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Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome Sequencing as a food safety management tool: A proof of concept

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ABSTRACT

Repeated *Listeria* outbreaks particularly associated with Ready-To-Eat (RTE) delicatessen meat products have been reported annually at global level. The most frequent scenario that led to foodborne outbreaks was the post-thermal treatment cross-contamination of deli meat products during slicing and modified atmosphere packaging (MAP). The precondition for such cross contamination is the previous introduction of *Listeria* into meat processing facilities and subsequent colonization of the production environment, associated with formation of biofilms resilient to common sanitation procedures regularly applied in meat establishments. The use of Whole Genome Sequencing (WGS) can facilitate the understanding of contamination and colonization routes of pathogens within the food production environment and enable efficient pathogen tracking among different departments. This study aimed to: a) provide a proof of concept on practical use of WGS in a meat establishment to define the entry routes and spread pattern of *L. monocytogenes*, and b) to consider the regular use of WGS in meat processing establishments as a strong support of food safety management system. The results revealed that *Listeria* spp. was present in slaughter line, chilling chambers, deboning, slicing, MAP, as well as in corridors and dispatch (53 positive samples, out of 240). Eight *L. monocytogenes* isolates (out of 53) were identified from the slaughterhouse, chilling chambers, deboning, MAP and dispatch. *L. monocytogenes* isolates were of three different serotypes (1/2a, 1/2c, 4b) and correspondingly of three MLST sequence types. Overall, two pairs of *L. monocytogenes* isolates were genetically identical, i.e. two serotype 4b isolates (ST1), isolated from water drain at dispatch unit and two isolates obtained from slaughterhouse (floorwall junction at the carcass wash point) and MAP (water drain). These findings indicated that *L. monocytogenes* isolates identified in meat processing units (MAP, chilling chamber and dispatch) originated from the slaughter line. Further, all eight *L. monocytogenes* isolates were confirmed to be biofilm producers on glass and stainless steel surfaces.

The identification of the main entry routes of *L. monocytogenes* into meat establishments and tracking the routes for spread of the pathogen are of essential importance to define appropriate risk mitigation strategies for *L. monocytogenes* in meat production environment. The routine use of WGS for bacterial characterization, as a strong support of food safety management system in meat establishments, will require the cost-effective approach. It may encompass in-house sequencing when sequencing equipment is used for multiple applications (e.g. WGS of pathogens, starter cultures and spoilage organisms).

1. Introduction

Over the last decade, listeriosis has become one of the most significant foodborne diseases with a high relevance for public health due to the high hospitalization rate (Iannetti et al., 2016; Scallan et al., 2011). Although the overall incidence of listeriosis is not high, the severity of symptoms, sequelae and high mortality rate can be significant (Goulet et al., 2012). Repeated *Listeria*-associated outbreaks particularly associated with Ready-To-Eat (RTE) delicatessen meat products were reported globally at annual level (Raheem, 2016), with > 1600 cases confirmed in the USA, including 1500 hospitalizations, 260 deaths and incidence of 0.26 cases per 100,000 individuals (US CDC, 2014) and 2206 cases (notification rate 0.46/100,000) in the EU (EFSA/ECDC, 2016). The implicated RTE foods included mainly meat

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products with relatively long shelf life, e.g. cooked sausages, cooked sliced ham, jelly products, and, on some occasions, fermented salami. *Listeria monocytogenes* was found in 3.71% of tested RTE batches of products made from pork meat, 2.26% from poultry meat, but only 0.17% from bovine meat, respectively, in the EU Member States (EFSA/ECDC, 2016). while 9.5% of tested deli meats in the USA harbored this pathogen (Simmons et al., 2014). The most frequent scenario that led to foodborne outbreaks was related to the post-thermal treatment cross-contamination of deli meat products during slicing and modified atmosphere packaging (MAP), i.e. in vacuum or gas mixtures (US FDA, 2009). The essential precondition for such cross contamination is the previous introduction of *L. monocytogenes* into the meat processing facility and subsequent colonization of production environment, associated with formation of biofilms resilient to common sanitation procedures regularly applied in meat establishments (Bolocan et al., 2016).

Pathogen can rarely be transmitted via animal-human contact, but is rather more common through consumption of contaminated food (Lakicevic et al., 2014). Therefore, listeriosis can be considered as a typical foodborne disease. In spite of vigorous implementation of good hygienic practices (GHP), standard sanitation operating procedures (SSOPs) and hazard analysis and critical control points systems (HACCP), the introduction and prolonged survival of *L. monocytogenes* in slaughterhouses and meat processing production environment continues to be expected. This is closely related to the type of production environment regularly found in meat establishments, where *Listeria* inevitably finds suitable niches (Lakicevic and Nastasijevic, 2017). Maintenance of the cold chain, humidity and damp spots are the factors that foster biofilm formation, survival and growth of the pathogen (Todd and Notermans, 2011), even boosting its competitiveness against other commensal microbiota (lactic acid bacteria, Total Viable Counts/TVC, *Enterobacteriaceae*/EC, *E. coli*). If SSOPs are not well designed and adequately implemented (i.e. including mechanical cleaning; disinfection and rinsing), the bacteria can easily form biofilms on food contact surfaces (FCSs), which are usually difficult to remove by the standard sanitation protocols. These biofilms can be a major source of contamination during food processing (Lakicevic and Nastasijevic, 2017).

