Detection of *Salmonella* spp. in ready-to-eat foods: use of the biomolecolar method BAX® in association with traditional microbiology

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Abstract

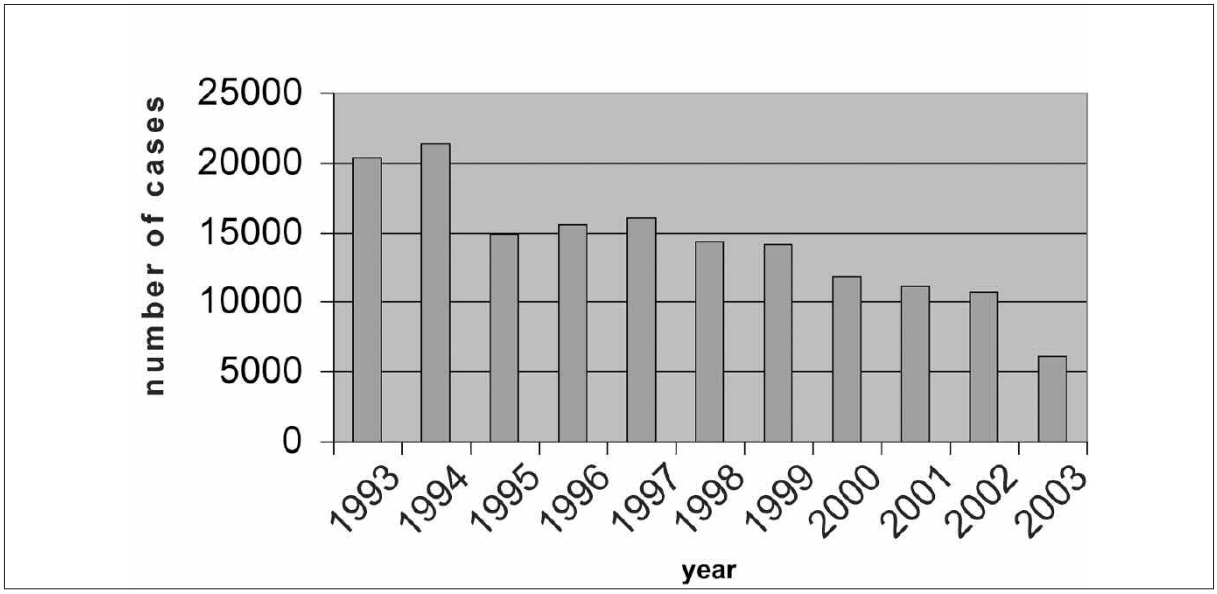
In order to evaluate the presence of *Salmonella* spp. in ready-to-eat foods, sixty-nine samples were analyzed using the BAX® system for Screening/*Salmonella*, a quick method based on PCR technology, and conventional culture procedures. Both methods showed the absence of *Salmonella* spp. in all samples and positive results for one sample artificially contaminated by a strain of *Salmonella* enteritidis, showing a full agreement. The biomolecolar system adopted appeared to be useful to supplement existing tests for the final identification of rough presumptive *Salmonella* isolates, for the rapid screening of food samples and the quick identification of *Salmonella* spp.

Key words: Salmonella, ready-to-eat foods, PCR

Introduction

Food-borne infections caused by members of the genus *Salmonella* continue to be a problem for public health all over the world as well as in Italy. These infections cause considerable morbidity, mortality and economic burden and are especially severe in the very young, the elderly and the immunocompromised.[1] In Italy, despite the introduction of the HACCP (Hazard Analysis and Critical Control Point), thousands of cases of Salmonellosis are notified each year, even though the trend is decreasing (Figure 1). According to the data available from the Epidemiology Bulletin of the Ministry of Health, an average of 15000 cases were notified for each year during the time-period 1993-2002,[2] however the underreporting of infectious diseases must be taken into consideration. Moreover, the emergence and

Figure 1. Notified cases of Salmonellosis in Italy from 1993 to 2003 (Source: Ministry of Health) [data relating to 2003 are not definitive].



persistence of highly virulent and antibiotic-resistant *Salmonella* strains in recent years are major public health concerns. The relentless human pandemic of *S. enteritidis* from the consumption of poultry and eggshells and the increasing prevalence of the pentavalent antibiotic-resistant *S. typhimurium* DT 104 in animal meats and in humans are disquieting.[3]

