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RESEARCH ARTICLE

Rapid Detection System of *Salmonella typhi* In Some Commercial Brands of Milk in Sulamani Of Iraq

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Abstract

Milk has many nutrients that make it an important food, and because Iraq is one of the developing countries who imports milk powder ,from deferent countries which many of them not fulfill the requirements of the quality control system including it s contamination with pathogenic bacteria ,this study conducted for detection of *Salmonella typhi* in some milk powder brands imported to Kurdistan region of Iraq ,by collecting seven Samples of milk powder brands from the Sulaimani market which include :Anchor , Mudhish, Maraey Al-khadra, Dielac (Ireland), Nido, Premier and Dielac (Neusland) and a fresh milk sample from cow, sheep and goat from private farm in a total of ten samples of milk . DNA extracted from these samples successfully using CTAB method and species specific PCR targeting the gene encoding CDP-tyvelose epimerase (rfbE), that is specific for typhoid fever agent in *salmonella typhi* was applied ,the result showed the expected size of amplified band specific for this strain of bacteria (615bp) was foun in four samples of the ten used in this study(40%) .Improving the quality control system is needed to use these modern techniques to protect the people from exposure to these pathogenic bacteria.

1.Introduction

Iraq is one of the developing countries who imports powdered milk and milk is one of the high-value food, which undergo a number of processing steps before they appear in the market ,so adulteration of these kinds of food become a big problem in recent years. Most studies revealed the transfer in the milk contaminated with pathogenic bacteria such as, Salmonella which reduce the quality of milk. *Salmonella* species have been linked with a major food borne pathogen for animals and humans **(1)**; in many countries, it is the leading cause of food borne outbreaks and infections .There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and three million deaths worldwide due to *Salmonella* **(2)**. In brief, *Salmonella* is facultative anaerobe, gram negative flagellated rod-shaped bacterium which is about 2-3 x 0.4-0.6 µm in size **(3)**. An outbreak of *Salmonella typhimurium* linked to milk contaminated post-pasteurization involving 96 illnesses from 2000-2007 described by Olsen *et.al* **(4)**, there were four outbreaks of salmonellosis in California which cased 329 illnesses from pasteurized milk **(5)**, it continues to be a major public health problem worldwide **(6)**.

It is expected that *Salmonella* may be present in any raw food materials **(7)**, in part because *Salmonella* is wide-spread in nature. The organism can colonize a wide variety of hosts (e.g., mammals, birds, reptiles, amphibians, and insects) and is widely disseminated in the environment (e.g., water, soil, plants). *Salmonella* can survive for weeks in water and for years in soil if environmental conditions such as temperature, humidity, and pH are favorable **(8)**. A high percentage of human salmonellosis occurs through consumption of raw milk or undercooked foods including dairy products (**9**). In a study conducted on 12 dairy farms from four state of USA, bulk tank milk were analyzed for *Salmonella*, it was found that 12.6% of samples were positive for *Salmonella* spp. **(10)**.

Salmonella outbreaks have also been linked to a number of food products with low water activity, including, dried milk **(11)**. Even pasteurized milk can be contaminated during bottling, shipment, and storage. Pasteurization only destroys the pathogens in the milk at the time of processing; if unsanitary conditions allow pathogens to re-enter the milk later, it will be contaminated again. An example of post-pasteurization contamination involving a multi-drug resistant strain of *Salmonella* typhimurium occurred in Pennsylvania and New Jersey in 2000 **(4)**.Currently, the official procedure for detection of *Salmonella* spp. is a cultural method, and this procedure could take from 3 to 5 days for confirmation **(12)** which is a disadvantage when the results are needed promptly **(13)**. Recently, nucleic acid amplification technologies have offered the potential for improved detection of Salmonella in the environment, providing greater sensitivity and dramatically speeding up detection, thereby improving the management of outbreaks through more rapid confirmation of the vehicle of infection (**12**). PCR has been gaining popularity as a tool in microbiological diagnosis due to the efficient, rapid and sensitive methods of detection, it used for detection of *Salmonella*, in different types of foods **(14)**.Several variations of standard PCR, such as multiplex PCR and real-time PCR, have recently been employed for Salmonella detection, and these methods have provided high sensitivity with some assays being able to detect as few as 30 cells per sample **(15)**. Species specific PCR was providing the chance of using primers for deferent specific genes in Salmonella ,while Croci *et.al* **(12)** designed specific PCR assay of the invA gene of Salmonella spp and showed that was highly specific and powerful tool for detection of Salmonella in 92 naturally-contaminated milk and meat samples, whereas Mousavi,*et.al* **(16)** developed a rapid and simple PCR-ELISA using primers specific for rfbE gene and found that the protocol was suitable for routine analysis of viable Salmonella typhi. So the aim of this study was to evaluate the PCR that used rfbE gene to detect Salmonella typhi in some commercial of milk powder brands imported to Iraq.

