Isolation of airborne *Listeria* spp in meat processing industry

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Review

Summary

Listeria monocytogenes and other Listeria species are microorganisms which can significantly affect the health of consumers transferring by meat and meat products. Special interest was emphasized due to the possibility of aerogene contamination of meat and products with microorganisms forming bioaerosol. In this paper the presence of Listeria monocytogenes and Listeria spp and in bioaerosol in air of meat processing plants and the importance of selecting the methods of air sampling as impaction or cyclone method were studied.

In experiment the cyclone method shows higher sensitivity for the detection of sampling L. monocytogenes and other Listeria spp in bioaerosols of the air in meat industry. With cyclone bioaerosol sampling method, we found the presence of Listeria spp in 41% of the sample, of which 24% of the sample confirmed on the presence of L. monocytogenes.

The results show a significant potential for aerogene contamination of meat and meat products with L. monocytogenes via bioaerosol in the meat industry. Cyclonic method indicated more reliable air sampling in detecting the presence of L monocytogenes in bioaerosols compared with the impact method.

Key words: Listeria monocytogenes, impaction and cyclonic air sampling methods, meat production

Introduction

Listeria monocytogenes is one of the most important foodborne pathogen causing *listeriosis* in human and animals. Although listeriosis is less common than campylobacteriosis or salmonelosis, 92 percent of listeriosis cases require hospitalization. Possibilities of air contamination by *Listeria* monocytogenes in meat processing industry has been considered as important due to risks of microbiological contamination. Many reports regarding *Listeria* isolations from a wide range of food products with special accent to the raw meat and its products have brought to attention to the possibility of different pathways of *Listeria* spp in the meat processing industry *Listeria* spp is ubiquitous and widely spread in the environment, thus the contamination of slaughtering plants likewise processing plants are now widely recognized as important transmission routes of *Listeria* food contamination (EFSA, 2006; Nesbakken, Kapperud and Caugant, 1996). The transfer of *Listeria* spp by the the animal fur and feathers should be primarily occurred thus in slaughtering plants all contact surfaces and carcasses can be exposed to *Listeria* contamination. *Listeria* spp can be disseminated by several environment sources like floors, drains, standing water, equipment, employees, animals, food additives, packing material and other (Griffiths, 2003). Moreover the treatment with animals at slaughtering and meat processing procedures, and sanitation processes always induces the generation of bio aerosols, which are contaminated by different species of bacteria and also by *Listeria* spp (Zhang et al., 2007; Kang and Frank, 1990). Bioaerosols pose a significant risk of contamination since they are easily spread with the air moving through different meat processing facilities including slaughtering line, chilling, processing, packing, meat, and meat dispatch departments, respectively (Spurlock and Zottola, 1991; Prendergast et al., 2004). Consequently the air saturated with bio aerosol contaminated with *Listeria* spp can be an important vector of this pathogen even after sanitation procedures; therefore sedimentation of bio aerosol droplets can recontaminate already washed and disinfected surfaces. Given the fact *Listeria* prefer humid and chill areas in meat processing plants with the strong affinity to form biofilms protecting the bacteria against disinfectants, is not surprising that numerous investigations tries to discover the paths of *Listeria* including the air.

Till this time most investigations regarding the airborne bacteria *Listeria* spp in the air of the meat industry have been made mostly in experimental conditions. Studies of airborne *Listeria* in real conditions are still very rare and do not show clear picture of this phenomena. Only few authors (Byrne et al., 2008; Zhang et al., 2007) report about investigations in meat industry, still the reasons for airborne *Listeria* dissemination in the air have not yet been sufficiently clarified and particularly no adequate methods for its sampling in real conditions were described. Classical methods of bio aerosol determination as sedimentation are not efficient since *Listeria* does not act like other bacteria. Thus the aim of our work was to compare the impaction and cyclonic method which might be potential reliable methods for diagnostics of airborne *Listeria* from the air and to investigate the potential presence of airborne *Listeria* during slaughtering and meat processing.

