimurium (St), Salmonella enteritidis (Se), Staphylococcus aureus (Sa) and Campylobacter jejuni (Cj) bacteria are potential infectious diseases causing pathogens[5,6]. The recommended biosafety laboratories mused be used to handle these bacteria. A schematic of the developed assay is shown in Figure 1A. The cotton swab was activated by sodium periodate oxidation to aldehyde in acidic solution followed by bacteria specific capture antibody immobilization by simple aldehyde and primary amine coupling. The polymeric dye coated nano beads or magnetic beads are immobilized with the specific secondary antibody by EDC/NHs coupling. The capture antibody immobilized on the cotton surface was swabbed on the St, Se, Sa and Cj spiked chicken surfaces with series of known bacterial cell counts and washed with PBS buffer. The antibody-bacteria complex was then dipped in the cocktail of magnetic beads or various colored polymeric nano-beads immobilized with antibodies specific to each bacteria to form sandwich type of immune complex with bacterial cell. After washing, only the color of beads with antibody specific to the bacteria will remain and the color of the cotton surface was changed. The color of the cotton surface indicate the type of pathogenic bacteria present on the chicken surface. The specificity of the sensors were tested by swabbing capture antibody immobilized cottons incubate with different bacteria contaminated chicken surfaces individually followed by incubating each cotton swabs with cocktail of mixed colored beads. A single color specific to the capture antibody specific bacteria and the rest of the three cottons are colorless indicating the specificity of the sensor.

Salmonella typhimurium specific monoclonal antibody was immobilized on the cotton to capture the Salmonella typhimurium from the contaminated chicken meat. Black magnetic beads linked with secondary antibody for the detection of Salmonella typhimurium. The sandwich complex of the Salmonella typhimurium between the two antibodies changed the color of the cotton into black as shown in the Fig.1B. The black color of the cotton surface increases with increasing concentration of the cell counts. The detection limit of the Salmonella typhimurium detecting cotton sensor from the chicken surface is 10^1 cfu/ml. In the case of Salmonella enteritidis, Salmonella enteritidis specific secondary antibodies were used as capture and detection steps. As blue color beads were used for this detection, the cotton surface turn to blue after complexation with Salmonella enteritidis. The cottons were treated with increasing concentration of the cells. From the visible change in the color of the cotton for 10^1 cfu/ml indicated the sensitivity of Salmonella enteritidis detection. Similarly the Staphylococcus aureus detection we used orange beads linked antibody for the sandwich complex formation. A change in the color of the cotton was observed upon treating with 10^1 cfu/ml Staphylococcus aureus, which is the sensitivity of this assay. Hoever, the lower detection limit of the Campylobacter jejuni was noticed only at elevated concentrations (10^2 cfu/ml) from the green colored cotton surface. Our simple portable use and through method can be used at the point of care and low cost. Figure 1C shows the specificity of the developed sensors.

We developed new colorimetric nano-bead based immune-biosensors for the detection of *Salmonella Typhimurium, Salmonella Enteritidis*, *Staphylococcus aureus* and *Campylobacter jejuni* using cotton-tips. The lower sensitivity of the each bacterial cells are determined to be 10 cfu/ml with a dynamic range of 10 to10^8 10 cfu/ml on the chicken surfaces. This simple portable, rapid and low-cost method can be used at the of poultry processing plant for the detection of pathogenic bacteria by nacked eye.