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Effect of inoculum size, bacterial species, type of surfaces and contact time to the transfer of foodborne pathogens from inoculated to non-inoculated beef fillets *via* food processing surfaces

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ABSTRACT

The objective of the present study was to determine the factors affecting the transfer of foodborne pathogens from inoculated beef fillets to non-inoculated ones, through food processing surfaces. Three different levels of inoculation of beef fillets surface were prepared: a high one of approximately 10^7 CFU/cm^2, a medium one of 10^5 CFU/cm^2 and a low one of 10^3 CFU/cm^2, using mixed-strains of *Listeria monocytogenes*, or *Salmonella* enterica Typhimurium, or *Escherichia coli* O157:H7. The inoculated fillets were then placed on 3 different types of surfaces (stainless steel-SS, polyethylene-PE and wood-WD), for 1 or 15 min. Subsequently, these fillets were removed from the cutting boards and six sequential non-inoculated fillets were placed on the same surfaces for the same period of time. All non-inoculated fillets were contaminated with a progressive reduction trend of each pathogen's population level from the inoculated fillets to the sixth non-inoculated ones that got in contact with the surfaces, and regardless the initial inoculum, a reduction of approximately 2 log CFU/g between inoculated and 1st non-inoculated fillet was observed. *S.* Typhimurium was transferred at lower mean population (2.39 log CFU/g) to contaminated fillets than *E. coli* O157:H7 (2.93 log CFU/g), followed by *L. monocytogenes* (3.12 log CFU/g; $P < 0.05$). Wooden surfaces (2.77 log CFU/g) enhanced the transfer of bacteria to subsequent fillets compared to other materials (2.66 log CFU/g for SS and PE; $P < 0.05$). Cross-contamination between meat and surfaces is a multifactorial process strongly depended on the species, initial contamination level, kind of surface, contact time and the number of subsequent fillet, according to analysis of variance. Thus, quantifying the cross-contamination risk associated with various steps of meat processing and food establishments or households can provide a scientific basis for risk management of such products.

1. Introduction

Food safety within the food chain constitutes a common obligation among food operators that have the primary responsibility, authorities that monitor this responsibility and consumers who must also recognize that they are responsible for the proper storage, handling and preparation of food (European Commission, 2000). The member states of the EU have developed an integrated approach to food safety intended to assure the protection of human health through national surveillance and monitoring programs for certain zoonotic and foodborne pathogens. On the other hand, food business operators are obliged to implement a hazard analysis and critical control points (HACCP) system for ensuring the safety and traceability of their products. Although, in the last two decades EU food legislation has been significantly enhanced in terms of consumer protection along the food chain (Official Journal European Communities L139, 2004; Official Journal European Communities L338/1, 2005), recent reports have indicated retail and domestic levels as significant sources of food poisoning outbreaks (Bloomfield, 2001; FDA, 2009; Neal et al., 2012).

More specifically, 35–90% of foodborne diseases and outbreaks have been attributed to occur in food service establishments and

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domestic areas due to inadequate food handling practices during processing (Catellani et al., 2014; Neal et al., 2012). For example, although raw meat (e.g. beef, pork, poultry) has not been incriminated for outbreaks despite the relative high occurrence of certain pathogenic bacteria such as *Escherichia coli* O157, *Salmonella* and *L. monocytogenes* (EFSA and ECDC, 2013; Rhoades et al., 2009), pathogen's transmission through cross-contamination often occurs when they are involved in food preparation areas and contact surfaces. Consequently, common factors throughout the food chain that might lead to cross-contamination and thus, contribute to foodborne illnesses include: (i) deficiencies in food handling such as using same or insufficient clean processing surfaces (ii) processing contaminated ingredients and (iii) infected food handlers who may also act as a source of contamination for foodstuffs (EFSA and ECDC, 2013).

The ability of microorganisms to survive on food processing surfaces for hours or days after contamination has been reported in several studies (Wilks et al., 2006; Zhao et al., 1998). However the risk of foodborne illness associated with cross-contamination is not only depended on the surface contamination, but also on the probability of transfer to other surfaces (Bloomfield and Scott, 1997). Thus several studies have conducted where different parameters/factors have been included in their experimental plan examining the transfer of bacteria from meat to surfaces and *vice versa* (Flores et al., 2006; Lin et al., 2006; Midelet and Carpentier, 2002; Rodríguez and McLandsborough, 2007; Vorst et al., 2006). Among the examined factors the type of bacteria species, strains, the type of contact surface, slicing or mincing food products, as well as fat and moisture content in foods have been reported to affect the transfer rates of microorganisms to surfaces (Aarnisalo et al., 2007; Chen et al., 2001; Keskinen et al., 2008; Kusumaningrum, 2003; Papadopoulou et al., 2012; Pérez-Rodríguez et al., 2007; Sheen, 2008; Vorst et al., 2006). To our knowledge there is not any information regarding the influence of more than two of the above-mentioned factors simultaneously.