2. MATERIALS AND METHODS

2.1 DNA extraction from powdered milk samples

This study was carried out between February and December 2011,by collecting seven samples of powdered milk , from the Sulaimani market including :Anchor , Mudhish, Maraey Al-khadra , Dielac (Ireland), Nido, Premier , Dielac (New Zealand) , and three fresh milk samples of Sheep, Goat and Cow milk. DNA from milk samples was extracted after pre enrichment with puffer pepton water, using CTAB method as described by He**,**et.al (17) with slight modification: 1ml of the milk sample was mixed with an equal volume of CTAB extraction buffer [1.4 M NaCl, 2% CTAB (cetyl tri methylammonium bromide), 100 mMTris, 20 mM EDTA, pH 8.0], 2% of 2-mercaptoethanol and 100 µg/ml of Protenase K for 1 h at 65ºC with shaking. Then the samples were extracted with 2 ml of chloroform, centrifuged for 30 min at 4000rpm.the supernatant precipitated with 2 volumes of CTAB precipitation buffer (40 mM NaCl, 0.5% CTAB) and incubated at room temperature for 60 min, and centrifuged for 30 min at 4000rpm.the pellet was dissolved in 300 µL of 1.2 M NaCl and extracted with an equal volume of chloroform, then the mix centrifuged for 30 min at 4000rpm.DNA in the aqueous phase was precipitated with 0.1 volume of Ammonium acetate and 2 volume of isopropanol .then the precipitated DNA was transferred into a fresh tube adding 500 µl of (70%) ethanol, and washed. Finally, it was dissolved in TE(Tris ,EDTA) buffer.

2.2 PCR Reaction

The sequences of specific PCR primers for Salmonella typhi ,which designed on previously published paper(**16)** which were as follows :forward; 5'-**:**GA GGAAGGGAAATGAAGCTTTT -3' and Reverse; 5'-TAGCAAACTGTCTCC CA CCATAC Which expected to produced a 615 bp fragment ,these primers were purchased from Bionerr Company (Korea) . PCR reactions were performed with 1×PCR buffer without MgCl2; 2.5 mM MgCl2;50 ng DNA, 0.3 µM each primer; 0.25 mM (each) dNTP; 25 U/ml Taq DNA polymerase (sigma). PCR reactions were carried out in PCR thermal cycler ((Biotech,U.K) . Amplification consisted of 35 cycles: 1 min at 94°C, 1 min, 1 min at 60°C, and 1 min at 72°C. The amplified products were electrophoresed in a 1.2% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining (18).

3. RESULTS AND DISCUSSION

3.1 DNA Extraction from milk powder

The full amount of DNA obtained in this study (Figure 1) refers to the successful method of DNA extracted from fresh and powder milk, which depend on using the protocol of He *et .al*(17). The efficiency of this method to obtain suitable amount of DNA is due mainly to the highly versatile cetyl trimethylammonium bromide (CTAB), because it is a detergent that helps lyses the cell membrane and make a complex with the DNA in the sample to protect it from the next steps of DNA extraction.

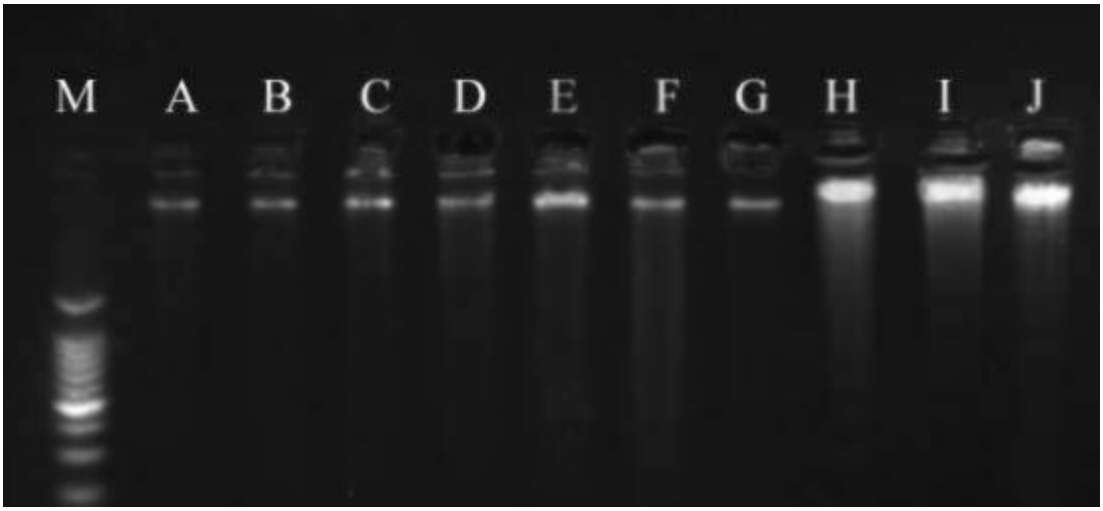

Fig 1: represent the results of DNA extraction from ten samples of milk, that was performed on (1.2 %) Agarose gel electrophoresis and run at 90 volt/cm for one hour.Lane (M) =1kb DNA ladder ,lane (A) Anchor (B) Mudhish (C) Maraey Al-Khadra (D) Dielac Ireland (E) NIDO (F) premier (G) Dielac New Zealand (H) Sheep milk (I) Goat milk (J) Cow milk.