Additional uncertainty lies with the lack of microbiological limits for *L. monocytogenes* on FCSs and the pathogen transfer rate from FCSs to RTE meat products. The only existing microbiological limit is defined for RTE products placed on the market. In the EU, *L. monocytogenes* must not be present in RTE products able to support the growth of *L. monocytogenes* (other than those intended for infants and for special medical purposes) in a 25 g sample "before the food has left the immediate control of the food business operator, who has produced it" and at levels above 100 cfu/g by the end of shelf life (product on the market) (European Commission, 2005). In the United States, a zero-tolerance policy requires that *L. monocytogenes* must not be present in RTE foods at any point (USDA FSIS, 2003; Anonymous, 2006).

For the purposes of assessment of good hygienic practice or sanitary measures implemented within meat establishments, it is also important to recover nonpathogenic *Listeria* spp., since these bacteria act as markers for the likely presence of *L. monocytogenes* (Lakicevic et al., 2010). Identification of the main entry routes for *L. monocytogenes* into meat establishments and tracking the routes for spreading the pathogen onto food contact surfaces (FCSs) are of essential importance to define appropriate risk mitigation strategies to prevent and control the presence of *L. monocytogenes* in production environments and its subsequent transfer to RTE meat products. The use of Whole Genome Sequencing (WGS) can facilitate the understanding of contamination/colonization routes of pathogens within the food production environment (FAO, 2016). It can also enable efficient pathogen tracking among different departments within a meat establishment and along the meat establishment–retail–consumer continuum, in order to facilitate foodborne outbreak investigations (Wang et al., 2016).Therefore, WGS is a novel and powerful tool for obtaining genomic data, which gives a higher level of resolution discrimination, i.e. better information about genetic similarity between isolates than conventional molecular typing such as fAFLP or PFGE. Such molecular methods only determine if isolates are the same or different but not how closely related they are genetically. Therefore, implementation of WGS is likely to be beneficial for many countries in the foreseeable future, in support of food safety management systems. In recent years, the cost of WGS has lowered significantly allowing its use in more routine applications (Kwong et al., 2016; Leong et al., 2016; FAO, 2016). For example, the price for bacterial genome sequencing fall to less than $50/isolate, in case that a considerable number of isolates is sequenced at the same time on a given instrument to achieve maximum economy of scale (Wiedmann, 2015). Hyden et al. (2016) stated that WGS is currently becoming the method of choice for characterizing *L. monocytogenes* isolates in national reference laboratories. However, the data in available literature on practical WGS usage in food production establishments to track the routes of contamination/colonization with *L. monocytogenes*, are scarce. This study aimed to: a) provide a proof of concept on practical use of WGS in a meat establishment to define the entry routes and spread pattern of *L. monocytogenes*, and b) to consider the regular use of WGS in meat processing establishments as a strong support of food safety management system.

2. Materials and methods

2.1. Company profile

Investigation of the presence and entry routes of *L. monocytogenes* on/in meats as well as tracking the routes of spread of the pathogen onto FCSs was carried out in a meat establishment approved for the export of meat and deli-meat products to the EU and Euro-Asian Customs Union markets. The meat establishment comprised three production units – slaughterhouse, deboning and meat processing. The capacity of the slaughter line was 30 cattle/h and 200 pigs/h, with two shifts per day; two slaughter lines, in the shape of "Y", were separated up to the point of evisceration (stunning, bleeding, bovine dehiding/pork scalding, singeing, polishing); from evisceration to the final wash step the one line is used for both – pigs and cattle dressing. The capacity of the deboning unit was 20 cattle/h and 140 pigs/h which was obviously 30% lower than the capacity of the slaughter line. Lastly, the capacity of the meat processing unit was 20 t/day of deli-meat products (cooked, sliced, packed in modified atmosphere), e.g. cooked sausages, bacon, ham, pork neck.

2.2. Environmental sampling

The sampling protocol encompassed the whole meat establishment, i.e. the slaughter line, deboning and meat processing units and included the most relevant critical sampling locations (CSLs) (Luning et al., 2011) in the establishment where *Listeria* could find suitable niches for survival, biofilm formation and growth. The sampling was carried out quarterly, throughout the year, covering four seasons, e.g. Q I (January–March), Q II (April–June), Q III (July–September) and Q IV (October–December). The 60 samples were collected per single visit for each quarter (10 samples per slicing unit, 10 samples per MAP unit and 8 samples per each: slaughter line, chilling chambers, deboning, corridors and dispatch unit). In total, 240 samples were taken within the whole year (60 samples per each of four quarters). Samples were collected in the early morning hours, before the beginning of production process and after regular sanitation had been conducted the previous day (pre-operational collection of samples). Each sample was taken from the total surface of 25 cm^2 and/or 100 cm^2, from easily and hardly accessible non-food and food-contact surfaces within the meat establishment (Table 1). Wet-dry swabs (Dryswab™, MWE, UK) were used for the sampling according to ISO 18593:2004 (2004) and were delivered to laboratory within 2 h, in a cold chain. The levels of Total Viable Counts (TVC) and *Enterobacteriaceae* (EC) were determined, as well as