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A 3-year hygiene and safety monitoring of a meat processing plant which uses raw materials of global origin

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ABSTRACT

A systematic approach in monitoring the hygiene of a meat processing plant using classical microbiological analyses combined with molecular characterization tools may assist in the safety of the final products. This study aimed: (i) to evaluate the total hygiene level and, (ii) to monitor and characterize the occurrence and spread of *Salmonella* spp. and *Listeria monocytogenes* in the environment and the final products of a meat industry that processes meat of global origin. In total, 2541 samples from the processing environment, the raw materials, and the final products were collected from a Greek meat industry in the period 2011–2013. All samples were subjected to enumeration of total viable counts (TVC), *Escherichia coli* (EC) and total coliforms (TCC) and the detection of *Salmonella* spp., while 709 of these samples were also analyzed for the presence *L. monocytogenes*. Pathogen isolates were serotyped and further characterized for their antibiotic resistance and subtyped by PFGE. Raw materials were identified as the primary source of contamination, while improper handling might have also favored the proliferation of the initial microbial load. The occurrence of *Salmonella* spp. and *L. monocytogenes* reached 5.5% and 26.9%, respectively. Various (apparent) cross-contamination or persistence trends were deduced based on PFGE analysis results. *Salmonella* isolates showed wide variation in their innate antibiotic resistance, contrary to *L. monocytogenes* ones, which were found susceptible to all antibiotics except for cefotaxime. The results emphasize the biodiversity of foodborne pathogens in a meat industry and may be used by meat processors to understand the spread of pathogens in the processing environment, as well as to assist the Food Business Operator (FBO) in establishing effective criteria for selection of raw materials and in improving meat safety and quality. This approach can limit the increase of microbial contamination during the processing steps observed in our study as well as the cross contamination of meat products.

1. Introduction

The safety of meat and meat products is controlled by applying proper hygiene practices throughout the food chain and decontamination interventions (if any) inside the meat processing plants. Nonetheless, despite the various safety control measures, outbreaks associated with meat products are still emerging with both severe long term health impact, as well as significant economic burden to the Food Industry and the society (Centers of Disease and Control, 2013). To optimize hygiene practices and assure product safety, it is of vital importance to identify and evaluate the contamination sources of foods at each processing step, from primary production to the final products at retail (Sofos, 2014).

Hygienic level of raw materials and processing environment may reflect the presence of pathogenic bacteria such as *Salmonella* spp. and *Listeria monocytogenes* (Pérez-Rodríguez et al., 2010), which have been associated with several outbreaks. According to the European Food Safety Authority (EFSA), 91,034 confirmed cases of human salmonellosis and 1642 of listeriosis were reported in 2012 in EU, with a case-fatality rate of 0.14% and 17.8% respectively. The highest prevalence of *Salmonella* regarding foodstuff was allocated to fresh broiler (5.5%), fresh turkey (5.5%), pig (0.7%) and bovine (0.2%) meat products. Likewise, the prevalence of *L. monocytogenes* in meat products was 2.1% (EFSA ECDC, 2014). The diversity and extent of pathogen presence in the plant environment are highly associated with the origin of raw materials, since strains of different geographical origin may show remarkable (genetic) biodiversity (Wasyl et al., 2012). In addition, cross contamination due to inadequate hygiene of personnel has been linked to several food-borne outbreaks (Michaels et al., 2004).

Salmonella inhabits the gastrointestinal tract of animals and may potentially contaminate the final meat products at several processing steps such as slaughtering, deboning, shredding, etc. *L. monocytogenes* is ubiquitous in both farm and plant environments Its ability to withstand and even grow under adverse conditions, such as low temperature, low moisture and limited nutrients explains why this

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organism is of high concern for the meat industry, especially that of Ready-To-Eat products (Valero et al., 2014). Based on the above, the risk posed by both pathogens is multifaceted not only due to the potential development of (ecological) niches and subsequent dispersion of microbial cells in various processing steps through cross-contamination, but also due to their ability to strongly attach to inadequately sanitized surfaces and create stable microbial aggregations (biofilms) (Brooks and Flint, 2008). Repeated isolation of pathogenic strains from the processing environment, despite the implementation of regular sanitation procedures suggests the potential persistence of certain strains and the intensive need for improvement of the sanitation protocols. The 'history' of persistent strains inside the plant, manifested by their exposure to inimical factors, may alter their phenotypic profile resulting not only in stress adaptation, tolerance to sanitizers, acidic or alkali conditions (gastrointestinal tract) and antibiotic resistance, but also to increased virulence (Alonso-Hernando et al., 2012; Belessi et al., 2011; Bosilevac et al., 2009).

Due to the fact that microbial transmission is caused unintentionally, the precise description of the vehicle of contamination is rather difficult. For this reason, the combination of traditional microbiological approaches with modern molecular methods is necessary. Polymerase Chain Reaction (Keeratipibul and Techaruwichit, 2012) and Pulse Field Gel Electrophoresis (Senczek et al., 2000) are powerful tools for the detection and tracking of persistent pathogenic bacterial strains inside the industry environment, enabling the identification of potential contamination routes, performing source-attribution and thus, assisting in the development of targeted pathogen reduction strategies.