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Materials and methods
Sample collection

The present research work was performed in poultry slaughterhouse determinating airborne *Listeria* spp with cyclonic sampling method, while results from our previous research on determination of airbone *Listeria* spp in red meat slaughterhouses and meat processing plants with the impaction method were required for methods comparation. Samples determined using cyclonic method (n=81) were collected in 14 periods during one year, meanwhile samples using impaction method (n=158) were collected in the period of one and half year. The management of tested plants was in accordance to EU standards (Directives 852, 853, 854/2004)

Airborne Listeria sampling - impaction method

As we reported in previous research impaction method was used for airborne *Listeria* determination (Dobeic et al, 2011). We used MAS 100 (Microbial Air Monitoring Systems ®, Merck) as one stage impaction sampler which operates on the principle of the Andersen air sampler (Anderson, 1958; Merck, 2001) and meets the requirements of the general regulatory instruments for medical equipment in the EU and EN ISO 14698-1/2 (Kelly, 2005).

Basic principle of MAS 100 operation is the impaction method as the active orientation of the air stream directly onto the agar plated Petri plates (56.72 cm^2 surface area). The sampler pump (MAS 100 Microbial Air Monitoring Systems ®) as a single stage air sampler draws a constant air flow with a capacity of 100 l/min directly to the board in accordance with EN ISO 14698-1/2 norm. Rate at which the air flows is 0.45 m/s in a laminar stream, however the velocity of the air spread directly to the medium does not exceed 20 m/s (Figure 1.)

Figure 1.: Air impactor - MAS 100 (Microbial Air Monitoring Systems ®, Merck)

Although the impaction method using MAS 100 on agar plated surfaces for the airborne mesophilic microorganisms (CFU) determination is adequate, this method was not reliable for the *Listeria* determination. The other environmental bacteria often overgrow the surface of nonselective media which are not suitable for isolation of *Listeria*.. In experiment instead of the agar media, the air filter (Universal filter for hoods thickness of 2-3 mm and air permeability 3600 l/m/s - (Etis d.o.o., Slovenia)) was inserted onto the surface of petri dish soaked by 2 ml of liquid broth as the mixture of Fraser medium (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom) and half concentration of antibiotic to prevent growth of other bacteria was used (Figure2.). Owing to porous filter structure the air circulates through the medium of Fraser broth inside of filter channels in which *Listeria* can accumulate or adhere on the filter channels structure. Round shape filters were (diameter of 9 cm) were, sterilized by autoclaving at 121 ° C for 15 minutes and after that put into Petri plates. Samples were represented by filters which were aerated with 1500 and 1000 l of air. Samples taken by MAS 100 were taken from slaughterhouses and meat processing plants 1 hour before and during the course of the manufacturing process. Before any air sampling the MAS 100 pump was sterilized (121°C/15 min.). During the sampling air sampler was disinfected with 70% ethyl alcohol.

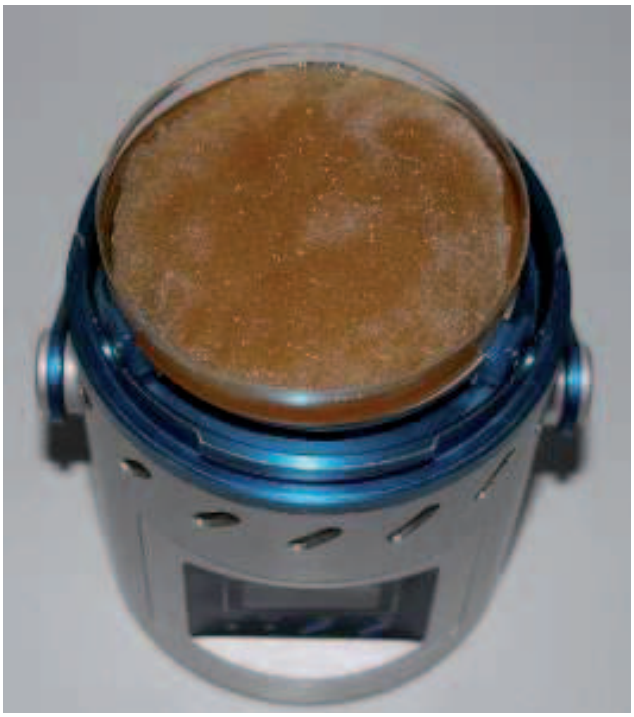

Figure 2.: Air filter soaked by Fraser broth (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom)

