Original Article

Escherichia coli and *Salmonella* spp. in meat in Jeddah, Saudi Arabia

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Abstract

Introduction: Food-borne pathogens are the leading cause of illness and death in developing countries, killing approximately 1.8 million people annually. In developed countries, food-borne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars. The objective of this study was to screen for two major food-borne pathogens, *Escherichia coli* and *Salmonella* spp., from meat samples obtained from different strata of the consumer market in Jeddah.

Methodology: A total of 60 meat samples, 20 each from large hypermarkets, groceries and small butcher shops were used in the study. Samples were transported to the laboratory in a cooler. They were macerated in peptone water and then seeded on selective media appropriate for each organism. Colonies were identified using conventional microbiological methods and suspected colonies were confirmed as *E. coli* and *Salmonella* spp. by polymerase chain reaction (PCR) using specific primers.

Results: The results indicated a high degree of contamination in samples from butcher shops as compared to those from groceries or hypermarkets (high scale supermarkets). Both pathogens *E. coli* and *Salmonella* spp. were found in higher rates in the samples from butcher shops. In small butcher shops, *E. coli* was found at an incidence of 65%, and *Salmonella* at 45%.

Conclusion: The results indicate an urgent need for applying proper food hygienic practices in food outlets, especially in small ones, to reduce the incidence of food-borne diseases. Vigilance by the right agencies must be implemented in order to prevent future food-borne outbreaks.

Key words: *Escherichia coli*; *Salmonella*; meat; PCR

J Infect Dev Ctries 2013; 7(11):812-818. doi:10.3855/jidc.3453

(Received 23 February 2013 – Accepted 31 May 2013)

Introduction

The broad spectrum of food-borne infections has changed over time; well-established pathogens are being controlled, and new ones are emerging. New pathogens may emerge as a result of changing ecology or changing technology that connects a potential pathogen to the food chain. They also can emerge *de novo* by transfer of mobile virulence factors, often through bacteriophages [1]. The burden of food-borne disease remains substantial. For instance, it is estimated that one in four Americans is affected by a significant food-borne illness each year [2]. Data indicating trends in food-borne infectious disease are limited to a few industrialised countries, and to even fewer pathogens [3]. Because outbreaks of food-borne illnesses may go underreported by as much as a factor of 30, the number of cases of gastroenteritis associated with food is estimated to be between 68 million and 275 million per year [4]. Although food production practices have changed, well-recognized food-borne pathogens such as *Salmonella* spp. and *Escherichia coli* seem to evolve and exploit novel opportunities, and to develop antimicrobial resistance to currently used agents. Constant vigilance, maintained by monitoring and surveillance, is necessary to sustain food safety standards [5]. Rapid detection of food-borne pathogens is a key step towards ensuring food safety. Since its advent in the 1980s, polymerase chain reaction (PCR) has become an indispensible tool in molecular diagnostics and can be very efficiently used in rapid detection of food-borne pathogens [6]. The high specificity of the primers designed to detect the

various pathogenic bacteria ensures that there is no ambiguity in the results. Moreover, the occurrence of these disease-causing pathogens in low numbers in food samples makes detection very difficult. The robustness of PCR lies in its ability to amplify even a single colony theoretically and make a definitive diagnosis. The conventional microbiological methods for detection of these bacteria, however, usually include multiple subcultures and biotype- or serotype-identification steps and thus are laborious and time consuming. Rapid detection of these pathogens in a multitude of samples simultaneously is necessary; this is very easily facilitated by PCR [7]. The main objective of this study was to screen meat samples from different consumer strata for the presence of food-borne pathogens. In this study, a total of 60 meat samples obtained from hypermarkets, groceries and small butcher shops in Jeddah were screened. The presence of both *E. coli* and *Salmonella* spp. are reported. Pathogens were detected by selective culture methods and confirmed with PCR using primers for specific genes of both pathogens.