Conventional detection methods for *Salmonella* cells can take 3 to 4 days to produce a negative result and up to 7 days for a confirmed positive result.[1] In recent years the lengthy nature of these procedures has led to the development of no fewer than 35 diagnostic systems for the rapid detection of *Salmonella* in foods and agricultural products: colorimetric and fluorimetric enzyme-linked immunosorbent assays (ELISA), DNA probe hybridization, immunoimmobilization of motile *Salmonella*, polymerase chain reaction (PCR), *Salmonella*-induced conductance changes in liquid media and systems for the identification of *Salmonella* based on generic biochemical reactions.[3] Among the biomolecular methods existing, the BAX® system for Screening/*Salmonella,* a quick method that uses PCR technology, is able to detect hundreds of *Salmonella* serotypes in milk, chicken, turkey, beef and pork; the system includes 99.7% of the purified DNA from 1800 strains of *Salmonella* and it demonstrates excellent exclusivity.[4]

The purpose of the present study is to evaluate the presence of *Salmonella* spp. in ready-to-eat foods, comparing the BAX® system for Screening/*Salmonella* with conventional culture procedures.

Methods

Sixty-nine samples of ready-to-eat foods collected in different refectories in the time period from September 2003 to September 2004 were analysed for the evaluation of bacterial load, the presence of *Salmonella* spp, total Coliphorms, *Escherichia coli* and *Staphylococcus aureus* using ISO methods. The same samples were also screened for *Salmonella* spp. using the BAX® system.

Different kinds of ready-to-eat foods were sampled: pasta, rice, meat, chicken, fish, eggs, cooked and raw vegetables, potatoes and sandwiches.

Sampling was carried out using sterile packages carried to the laboratory in a refrigerated thermos bag in order to avoid bacterial proliferation.

Standard culture methods used for the detection of *Salmonella* spp. is based on five distinct steps, as reported in ISO 6579 [5]: pre-enrichment, selective enrichment, plating on differential agar, biochemical screening and serological confirmation.[3]

The reference limit for microbial count for *Salmonella* spp. in cooked preparations, according to the Guidelines of the Lazio Region, edited in 1998, is "absent in 25 g".[6]

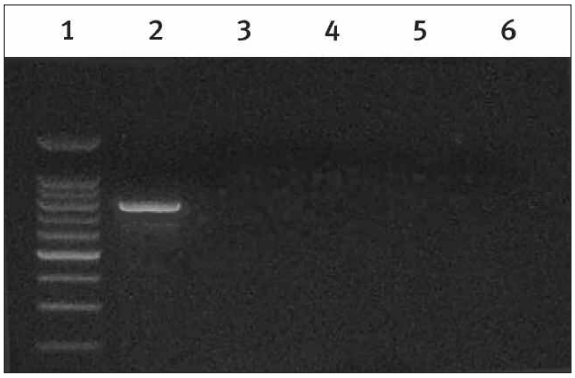
The biomolecolar method used, the BAX® system for Screening/*Salmonella* (DuPont Qualicon), is a quick method for accurately detecting *Salmonella* in food and environmental samples; it is a qualitative screening tool that uses PCR technology to provide results the day after sampling. The BAX® system involves a basic three-step process: DNA preparation, amplification and detection. Bacterial DNA is released from organisms in enriched samples through a lysing procedure, as recommended by the manufacturer. Then 50µL of the lysed sample is transferred into new tubes with a tablet inside, containing all the components for the PCR (primers for a specifically targeted DNA region, Taq polymerase, buffer, dNTPs). DNA amplification is performed in a programmable DNA thermal cycler (Eppendorf). The cycling parameters are initial denaturation at 94°C for 2 minutes, followed by 35 cycles each consisting of 15 seconds at 94°C and 3 minutes at 72°C. The amplification product is then visualized by gel electrophoresis and the results are indicated by the presence or absence of fluorescent bands, at a specific molecular weight, in each lane. The specific targeted fragment of bacterial DNA is unknown (registered trademark), however it is stable, unaffected by growth environment and unique to *Salmonella* spp. Thus it provides a highly reliable indicator of the presence of *Salmonella*. PCR technology enables the BAX® system to provide such rapid and specific DNA amplification that results are available about seven hours after enrichment; if the target sequence is not present, no detectable amplification takes place. The procedure takes about one hour of user time.[4] In order to verify the validity of both culture methods and the biomolecular one, a food sample was also artificially contaminated by a well characterized strain of *Salmonella enteritidis*.

