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Transfer of foodborne pathogenic bacteria to non-inoculated beef fillets through meat mincing machine

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ABSTRACT

The aim of this study was to evaluate the transfer of pathogens population to non-inoculated beef fillets through meat mincing machine. In this regard, cocktails of mixed strain cultures of each *Listeria monocytogenes*, *Salmonella enterica* ser. Typhimurium and *Escherichia coli* O157:H7 were used for the inoculation of beef fillets. Three different initial inoculum sizes (3, 5, or 7 log CFU/g) were tested. The inoculated beef fillets passed through meat mincing machine and then, six non-inoculated beef fillets passed in sequence through the same mincing machine without sanitation. The population of each pathogen was measured. It was evident that, all non-inoculated beef fillets were contaminated through mincing with all pathogens, regardless the inoculum levels used. This observation can be used to cover knowledge gaps in risk assessments since indicates the potential of pathogen contamination and provides significant insights for the risk estimation related to cross-contamination, aiming thus to food safety enhancement.

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1. Introduction

Cross-contamination contributes to foodborne illnesses due to the potential transfer of pathogens to food products. The common routes for cross-contamination are summarized to the indirect contact from air, the direct contact from hands to foods and the direct contact from equipment and utensils to food (den Aantrekker, Boom, Zwietering, & van Schothorst, 2003). Nowadays, ready-to-eat food products need more attention, since cross-contamination during handling at food processing points and retail has been recognized as a causative agent of human illnesses (Aarnisalo, Sheen, Raaska, & Tamplin, 2007; Perez-Rodrıguez et al., 2007, 2010; Sheen & Hwang, 2010; Vorst, Todd, & Ryser, 2006). Furthermore, bad hygiene practices and improper food handling might result to cross-contamination in domestic kitchens too, leading some times to infections, in cases of contamination with foodborne pathogens. Redmond and Griffith (2003) reported that foodborne diseases are three times more frequent from private kitchens than those occurring from food serving points.

Pathogenic bacteria such as *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* are highly associated with outbreaks related to meat consumption, leading to human diseases and deaths worldwide (Rhoades, Duffy, & Koutsoumanis, 2009). According to data collected from European countries for zoonotic diseases in humans (EFSA, 2006), *Salm.* ser. Typhimurium and *Salm.* ser. Enteritidis were the most frequent serovars related to human illnesses, whereas *Salm.* ser. Typhimurium was more often associated with the consumption of contaminated poultry, pork and bovine meat. For the year 2005, 177963 outbreaks of salmonellosis reported for 26 European countries. According to Global *Salmonella* Surveillance Progress Report (WHO, 2005), *Salmonella* ser. Typhimurium was found to be the second most common human serotype emerging in Europe. Likewise, verotoxigenic *E. coli* O157 has been linked with severe outbreaks and is broadly recognized as an important and threatening pathogen since 1980's (Davis & Brogan, 1995; Duffy, Cummins, Nally, O'Brien, & Butler, 2006). 3314 outbreaks were reported for *E. coli* O157 on 2005 (EFSA, 2006) and is one of the main threatening bacteria of beef, that could be potentially transferred from gut or hide during slaughtering (Duffy et al., 2006). Additionally, *E. coli* O157 can survive for hours or days on hands, cloths or utensils, leading to a potential cross-contamination if bad hygiene practices are followed (Chen, Jackson, Chea, & Schaffner, 2001; Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003). In the case of listeriosis, recorded data showed 0.3 confirmed cases per 100000 populations (EFSA, 2006). The high mortality and high hospitalization rates caused by *L. monocytogenes* in tandem with its ability to grow on refrigerated temperatures have increased the interest in this bacterium as a serious post processing contaminant pathogen

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(Perez-Rodrıguez et al., 2010; Rhoades et al., 2009; Vorst et al., 2006). Several studies indicated that *L. monocytogenes* might contaminate ready-to-eat food products at post processing points (Aarnisalo et al., 2007; Lin et al., 2006; Vorst et al., 2006; Wilks, Michels, & Keevil, 2006).

Nowadays, the consumption of minced meat is increasing worldwide, so and the risk for contamination. This risk becomes even more serious because the consumption of raw minced meat (like Tartar) or undercooked meat products is now very frequent (Rhoades et al., 2009). In addition, contaminated minced meat could lead indirectly to cross-contamination because of the transfer of pathogenic bacteria to food through the equipment in household or/and food serving kitchens, such as mincing machine. To our knowledge, there is limited (if any) information related to cross-contamination caused by meat mincing machine. However, knowledge gaps related to the transfer of foodborne pathogens caused by meat mincing machine need to be addressed for risk assessments.

The aim of the current study was to evaluate the transfer of *Listeria monocytogenes, Salmonella* ser. Typhimurium and *Escherichia coli* O157:H7 from inoculated beef fillets to subsequently non-inoculated fillets, through their passage from mincing machine. Such research provides significant insights in order to estimate the risk related to cross-contamination and thus enhancing food safety.