Airborne Listeria sampling – cyclonic method

Using cyclonic air sampler (Coriolis Delta Air Sampler) several advantages were achieved (Figure 3.). Instead of collecting bacteria onto the flat surfaces, the air is suckled through the liquid and whirls along rounded wall sample bottle in turbulent flow. As the cyclonic air sampler draws a turbulent air flow with a capacity of 100 l/min, microorganisms accumulate onto the lateral surface of the of sample bottle. As a principle of air sampling in liquid, distilled water or any broth can be used as collection media, what improve bacterial living conditions during the time of sampling and transport to the laboratory. Samples for *Listeria* cultivating were collected using cyclonic air sampler in liquid broth as the mixture of Fraser medium (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom) and halfconcentration of antibiotic to prevent growth of other bacteria. 10 ml of Fraser broth was poured in sterilee sampling bottles. The liquid broth was aerated by 3000 l of the air for each sample taken in poultry slaughterhouse during slaughtering and meat processing. Samples were transported to the laboratory within a few hours. Before any air sampling the pump was sterilized (121°C/15 min.). During sampling sterile sample bottles were changed regularly. The validation of the Coriolis® technology has been performed by Health Protection Agency (HPA) in Porton Down (UK). Physical and biological efficiency have been determined, in accordance with ISO14698-1 norm, by comparison with reference methods of impaction on agar dishes or filters. Coriolis® equipment also conforms to CE/UL/CEM norms and requirements.

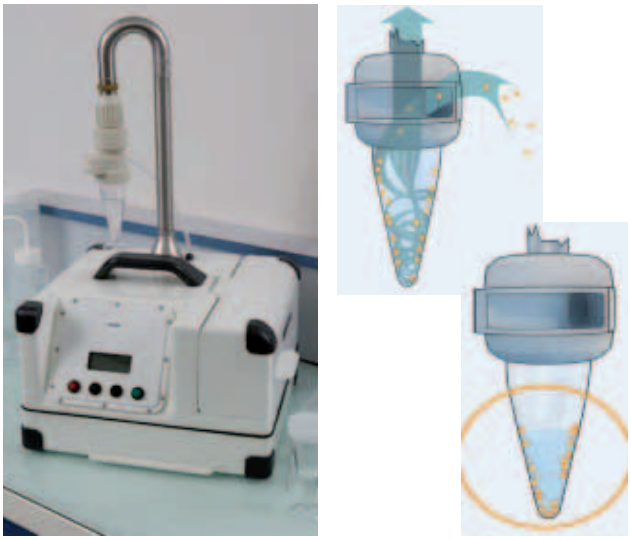

Figure 3.: Cyclonic air sampler (Coriolis Delta Air Sampler) as turbulent air flow pump

Laboratory Listeria spp determination

Before incubation an additional amount of 8ml of half Fraser broth was added into each dish and the contents were shaken gently. Cultures were incubated at 30°C for 24h. Later on, 0.1ml of culture from F1 was transferred into 10 ml of the second enrichment broth F2 (Fraser broth, with full concentration of antibiotics, Oxoid). From the primary enrichment one loop was also taken for each of the selective plating media: ALOA agar (Biolife Italiana, Milan, Italy) and Palcam agar (Oxoid, Basingstoke, England) and incubated at 37°C for 24-48h. The same procedure was repeated with a culture, obtained on the secondary enrichment medium after 48h of incubation. Up to five typical colonies of *Listeria* sp. grown on ALOA and Palcam agar were transferred onto the blood agar for pure culture and to determine the haemolytic activity. Final identification was performed with the commercial biochemical kit API *Listeria* (Bio Merieux, France) following the producer's instructions.

Results evaluation

Statistical evaluation of results was carried out by ANOVA, t-test and correlation analysis using the GraphPad Prism 6 computer programme (GraphPad Software, Inc., USA, 2014). The Pearson product-moment correlation and linear regressions ABS versus time were accepted for $r > 0.95$, and values of the slopes less than $P<0.05$ were considered statistically significant.

Results and discussion

Airborne Listeria

In previous investigations (Dobeic et.al., 2011) the modified impaction method of air sampling was used. In experiment airborne *Listeria* spp in the red meat slaughterhouses in 4,6 % (7/151) samples and processing plants in 14,3 % (1/7) samples were determined. Airborne L. monocytogenes, were not isolated despite of using the modified impaction method which is suitable to determine airborne *Listeria* spp.