Methodology

Sample preparation

A total of 60 meat samples (20 from each outlet) were collected from three types of food outlets: hypermarkets, groceries, and butcher shops. All samples were within their respective expiry dates. Meat samples collected from the hypermarkets and groceries were frozen samples, while the samples from the butcher shops were fresh. Meat samples of five grams each were macerated with 10 mL of sterile peptone water and filtered through a sterile muslin cloth to remove the particulate matter. Out of the filtrate, 1 mL was then boiled with 1 mL of lysis buffer made up of 1% Triton X-100, 10 mM Tris, and 1 mM EDTA, for 30 minutes at 95ºC. The suspension was centrifuged at 10,000 rpm for 10 minutes. Ten µL of the supernatant was used directly as the template for PCR.

Culture methods

After filtering the homogenates, 1 mL of the filtrate was taken and serial dilutions ranging from 10^{-1} to 10^{-6} were prepared by adding 1 mL of homogenate to 9 mL sterile water (1:10 dilution factor). Bacteriological analyses were performed by plating 0.1 mL of each dilution on agar plates. To detect E. coli, samples were inoculated on MacConkey's agar (HiMedia Laboratories, Mumbai, India) and incubated at 37ºC overnight; lactose-fermenting pink colonies were identified as E. coli. Biochemical tests were performed to confirm E. coli using Gram negative staining, catalase test, indole, methyl red, Voges-Proskauer test, nitrate reduction, and urease production. For Salmonella spp., the samples were plated on a selective medium such as SS agar (HiMedia Laboratories, Mumbai, India) for 18-24 hours at 37°C. For identification of Salmonella colonies, samples were subjected to biochemical tests such as triple sugar iron (TSI), sulfide-indole-motility medium (SIM), methyl red, Voges-Proskauer (MRVP), urea, and catalase, and finally reconfirmed as negative-bacilli or cocco bacilli by optical microscopy.

PCR primers

For *E. coli*, the primer sequence was based on the gene sequence of afa. This gene is responsible for pathogenicity and is specific to E. coli [8]. The primer sequence for the amplification of the afa gene from E. coli is: forward primer, 5' GCT GGG CAG CAA ACT GAT AAC TCT C 3'; reverse primer, 5' CAT CAA GCT GTT TGT TCG TCC GCC G 3'. The fimA gene in *S.* Typhimurium encodes the major fimbrial subunit. This gene has been cloned and sequenced from *S.* Typhimurium, and a particular region was found to be specific for Salmonella spp. The sequence of the primers used is: forward primer, 5' CCT TTC TCC ATC GTC CTG AA 3'; reverse primer, 5' TGG TGT TAT CTG CCT GAC CA 3'[4]. The afa gene PCR yields a product of 400 bp while the fim gene PCR yields a product of 120 bp. Strains identified as pathogenic E. coli by afa gene PCR were further characterized to determine the presence of the Shiga toxin gene (stx1). Primers used for stx1 gene were 5'CAGTTAATGTGGTGGCGAAG3' and 5' CTGTCACAGTAACAAACCGT 3' [8]. These primers were used to amplify a 513 bp product specific to the stx1 gene.

PCR conditions

PCR was conducted using a thermal cycler (Biorad, Hercules, USA). The reaction mixture consisted of an assay buffer, dNTP mix, 0.075 µM of each of the primers, 0.65 units of Taq DNA polymerase. Ten µL of the supernatant from the processed homogenates was taken in a sterile 0.5 mL microcentrifuge tube. The reaction volume was adjusted to 25 µL with sterile nuclease-free water. The reaction mixture tubes were placed in a thermal cycler and the reaction was performed for 40 cycles of PCR, with each cycle consisting of 1 minute at 94.8ºC

Table 1: Percentage distribution of pathogens in meat samples from different outlets.

	Hypermarkets (Total samples 20)	Groceries (Total samples 20)	Butcher shops (Total samples 20)
E.coli	4 (20%)	8 (40%)	13 (65%)
Salmonella spp.	1 (5%)	5 (25%)	9 (45%)
E.coli & *Salmonella* spp.	0	2 (10%)	4 (20%)

Figure 1: Detection of *E.coli* in meat samples from various outlets.

