Evaluation of the BAX® system for the detection of *Salmonella* spp. in naturally contaminated chicken meat

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Abstract

The aim of this study was to verify the efficiency of the BAX® system for the detection of *Salmonella* spp. in raw chicken meat. The conventional culture method (IN 62, MAP) was used as a reference method. A total of 8,813 chicken carcass samples were analyzed. In the first part of the study, 1,200 samples were analyzed using the BAX® System and the conventional culture method. In the second part, 7,613 samples were analyzed by the BAX® system, and the conventional method was used only for samples that tested positive for *Salmonella* spp. by the BAX® system. The sensitivity, specificity, relative accuracy, positive predictive value, and negative predictive value obtained in the first part of this study were 100%, 92.3%, 96.4%, 53.3% and 100%, respectively. The BAX® system showed no false-negative results and reduced the time to obtain presumptive positive results. It is a suitable method for use in laboratories that perform a large number of food samples analyses daily. However, the conventional method is still required to confirm the presence of *Salmonella* spp. in samples that test positive using the BAX® system.

Keywords: molecular methods; PCR; rapid methods.

1 Introduction

Brazil is the world's largest exporter and the third largest producer of poultry meat. In 2011, Brazil produced approximately 13.05 million tons of chicken, approximately 30.2% of which was exported (UNIÃO..., 2012). The state of Parana is responsible for the largest proportion of domestic production accounting for 28.36% of the Brazilian production in 2011. Brazil per capita consumption of chicken increased from 29.91 kg in 2000 to 47.38 kg in 2011 (UNIÃO..., 2012).

Salmonellosis is the most common food-borne disease in Brazil, and *Salmonella* spp. were responsible for 1,660 (42.27%) of the 3,927 confirmed etiology outbreaks that occurred from 2000 and 2011. Its main vehicle of transmission to humans is foods of animal origin, especially chicken products (BRASIL, 2011).

The control of *Salmonella* spp. in chicken meat has been increased as a demand of international trade, and the prevention of contamination is related to control measures throughout the production chain. Conventional methods for the detection of *Salmonella* based on culture are time consuming and laborious. Corrective and preventive measures during food processing are more effective when laboratory results can be obtained rapidly. Different immunological and molecular methods have been developed for the rapid detection of *Salmonella* spp. as an alternative to traditional culture method (ALVES et al., 2012; SILVA et al., 2011).

Polymerase chain reaction (PCR) is a sensitive and specific method for the detection of *Salmonella* that can provide accurate results within approximately 24 hours (SILVA et al., 2011). The automation of this method is required to facilitate the analysis of a great number of food samples. The BAX® system (Qualicon DuPont, Wilmington, DE, USA) for detection of *Salmonella* spp. in food is an automated PCR method that is widely used by Brazilian food quality control laboratories. The BAX® system combines speed with easy and simple handling. All reagents are provided in a single tablet and pre-packed in PCR tubes eliminating several reagent transfer steps diminishing analysis errors. In addition, this method does not require gel electrophoresis or photodocumentation.

Although the BAX® system has been approved by the Ministry of Agriculture and Food Supply (MAPA) (BRASIL, 2004), few studies validating this system have been performed in the country. The objective of this study was to evaluate the efficiency of the BAX® system to detect *Salmonella* spp. in samples of naturally contaminated raw chicken meat.

2 Materials and methods

2.1 Sampling

A total of 8,813 chicken carcass samples were analyzed. In the first part of the study, 1,200 samples were analyzed in March and April 2009 by the BAX® system and the conventional method. In the second part of the study, 7,613 samples were analyzed from May 2009 to June 2011 using the BAX® system, and the conventional method was used only with samples that tested positive by the BAX® system.

The chicken carcass samples were collected from slaughterhouses located in different regions of Parana state under the Federal Inspection of the Ministry of Agriculture

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and Food Supply (MAPA) of Brazil. The samples were sent to the laboratory in sterile plastic bags under refrigeration at temperatures between 2 and 8 °C. The laboratory received the samples within 24 hours after collection, and the analyses were carried out on the same day.

2.2 BAX® system

The BAX® system was used to detect *Salmonella* according to the manufacturer's protocol (DuPont Qualicon). Twenty-five grams of each sample were homogenized in 225 mL of buffered peptone water (BPW) (OXOID, Basingstoke, Hampshire, UK; CM: 509) and the rinses were incubated at 36 °C for 16-20 hours. Aliquots of 1 mL of the pre-enrichment broths were inoculated in 10 mL *Salmonella* Xpress 2 broth (SX2) (bioMerieux, Brasil) and incubated at 41.5 ± 0.5 °C for 24 hours. Aliquots of the selective enrichment broths (3 mL) were added to sterile tubes and heated at 95 °C for 15 minutes, and aliquots of 5 µL were added to cluster tubes containing 200 µL of lysis reagent (protease and lysis buffer). The mixture was heated for 20 minutes at 37 °C and for 10 minutes at 95 °C and then cooled at 4 °C for 5 minutes The PCR tablets in the PCR tubes were dissolved in 50 µL of the cooled lysate and loaded into the BAX® system cycler detector (BAX® System Classic) for a full process run. The results were obtained after approximately 3.5 hours.