The objectives of the study were; (i) to evaluate the hygienic level of a large-scale meat industry using classic microbial indicators, (ii) to determine the occurrence and persistence of *Salmonella* and *L. monocytogenes* in the processing environment, (iii) to characterize the antibiotic resistance of the isolates and, (iv) to identify sources of contamination or map potential routes of cross-contamination in order to improve the correct handling of products.

2. Materials and methods

2.1. Description of the industry

The meat processing plant that was used as a case study (model-industry) here is located in central Greece. The selection of the particular plant was made due to its capability to process large amounts of meat (approximately 50–80 tons of meat *per* day), the different processing lines (beef, pork, poultry) and the variety of final products (ground meat, steaks, burgers, fillets, etc.). In addition, it is of high importance that the raw materials of the industry are imported from different European or non-European countries, including France, Poland, Netherlands, Germany, Bulgaria, Romania, Hungary, Spain, Italy, Namibia, Botswana, New Zealand, USA, etc., while in some cases the meat originated from different slaughterhouses of the same country. Eighty percent (80%) of the production is ground meat (60% beef, 15% pork, 15% mixed meat, 5% chicken, 5% turkey), while the remaining 20% refers to whole cuts, kebab, patties, steaks, sausages, pork or chicken rolls, marinated products, etc. Due to its high production capacity, the plant has also exporting activities to many European countries.

2.2. Sampling plan

In total, 2541 samples were collected over almost a 3-year period (03/2011 to 12/2013), through 167 independent sampling times, with a frequency of approximately once a week. Based on the average production volumes of the industry in the above period, the collected samples were grouped into five broad categories; (i) raw materials (n = 202), (ii) ground meat (beef, pork, poultry, mixed ground meat; n = 1668), (iii) other final meat products (e.g., steaks, fillets, patties, sausages, kebabs, gyros, marinated rolls; n = 519) of the aforementioned types of meat, (iv) personnel (n = 42) and (v) others (surfaces, packaging materials, air, water; n = 110). At each sampling, 15 samples were collected; 10 ground meat, 2 to 3 final products, 1 to 2 raw materials and 1 environmental sample (i.e., food-contact surfaces, personnel, packaging). The raw materials were collected at the beginning of the production line, while ground meat and meat products were obtained as freshly produced in commercial packages. Personnel (hands, clothes) and surfaces (10 × 10 cm) were sampled using sterile gauze swabs pre-moistened with 5 ml buffered peptone water (BPW; LAB M, Lancashire, UK). Sampling of surfaces was conducted either during production or after cleaning/sanitation in order to monitor the microbial spread during production or the effectiveness of the disinfection plan, respectively. All samples were transferred and stored in the laboratory under refrigeration and the analysis took place within 4 h post sampling.

2.3. Microbiological analysis of microbial hygiene indicators

All samples were analyzed to determine the levels of total aerobic viable counts (TVC), total coliforms (TCC) and *Escherichia coli* (EC), which serve as hygiene indicators, as it is being described in the corresponding International Standards (i.e., ISO 4833-1:2013, ISO 4832:2006 and ISO 16649-2:2001, respectively). In particular, 25 out of 500–1000 g of each sampled product was mixed with 225 ml of BPW and stomached for 2 min. Similarly, gauze swabs taken from surfaces were diluted in 100 ml of BPW and thoroughly mixed in a stomacher for 1 min. Following serial decimal dilutions in 9 ml BPW, 1 ml of the proper dilution was poured into the appropriate medium and the colonies formed were enumerated following the suggested incubation periods.

2.4. Detection and identification of Salmonella spp. and L. monocytogenes

Detection of *Salmonella* spp. took place according to ISO 6579:2002 in all analyzed samples (n = 2541). Presumptive colonies on the selective medium (xylose lysine deoxycholate agar; LAB M) were subjected to *Salmonella* latex test (Microgen Bioproducts, Surrey, UK). Positive agglutination was evident within 2 min, while delayed or partial agglutination was considered as negative, against a positive control. Isolates were further identified by API 20 test (bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions.

Even though *L. monocytogenes* is not of direct safety concern for meat products (non-Ready to Eat) that are not intended to be eaten raw (EU Reg. 2073/2005), it may still constitute a significant hygiene indicator. Therefore, the detection of this organism was conducted occasionally and in samples of the highest interest for the industry at the period of the survey, e.g., based on the requirements of the self-control system in place. In particular, 435 meat products (i.e., beef, pork or poultry product), 121 raw materials and 41 environmental samples were analyzed, while ground meat samples were analyzed mostly during 2012, due to the increased production needs. The detection of the pathogen in all samples was conducted according to the corresponding ISO 11290-1:1996. Positive isolates were further confirmed by conventional colony-PCR (Polymerase Chain Reaction) according to D'Agostino et al. (2004). The *prfA* specific primers LIP1 (5′-GATACAGAAACATCGGTT GGC-3′) and LIP2 (5′-GTGTAACTTGATGCCATCAGG-3′) were used in a reaction mix (25 μl total reaction volume) that contained a final concentration of 1× buffer, 2.5 mM $MgCl_2$, 200 μM dNTPS, 300 nM of each primer and 0.5 unit of Taq polymerase (M0273S, New England Biolabs, USA). The PCR running program conditions were 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, while a final step of 7 min at 72 °C was followed. The PCR products were then analyzed by electrophoresis in a 1.5% w/v agarose gel stained with (5 μg/ml) ethidium bromide.