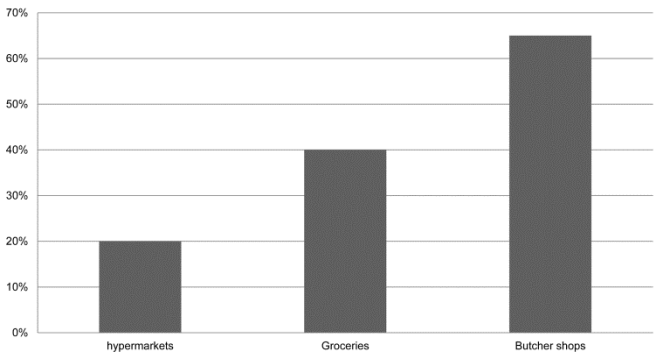
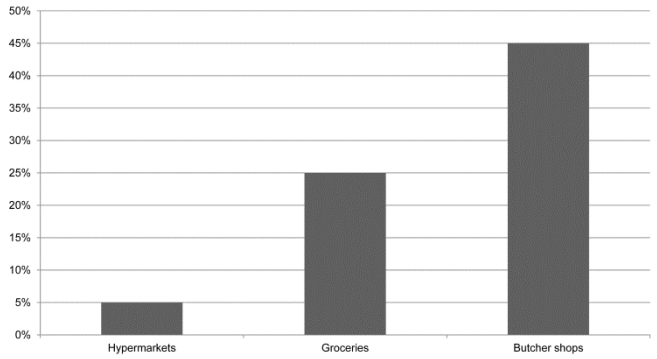


Figure 2: Detection of *Salmonella* spp. in meat samples from various outlets.



(denaturation), 30 seconds at 58ºC (annealing) and 1 minute at 72ºC (primer extension). An additional step of 5 minutes at 72ºC was also included for primer extension at the end of the reaction. After the reaction was complete, the PCR products were detected on agarose gel by electrophoresis, followed by visualization under a UV transilluminator. For the 400 bp afa gene product and the 513 bp stx gene product, 1% agarose gel was used; for the 120 bp fim gene product, 3% agarose gel was used.

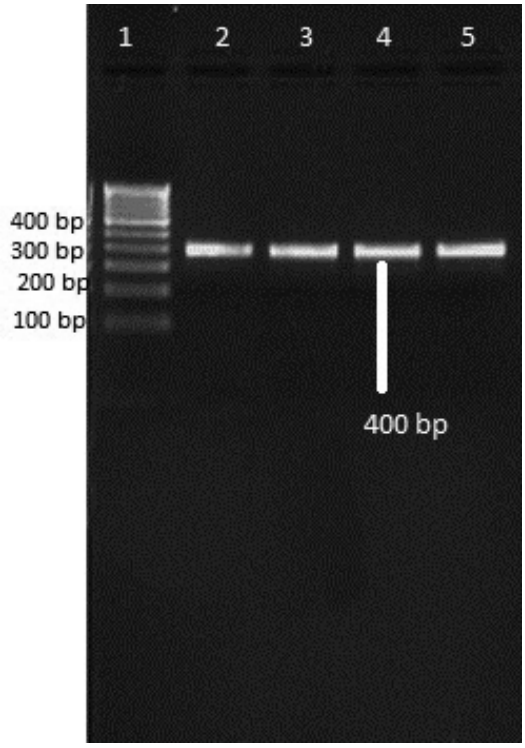
Statistical analyses

The chi square test was used to analyze if there was any significance in the pathogens isolated from the different outlets (hypermarkets, groceries and butcher shops). P values less than or equal to 0.05 were considered significant.