Thus the objectives of the present study were to investigate: (i) species and strain variability of 3 different pathogenic bacteria (*L. monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium), (ii) inoculum size (ca. 10^3, 10^5 and 10^7 CFU/cm^2), (iii) the different type of surfaces (polyethylene-PE, wood-WD and stainless steel-SS) and (iv) period of contact time (1 and 15 min) of beef with surfaces, as factors that may affect microbial transfer. The reported data of this study may contribute in covering gaps associated with risk assessments at different levels of meat processing such as retail shops and domestic environments. Based on the above, assessment of the transfer potential of foodborne pathogens from contaminated to non-contaminated foods *via* processing surfaces, is expected to be of great importance, since quantification of cross-contamination risk associated with various steps from food production to food consumption, provides a scientific basis for risk management (Sheen and Hwang, 2010).

2. Materials and methods

2.1. Bacterial strains and cultural conditions

The bacterial strains used in this study are presented in Table 1. Totally, 6 strains of *L. monocytogenes*, 3 strains of *S.* Typhimurium and 3 strains of *E. coli* O157:H7 were selected. Cocktail strains were used to include the uncertainty of phenotypic variability among single bacterial cells that has been reported to occur among strain of the same species (Lianou and Koutsoumanis, 2010). Stock cultures of the strains were stored frozen (−80 °C) onto Microbank™ porous beads (Pro-Lab Diagnostics, Austin, TX, United States) and were activated by transferring a bead from each culture into 10 ml of Tryptone Soy Broth (TSB; LAB M Limited, Lancashire, United Kingdom). Each bacterial culture was incubated overnight at specific temperature for each strain (30 °C for *L. monocytogenes*, 37 °C for *S.* Typhimurium and *E. coli*). A 100 μl portion of the activated culture was transferred to 10 ml of fresh TSB and incubated for 18 h at appropriate temperatures, as described above. Bacteria were harvested after centrifugation at 5000×*g* for 10 min at 4 °C (Heraus Multifuge 1S-R, Thermo Electron Corporation, Langenselbold, Germany). The cell pellet was washed twice in 10 ml of ¼ Ringer solution (TSB; LAB M Limited, Lancashire, United Kingdom), before inoculation. The 6 strains of *L. monocytogenes*, the 3 strains of *E. coli* and the 3 strains of *S.* Typhimurium were combined to provide a population of approximately 10^9 CFU/ml.

2.2. Surfaces and samples

Clean and sterilized surfaces of various material types (stainless steel-SS, polyethylene-PE and wood-WD) were used. SS and PE are commonly encountered in slaughterhouses, food processing industries, catering and domestic kitchens. On the other hand, WD represents a type of surface that is commonly utilized by consumers. Fresh beef meat was obtained from the central market of Athens, and transported to the laboratory within 30 min at 4 °C. Then the beef meat was cut (vertically to muscle fibers) in fillets of a total surface of 25 cm^2 (5 cm × 5 cm × 1 cm) in a laminar flow cabinet.

Table 1
Bacterial strains used in this study.

Species	Strain num	Strain characteristics	Origin
Listeria monocytogenes	NCTC 10527	Serotype 4b	Spinal fluid of child with meningitis, Germany[a]
	ScottA	Serotype 4b	Human isolated[b]
	FMCC B-126		Meat isolated[c]
	FMCC 21085		Soft cheese isolated[c]
	FMCC 21350		RTE frozen meal – minced meat based[c]
	FMCC 21411	Serotype 4b	Conveyor belt of ready-to-eat frozen foods[c]
Salmonella ser. Typhimurium	DT 193		Human isolate epidemic [d]
	4/74		Isolated from calf bowel [d]
	JH3298		Mutant from S. Typhimurium 4/74[d]
E. coli O157:H7	NCTC 13125	Verocytoxins negative	Human faeces[a]
	NCTC 12079	Produces verocytoxins V1–V2	Human faeces[a]
	NCTC 13127	Verocytoxins negative	Human faeces[a]

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[c] Food Microbiology Culture Collection of Agricultural University of Athens.
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