2. Materials and methods

2.1. Inoculum preparation

Listeria monocytogenes, *Salmonella* ser. Typhimurium and *Escherichia coli* O157:H7 were the tested bacteria of the study. For each bacterium, a cocktail of strains was prepared. More specifically, six strains of *L. monocytogenes* (NCTC 10527, serotype 4b, isolated from spinal fluid of child with meningitis, Germany, kindly provided by Dr. E. Drosinos; ScottA, serotype 4b, epidemic strain, human isolate, kindly provided by Dr. E. Smid, ATO-DLO, Netherlands; FMCC B-126, isolated from meat, Food Microbiology Culture Collection of Agricultural University of Athens; FMCC 21085, isolated from soft cheese, Food Microbiology Culture Collection of Agricultural University of Athens; FMCC 21350, isolated from ready-to-eat frozen meal — minced meat based, Food Microbiology Culture Collection of Agricultural University of Athens; FMCC 21411, serotype 4b, isolated from conveyor belt of ready-to-eat frozen foods, Food Microbiology Culture Collection of Agricultural University of Athens), three strains of *Salm.* ser. Typhimurium (DT 193, human isolate-epidemic; 4/74, isolated from calf bowel, kindly provided by Dr. P. Skandamis; JH3298, a mutant derived from *Salmonella enterica* subsp. e*nterica* serovar Typhimurium strain 4/74, kindly provided by Dr. P. Skandamis;) and three strains of *E. coli* O157:H7 (NCTC 12079, serotype O157:H7 / Produces Vero cytotoxins VT1 and VT2, isolated from human faeces, kindly provided by Dr. E. Drosinos; NCTC 13125, serotype O157:H7 / Vero cytotoxins negative; NCTC 13127, serotype O157:H7 / Vero cytotoxins negative) were activated from a stock culture stored at −80 °C, subcultured into 10 ml Tryptone Soy Broth (TSB, LabM, LAB004) and incubated overnight at the appropriate temperature for each bacterium (30 °C for *L. monocytogenes* and 37 °C for *E. coli* and *Salm.* ser. Typhimurium). A second subculture was prepared in fresh 10 ml TSB and incubated for 18 h at appropriate temperatures for each strain. Cells were then harvested by centrifugation (5000 g, 10 min, 4 °C, in Multifuge 1S-R, Thermo-Electron Corporation), washed twice with sterile 10 ml Ringer solution (LabM, 100Z), resuspended in Ringer solution and combined to provide a population of approximately 10^9 CFU/ml.

2.2. Mincing machine

A domestic meat mincing machine was used. For the disinfection of the machine's parts that were coming in contact with the meat, chlorine at concentration of 1000 ppm (sodium hypochlorite at 1000 mg/L of free available chlorine determined by titration with sodium thiosulfate) was applied for 6 min. Then, the parts were washed with detergent and hot water. Subsequently, the parts were rinsed with pure ethanol, burned to let ethanol evaporated, rinsed well with sterile distilled water and let dry.

2.3. Inoculation and treatment of the samples

Fresh beef was purchased from the central meat market of Athens and transported under refrigeration to the laboratory within 30 min. A factorial experiment 3X3 (3 inoculum levels by 3 pathogenic bacteria) was designed and performed. The meat was divided in portions of 100 g in a laminar flow cabinet. Beef fillets were inoculated with 3, 5, or 7 log CFU/g population level of each of *L. monocytogenes*, *Salm.* ser. Typhimurium or *E. coli*. In detail, 1 ml from 10^5, 10^7, or 10^9 CFU/ml dilution was added to the fillets (fillet sample) providing a population approximately of 3, 5, or 7 log CFU/g, respectively. 30 min after inoculation, mincing of each inoculated fillet was performed using mincing machine (sample 1). Then, six additional non-inoculated fillets (samples 2 to 7) were minced in sequence using the same mincing machine without sanitation.

2.4. Microbiological analysis

To estimate the number of viable cells transferred during the mincing process, 25 g of meat was placed in stomacher bag with 50 ml Ringer solution (1:2; Sample weight: Volume Ringer) and homogenized in the Stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 sec at room temperature. In addition, control samples (fillets without pathogen inoculation) were tested to confirm the absence of pathogens in the raw meat. Serial dilutions were prepared with the Ringer solution and duplicate 0.1 or 1 ml samples of the appropriate dilutions were spread or mixed on the following media: Palcam Listeria Agar Base (Biolife, 4016042) for *Listeria monocytogenes*, incubated at 30 °C for 48 h; Harlequin Tryptone Bile Glycuronide Agar (LabM, HAL 003) for *Escherichia coli*, incubated at 37 °C for 4 h and then transferred to 44 °C for 18–24 h; Xylose Lysine Deoxycholate Agar (Merck, 1.052.87.0500) for *Salmonella* spp, incubated at 37 °C for 24 h; Plate Count Agar (Biolife, 4021452) for total viable counts, incubated at 30 °C for 48 h; Pseudomonas Agar Base selective supplement (Biolife, 401961) for *Pseudomonas* spp., incubated at 25 °C for 48–72 h; Streptomycin Thallous Acetate-Actidione Agar (Biolife, 402079) for *Brochothrix thermosphacta,* incubated at 25 °C for 72 h; Violet Red Bile Glucose Agar (Biolife, 402185) for *Enterobacteriaceae* counts, incubated at 37 °C for 18–24 h; de Man-Rogosa-Sharpe medium with pH adjusted at 5.7 (Biolife, 4017282) for lactic acid bacteria, incubated at 30 °C for 48–72 h. The detection limit of the enumeration method was 0.48 log CFU/g.

2.5. Data analysis

Each experiment was replicated two times (two different batches of meat for each pathogen–totally six batches) with three samples analyzed each time for each pathogen (six replicates). A multifactor analysis of variance (ANOVA) was performed to evaluate the effect of different meat samples (fillet, 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th) on pathogen counts and on total bacterial counts. The multiple range test (MRT) was applied to determine which level of each factor was perceptibly different ($p<0.05$). In the MRT, the F-distribution (LSD) was used to check equality of variances. All the statistical analyses were done with XLSTAT. ® v2006.06 (Addinsoft, Paris, France).

3. Results

Beef fillets were inoculated with *ca* 3, 5, or 7 log CFU/g for each pathogen and passed through meat mincing machine. Then, six non-

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