Results

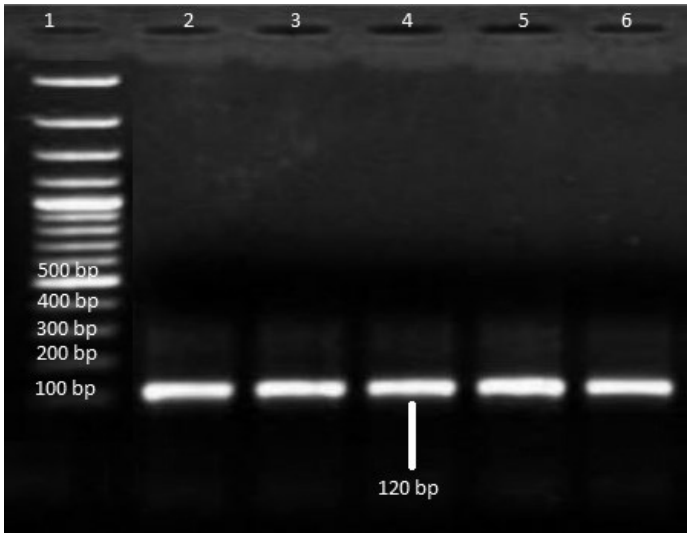
Culture tests from the meat homogenates showed the presence of both pathogens *E. coli* and *Salmonella* spp., and PCR confirmed these findings. The distribution of the occurrence of both pathogens in meat samples from various sources is listed in Table 1. We found a high incidence of *E. coli* in open butcher shops (65%) when compared to groceries (40%) and hypermarkets (20%). With respect to *Salmonella*, the rate of incidence was 45% from butcher shops, 25% from groceries, and 5% from hypermarkets. Figures 1 and 2 are the graphical representations of the rate of incidence of *E. coli* and *Salmonella* spp. from the different outlets. A high rate of incidence of both pathogens was found in open butcher shops compared to hypermarkets and groceries. PCR conducted on the samples that tested positive by culture also reiterated these findings. Figure 3 shows the PCR products of the afa gene, confirming the presence of pathogenic *E. coli*, while Figure 4 shows the *fim* gene product, indicating the presence of *Salmonella* spp. It is interesting to note that all the samples in which pathogens were detected using microbiological methods showed the presence of the respective gene PCR products, confirming our findings. Some of the isolates identified as *E. coli* by PCR and culture methods were also found to harbour the stx1 gene, further confirming the toxicity of the *E. coli* strains. Some of the *afa*-positive *E. coli* strains were stx-negative as indicated in Figure 5. Chi square analysis showed a significant difference associated with the presence of pathogens in butcher shops as compared to hypermarkets and groceries, with a p value of < 0.05. The observation that PCR conducted on the direct homogenate extracts confirmed the results obtained by the culture methods strongly asserts that direct PCR can be a very good method for rapid detection of food-borne pathogens.

Figure 3: afa gene PCR products from pathogenic *E. coli*.



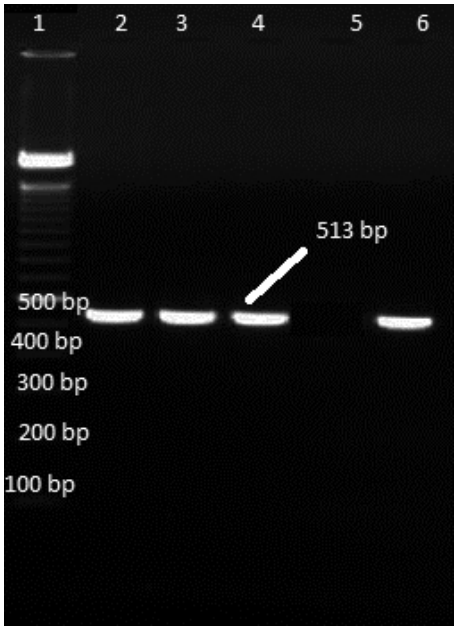
Lane 1: 100 bp marker
Lanes 2 & 3: Sample from butcher shops
Lanes 4: Sample from hypermarkets
Lane 5: Sample from groceries

Figure 4: fim gene PCR products from *Salmonella* spp.



Lane 1: 100 bp marker
Lanes 2 & 3: Samples from butcher shops
Lanes 4 & 5: Samples from supermarket
Lane 6: Sample from grocery

Figure 5: stx1 gene PCR products from pathogenic *E. coli*.