2.3 Conventional cultural method

The conventional method for *Salmonella* spp. detection was carried out according to the Normative Instruction 62 of MAPA (BRASIL, 2003), which is based on ISO 6579 (INTERNATIONAL..., 2002).

Aliquots of 0.1 and 1 mL of the pre-enrichment broths obtained as described above were inoculated into 10 mL of Rappaport-Vassiliadis medium (RV) (OXOID) and 10 mL of selenite-cystine broth (SC) (DIFCO Microbiology, Lawrence, Kansas, USA), respectively. The RV and SC broths were incubated at 42 °C for 24 hours. The selective enrichment broths were plated onto Brilliant Green Phenol Red Lactose Sucrose agar (BPLS) (OXOID) and Mannitol Lysine Crystal Violet Brilliant Green agar (MLCB) (OXOID), and the plates were incubated at 36 °C for 24 hours. The API 20E system (bioMerieux, Brasil) was used for the biochemical identification of suspect colonies of *Salmonella* and the bacterial contaminants isolated in BPLS and MLCB. The confirmation of *Salmonella* spp. was carried out using specific *Salmonella* O and H agglutinating antisera (Probac do Brasil, São Paulo, SP, BR).

2.4 Analysis of results

The conventional method is one of the official methods used to detect *Salmonella* spp. in foods in Brazil, and it is considered the gold standard for evaluating new detection techniques. The sensitivity, specificity, relative accuracy, positive predictive value, negative predictive value and *kappa* coefficient of the BAX® system were calculated considering the conventional method as the reference method (MÄDE et al., 2004).

3 Results

The results obtained in the first part of this study in 1,200 chicken carcass samples analyzed by the BAX® system and the conventional method are shown in Table 1. The sensitivity, specificity, relative accuracy, positive predictive value and negative predictive value of the BAX® system were 100%, 92.3%, 96.4%, 53.3% and 100%, respectively. Since no false-negative results were obtained using the BAX® system and conventional method, the conventional method was only performed in the beginning of May 2009 to confirm the presence of *Salmonella* spp. in the samples that tested positive using the BAX® system.

The results obtained in the second part of this study, comprising 7,613 chicken carcass samples, are shown in Table 2. Samples that tested positive for *Salmonella* spp. in the BAX® system were considered contaminated when the pathogenic bacterium was also isolated using the traditional method. Samples were considered not contaminated and the result was considered a false positive when *Salmonella* spp. was not isolated in culture.

Table 1. Results of chicken carcass samples analyzed in March and April 2009 by the BAX® system and the conventional method.

	Results
Total samples analyzed	1,200
Total positive samples (BAX® system) (%)	92 (7.7%)
Total positive samples (BAX® system and conventional method) (%)	49 (4.1%)
Percentage of false-positive results	3.6% (n = 43)
Percentage of false-negative results	0% (n = 0)
Sensitivity[a]	100.0%
Specificity[b]	92.3%
Relative accuracy[c]	96.4%
Predictive positive value (VPP)[d]	53.3%
Predictive negative value (VPN)[e]	100.0%

[a] Sensitivity: samples that tested positive by both methods / total samples that tested positive by the conventional method x 100. [b] Specificity: samples that tested negative by both methods / total samples that tested negative by the conventional method x 100. [c] Relative accuracy: samples that tested positive by the BAX® system and were confirmed by the conventional method / total samples analyzed x 100. [d] VPP: samples that tested positive by both methods / total samples that tested positive by the BAX® system x 100. [e] VPN: samples that tested negative by both methods / total samples that tested negative by the BAX® system x 100.

Table 2. Results of chicken carcass samples analyzed from May to April 2009 using the BAX® system and the conventional method.

	Results obtained
Total samples analyzed	7,613
Total positive samples (BAX® system) (%)	845 (11.1%)
Total positive samples (BAX® system and conventional method) (%)	400 (5.3%)
Percentage of false-positive results	5.8% (n=445)
Specificity[a]	93.8%

[a] Specificity: samples that tested negative by both methods / total samples that tested negative by the conventional method x 100.

4 Discussion

Limiting the time required for the completion of microbiological analysis is essential to reduce the storage time of food products and therefore reduce the production costs, which are passed on to the consumer. New analytical methods and careful adjustments of existing methods have been proposed as strategies for reducing the analysis time; however, these methods must be validated.

Automated systems and commercial kits are available for the rapid detection of *Salmonella* spp. in foods. Many of these alternative methods have been validated by international organizations, but most of these validations are carried out on artificially contaminated samples, and these methods might produce different results when naturally contaminated foods are tested (MALORNY et al., 2009).

The BAX® system is adopted by the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to detect *Salmonella* spp. in food industries that process raw meat (UNITED..., 2013). The BAX® system was also approved in 2003 by the Ministry of Agriculture in Brazil as an official reference method to detect *Salmonella* spp. in food, water, and environmental samples (BRASIL, 2003).