3.2 Results of PCR Reactions

The gene encoding CDP-tyvelose epimerase (rfbE), that is specific for typhoid fever agent, was selected as the target of this assay, to detect the Salmonella typhi. Alternative conditions for PCR amplification were tested which include higher annealing temperatures as well as higher primer concentrations. The before mentioned conditions were, therefore, found to be the optimum reaction conditions. Standard amplification with the primer pair resulted in a 615-bp band, as seen in the Ethidium Bromide stained agarose gel electrophoresis (Figure 2). In line A, B, C, D which represent the powder milk of Anchor, Mudhish, Maraey Al-Khadra and Dielac (Ireland) respectively, these primer also used by, Hirose *et.al* (19) and Mousavi,*et.al* (16) for detection of Salmonella typhi in food. One of the critical points of the PCR used, is the choice of the sequence to be amplified, which must be present in bacterial strain and do not present homology with other organisms.

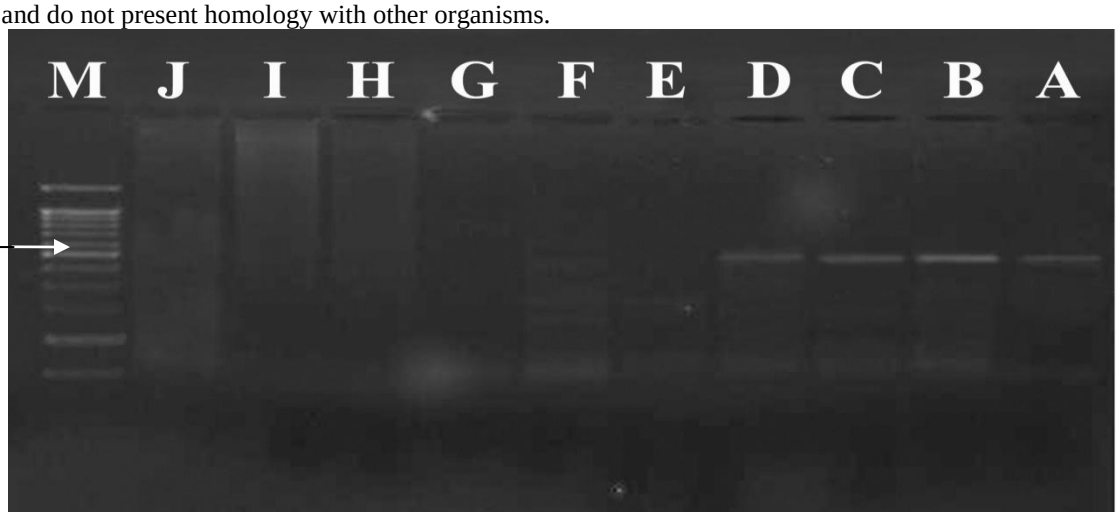

Figure 2: Represents amplified product with 615bp using salmonella specific primer in 10 sample of milk. Lane (M) = 1kb DNA ladder ,lane (A) Anchor (B) Mudhish (C) Maraey Al-Khadra (D) Dielac Ireland (E) NIDO (F) premier (G) Dielac New Zealand (H) Sheep milk (I) Goat milk (J) Cow milk, and Gel electrophoresis was performed on (1.2 %) Agarose gel and run at 90 volt/cm for one hour.

Salmonella was the second most commonly reported pathogen overall, and the most frequent agent linked to pasteurized milk outbreaks **(6).** Pasteurized milk can be contaminated during bottling, shipment, and storage. Pasteurization only destroys the pathogens in the milk at the time of processing; if unsanitary conditions allow pathogens to re-enter the milk later, it will be contaminated again. Entry of food borne pathogens via contaminated raw milk into dairy food processing plants can lead to subsequent contamination of processed food products. Good monitoring and screening programs are required in order to prevent salmonella infections **(12).**The detection of food borne pathogens is challenging for several reasons: target bacteria are often present in low numbers in foods and

bacteria may be sub-lethally injured due to food processing. Thus, most methods use sample enrichment to increase the number of live target bacteria in the matrix to detectable levels prior to analysis. This was also done in this study as the sample treatment comprised non selective pre enrichment in peptone water (BPW),before using the selective media for *Salmonella typhi* .Increased public awareness related to the health and economic impact of food-borne contamination and illness has resulted in greater efforts to develop more sensitive methods of pathogenic detection and identification, also it is importance of using the appropriate methodology to detect the strains of Salmonella that were causing illnesses. Therefore, efforts have been made by prior investigators to reduce the required time and increase the sensitivity of detection technique **(19)**. Due to its high sensitivity, specificity, and rapid results, PCR is an efficient alternative to conventional microbiological culture methods to detect specific types of microorganisms in foods **(20)**. Prevalence rates of Salmonella spp. in milk vary considerably depending on many factors such as geographical location, season, farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling, variation in types of samples evaluated, and differences in detection methodologies used. However, in spite of the variation, all of these surveys demonstrated quite clearly that milk can be a significant source of food borne pathogens of human health significance.

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