Lane 1: 100 bp ladder DNA marker
Lanes 2 & 3: Sample from butcher shops
Lane 4: Sample from hypermarkets
Lane 5: *E. coli* strains negative for stx 1 gene
Lane 6: Sample from groceries

Discussion

Food-borne pathogens are very diverse in their nature and are of major concern to public health worldwide. Many high-risk pathogens that cause diseases in humans are transmitted through various food items or water. Therefore, the microbiological safety of food has become an important issue for consumers and industry and regulatory agencies [9]. The Centers for Disease Control and Prevention (CDC) estimates that 48 million cases of food-borne illnesses occur in the United States every year, many of them caused by *Salmonella* spp. and *E. coli*. *Salmonella* spp. is now estimated to cause more than one million illnesses and 378 deaths annually [10,11]. *E. coli* toxins are estimated to cause 176,000 illnesses and 20 fatalities a year [12]. Hence, these two pathogens are a major cause of concern and were therefore selected for our study.

As meat consumption around the world increases concerns and challenges to meat hygiene and safety also increase. These concerns are mostly of a biological nature and include bacterial pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *Campylobacter* in raw meat and poultry, and *Listeria monocytogenes* in ready-to-eat processed products, while viral pathogens are of major concern in foodservice [13]. In 2012, Saleh *et al.* [14] reported isolation of *Yersinia* that was highly resistant to antimicrobials from dairy-based products in Lebanon, while Harakeh *et al.* [15] reported the presence of highly resistant *Listeria* in dairy-based foods. A major goal of scientists, industry, public health and regulatory authorities is to control pathogenic microorganisms and to improve meat product hygiene and safety within countries and internationally [16].

In recent years, contamination of meat with pathogens has been reported in Saudi Arabia. A 2012 study by Bharathirajan *et al.* [17] examining the isolation of pathogens from domestic refrigerators to determine the prevalence of pathogenic microorganisms showed the presence of pathogens such as *E. coli, Salmonella* spp*., Campylobacter* and *Listeria*. This clearly indicates very poor standards of consumer refrigerator management and hygiene, posing risks to consumer health. Another study by Altalhi *et al.* in 2009 [18] that examined meat samples from various outlets in Taif, Saudi Arabia, showed significant contamination with *E. coli* strains that were highly resistant to a wide range of antibiotics. Another study by Dughaym and Altabari [19] on chicken meat from Al-Ahsa markets in Saudi Arabia showed a high incidence of pathogens such as *E. coli*, *Salmonella* spp. and *Staphylococcus aureus*. In this study, meat samples from three different strata of the consumer market (large hypermarkets, small groceries and small butcher shops) were examined to determine if the handling, packaging, storing and distribution variables in these different strata were associated with the isolation of pathogenic *E. coli* and *Salmonella* spp. Using microbiological testing and biochemical characterization, both pathogens with varying incidence rates from the different outlets were found, with a maximum occurrence in the small butcher shops. Cross-verification for confirmation with PCR of the meat homogenates as direct templates confirmed these findings. Since the mid-1980s, PCR technology has been proven to be an invaluable tool for the detection of pathogens in food. Numerous papers have published on PCR detection of different food-borne pathogens including *E. coli, Salmonella* spp*.*, *Shigella, Yersinia, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Listeria monocytogenes*, and *Staphylococcus aureus* [13,20]. Rapid detection of these pathogens is an important step towards the prevention of food-borne epidemics. The most commonly used molecular methods are the conventional PCR, real time PCR and multiplex PCR, which are very rapidly aiming to replace conventional culture methods [21]. Although new technologies like biosensors show potential approaches, further research and development is essential before biosensors become a real and reliable choice. New bio-molecular techniques for food pathogen detection are being developed to improve the biosensor characteristics such as sensitivity and selectivity, which are also rapid, reliable, effective, and suitable for *in situ* analysis [22].