Brazil and the United States are the world's largest exporters of chicken meat (UNIÃO..., 2012). Although the BAX® system was approved by these countries some years ago for the detection of *Salmonella* spp. in chicken meat, only few studies have been performed to analyze the efficiency of this method in naturally contaminated chicken meat (FRANCHIN et al., 2006; TOMAZELLI et al., 2008). The present study is the first to analyze a significant number of samples (n = 8,813) in Brazil.

The *kappa* coefficient between the BAX® system and the traditional method was not calculated because no false-negative results were produced by the BAX® system.

The sensitivity of the BAX® system was 100% (Table 1), and the percentages of specificity of 92.3% (Table 1) and 93.8% (Table 2) indicated that the method can be recommended for the detection of negative results; the concept of specificity is related to the proportion of correct negative results and the total of the non-contaminated samples. The negative predictive value of 100% indicated a high probability that a negative result obtained by the BAX® system is in fact a negative result (Table 1).

The false-positive rates found in the first and the second parts of this study were 3.6% and 5.8%, respectively (Tables 1 and 2). The false-positive results might occur due to the amplification of DNA from dead cells that could be present in the samples (SHERIDAN et al., 1998; DUPRAY et al., 1997; HERMAN, 1997; WOLFFS; NORLING; RADSTRÖM, 2005). Therefore, this method cannot be used to differentiate viable from nonviable food-borne pathogens (VELUSAMY et al., 2010).

Few studies have been conducted in Brazil to evaluate the efficiency of the BAX® system. Franchin et al. (2006) compared the BAX® system with the Modified Semi-Solid Rappaport-Vassiliadis method (MSRV) for the detection of *Salmonella* spp. in chicken carcasses (n = 762) and pork meat (n = 566). The conventional method was not included in the experiment, and the authors found no statistically significant differences between the two methods tested. In 2008, Tomazelli et al. analyzed 1,988 samples of 70 different food products produced in Brazil, including 235 samples of raw poultry meat, using the BAX® system and the traditional method. The sensitivity and false-negative rates of the BAX® system were ≥ 99.0% and ≤ 1.1%, respectively. The specificity (≥ 97.2%) was higher, and the false-positive rate (≤ 2.8%) was lower than the values obtained in the present study. Mata e Vanetti (2012) found no difference between the traditional method and the BAX® system in the analysis of 63 samples of Minas cheese produced in Minas Gerais, Brazil.

Studies carried out in other countries comparing the BAX® system with the traditional method for detecting *Salmonella* spp. in various food matrices also suggested good performance of the BAX® system. Studies conducted on produce, beef, and soy protein isolate indicated that the BAX® system performed as good as or better than the reference method for the detection of *Salmonella* spp. after 8-24 hours of enrichment (PENG et al. 2011). A multilaboratory study to detect *Salmonella* in peanut butter indicated that the BAX® system detected *Salmonella* in 10/60 low-spike samples (1.08 most probable number/25 g) and in 58/60 high-spike samples (11.5 most probable number/25 g), results similar to those obtained with the reference FDA-BAM method (Food and Drug Administration's Bacteriological Analytical Manual) (TICE et al., 2009).

According to Mattick et al. (2002), one of the main problems in the isolation of *Salmonella* in foods is the small number of these bacteria considering the rate of bacterial contamination. According to Bennett et al. (1998), the BAX® system always produced a positive result when the cell count of *Salmonella* spp. in BPW was 5.0×10^3 CFU / ml. Wu et al. (2003) reported that *Salmonella* spp. was detected by the BAX® system on pork carcass sponge samples with an initial inoculum (prior to enrichment) of 1.4×10^1 CFU / ml in the presence of 3.0×10^6 CFU / ml of other microbial contaminants.

Lots of foods, especially raw meat, are contaminated with *Proteus* spp. and *Citrobacter freundii*, which produce colonies similar to those of *Salmonella* spp. in the selective and differential media used in the conventional method. According to the São Camilo Laboratory, Maringa, Parana (personal communication), a total of 50,481 food samples were analyzed using conventional method between 2008 and April 2011, including 21,370 samples of raw chicken meat (42.3%). Approximately 100% of the samples of raw chicken meat analyzed required the biochemical identification of the colonies, which greatly increased the time and cost of analysis. Although the percentages of false positives found in the first (3.6%) and second parts (5.8%) of this study are higher than those observed in other studies, these results demonstrate that the BAX® system is a good alternative routine technique for laboratories that analyze a large number of samples on a daily basis.

The majority of published studies using alternative methods for the detection of *Salmonella* spp. analyzed a much smaller number of food samples than that used in this study. Therefore, the results obtained provide an accurate evaluation of the BAX® system for the analysis of raw chicken meat produced in Parana, Brazil.

5 Conclusion

The BAX® system used to detect *Salmonella* spp. in raw chicken meat produced no false-negative results and reduced the time required to obtain presumptive positive results. The method is suitable for use in laboratories, especially those that perform a large number of food samples analyses daily. However, this method should be considered a screening test, and the presence of *Salmonella* spp. must be confirmed by the conventional method.

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