Results

In 21% (15/69) of the food samples the bacterial load was <10 CFU/g; in 24% (17/69) it was > 10^4 CFU/g. In 52.1 % (36/69) total Coliphorms were present; in 27.5% (19/69) *Escherichia coli* were present; in 2.8% (2/69) *Staphylococcus aureus* were present (10^2 CFU/g).

Both standard culture methods and biomolecular methods used for the detection of *Salmonella* spp. showed an absence of *Salmonella* spp. in all of the

Figure 2. Analysis of PCR-amplified 600-bp fragments by 1,5 % agarose gel electrophoresis. Lane 1: 100 bp (marker); lane 2: positive control; lanes 3-5: negative samples; lane 6: negative control (distilled water).



samples analysed (Figure 2). The artificially contaminated sample yielded positive results for both methods.[7]

Discussion

Salmonella spp. still remains the main cause of food-borne infections notified in Italy.[2] The risk of infection is high if contaminated foods are "ready-to-eat products", because they are not subjected to further cooking at high temperatures. Moreover, in addition to the importance of raw and undercooked meat, poultry, eggs and dairy products as potential vehicles of human Salmonellosis, there are increasing reports of outbreaks associated with fresh fruit and vegetables.[8] New foods such as vegetable sprouts, fresh fruits and fruit juices have been incriminated in recent years as vehicles of human *Salmonella* infections.[3] The problem of Salmonellosis is further compounded by the massive and unrestricted movement of foods through international trade, the national disparities in the hygienic agricultural and aquacultural production of foods and the non-uniform government and industry food safety controls implemented during the processing, distribution and marketing of fresh and processed food products.[3] Furthermore, the survival of *Salmonella* spp. on dry stainless steel surfaces, as demonstrated by recent studies,[9] points out the risk of cross-contamination of ready-to-eat foods.

For these reasons, it is evident that there is a necessity for quick methods to evaluate the presence of *Salmonella* spp. in food samples, considering that current conventional methods of *Salmonella* spp. detection require 96 hours. For example, the rapid distribution of fresh fruit and vegetables to retail outlets pre-empts their premarket testing for *Salmonella* spp. because detection methods require 3-4 days to ascertain the absence or presumptive presence of the pathogen.[3] The evaluation of the presence of *Salmonella* spp. using the biomolecular method offers the advantage of rapid identification (one day);[10] moreover, it may be useful in order to ensure the early arrest of the productive cycle.

In the present study, the results of the BAX® system were always in agreement with the conventional plating results.

One of the most important advantages of the PCR kit used is that it does not require further investment in amplicon detection equipment, outside those already available in a conventional PCR laboratory: it is a ready-to-go *Salmonella* PCR test; moreover, due to the flexibility of the sample set-up, it can be used by both small and large laboratories and it can be easily implemented in accredited laboratories with limited experience in molecular biology.[11]

Furthermore, the BAX® system is able to simplify the PCR assay by including all of the reagents necessary for the PCR, such as primers, enzyme and deoxyribonucleosides and positive controls into a single sample tablet, already packaged inside the PCR tubes.[1,4]

However, the biomolecular system utilised has been tested against many, but not all, strains of *Salmonella* within the sample types specified (milk, chicken, turkey, beef and pork) [4] so there is no warranty that this product is capable of detecting every bacterium within the *Salmonella* genus. Moreover, the BAX® system for Screening/ *Salmonella* is designed to test only for the presence of *Salmonella* and its positivity cannot exclude human or faecal contamination. In fact, in our study *Salmonella* spp. was not detected in any of the food samples analyzed, even if in some samples Coliphorms bacteria or *Staphylococcus aureus* were present. Furthermore, a positive test shows the presence of the DNA of the microrganism, but it is not able to demonstrate the presence of viable bacterial cells.

For all of these reasons, the biomolecular method should not be used as the sole basis for determining product safety, nor should it be used as the sole test for the release of a product.[4] Considering that one of the most important potential applications of PCR is identification testing, this new system is useful in supplementing, rather than replacing, existing tests for the final identification of, often problematic, rough presumptive *Salmonella* isolates,[11] for the rapid screening of samples and for the quick identification of *Salmonella*.

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