In the present study, direct PCR was shown to be very effective in the detection of the pathogens from meat sample homogenates; these findings were confirmed by culture methods, indicating that direct PCR is a robust method for rapid detection in comparison with culture methods. This indicates that direct PCR can actually be used for routine screening of large batches of meat products to ensure food safety in the meat packaging and processing industry. For effective detection of *Salmonella* spp., *E. coli* O157, and *Listeria monocytogenes* in meat products, a multiplex real-time PCR coupled with a multi-pathogen enrichment strategy was developed by Suo *et al.* in 2010 [21]. They proved that the multiplex real-time PCR assay combined with multi-pathogen enrichment is a rapid and reliable method for

effectively screening single or multiple pathogen occurrences in various meat products. Another study by Hill *et al.* in 2011 [23] used PCR to effectively screen for *Salmonella* spp. and enterohemorrhagic *E. coli* in beef products in processing establishments.

As clearly indicated by the present results, small butcher shops had a higher incidence of both pathogens. This can be attributed to the fact that these small butcher shops do not practice proper sanitation methods while slaughtering and packing meat products. A 2012 study by Aftab *et al.* [24] in Pakistan was designed to determine the level of *Salmonella* spp. contamination on cattle body coats, on fresh carcasses, and on beef after transportation and display at butcher shops. The results suggested that samples collected after being on display at the shop between six to seven hours showed a higher degree of *Salmonella* spp. contamination, and the isolation was significantly affected by the duration and washing of the samples, clearly indicating that the extent of practicing good sanitation methods is a key factor in the prevalence of pathogens in butcher shops. Some of the pathogenic *E. coli* in the present study tested positive for the presence of the Shiga toxin gene, indicating a higher pathogenecity and a higher risk of causing epidemics of gastroenteric disorders. A similar study by Hassan Ali *et al.* in 2010 [25] on the microbial contamination of raw meat and its environment in retail shops in Pakistan showed a high prevalence of a wide range of food-borne pathogens such as *E. coli* O157:H7, *Listeria*, *Salmonella* spp., *Enteritidis* and *Shigella* species. Packaging and storage are better in groceries, as proper refrigeration methods are used to store the meat. A study by Kegode *et al.* in 2008 [26] on pathogens prevalent in raw meat from groceries in North Dakota showed a high incidence of *E. coli, Salmonella* spp., and *Campylobacter*, indicating the need for proper storage and handling practices. That study also suggested that raw retail meats may be vehicles for transmitting food-borne diseases and signified the importance of sustained surveillance of food-borne pathogens in retail meats. In comparison to small butcher shops and groceries, hypermarket meat samples had a very low incidence of these pathogens. This can be explained by the fact that bigger hypermarkets have better storage conditions and have more rigorous and strict means of ensuring safety at all levels of handling meat products. Harakeh *et al.* in 2005 [13] characterized at the molecular level the different strains of *Salmonella* spp. and *E. coli* that were isolated from meat-based fast food in Lebanon by using PCR; the researchers also evaluated the resistance of those strains to different antimicrobials that are commonly used. They found that the isolates were highly resistant to multiple antibiotics, indicating the need for implementing better handling and sanitation practices in the meat industry.

Conclusions

In conclusion, the present study has clearly established two facts. First, that direct PCR is very effective in detecting both pathogens; the positive ones were confirmed by routine microbiological methods. This finding serves as a platform to design regular screening programs in the food safety and processing units to ensure early and rapid detection of these food-borne pathogens. Second, this study has clearly shown the difference in levels of contamination of meat from different sources, suggesting that safety and sanitation practices at different consumer market strata are strong contributing factors to the level of contamination. This study demonstrates the importance of hygienic handling of foods. Though this study included a small number of outlets, we are sure that, if extended to a wider range of outlets, the results would support our findings. Encouraged by these results, we intend to extrapolate the study to a greater number of samples and to use techniques such as real time, multiplex, and qPCR to make the study more effective in not only rapid detection of food-borne pathogens, but also in formulating safety protocols to curb the incidence of diseases caused by these pathogens.

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Conflict of interests: No conflict of interests is declared.