In the present experiment for the isolation of *Listeria* spp cyclonic method in poultry slaughterhouse was used. Altogether 40.8% (33/81) of samples were positive on airborne *Listeria* spp. (Figure 4.) Among them 23.5% (19/81) of samples were positive on *L. monocytogenes* (0.24 ± 0.43) and 17.3% (14/81) of samples on *L. innocua* (0.18 ± 0.39) (Figure 5.). The differences between *Listeria* species were insignificant ($r = 0.24$). Highest number ($n=18$) of positive samples was isolated in the air of evisceration room, which was saturated with aerosol and in the packing room ($n=7$) what is even more important due to the food safety (Byrne et al.; 2008).

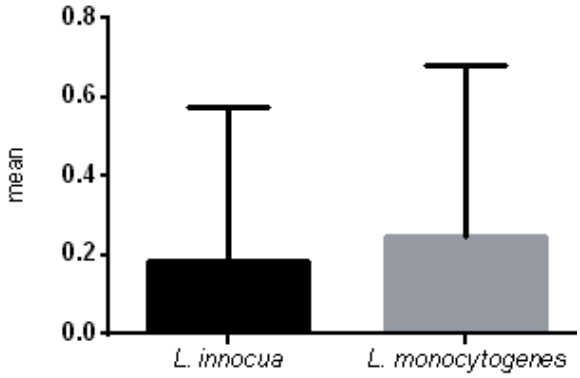

Figure 5.: Mean of positive samples number of *L. monocytogenes and L. innocua using cyclonic method in poultry slaughterhouse*

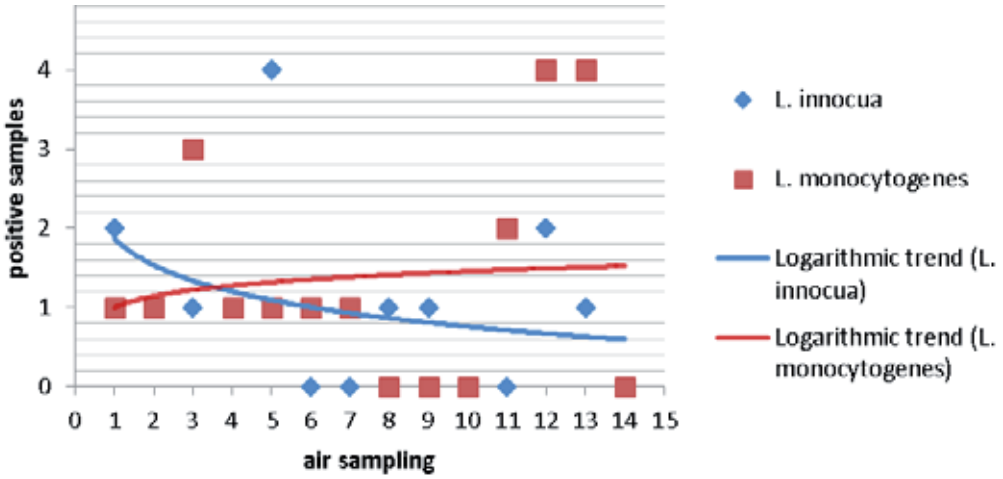

Figure 4.: Positive samples of *L. monocytogenes* and *L. innocua* using cyclonic method in poultry slaughterhouse, during 14 periods

From this experiment we found that the relatively large number of airborne *Listeria* can be determined in poultry slaughterhouses, as well as in red meat slaughterhouses and meat processing industry what was gathered from previous experiments. The methods by which we can establish the presence of aerobic bacteria are still developing, while among the tested methods the cyclone sampling method was presumably more efficient since we identified a significantly higher percentage of *Listeria* in the air compared by impaction method. In addition using cyclonic method the major share of *L. monocytogenes* was identified while none of this *Listeria* species can be determined using impaction method. Still it should be noted that the results gathering by impaction or cyclonic method in our experiments are difficult to compare owing to different meat processing plants, sampling conditions and different sampling air volumes. However for *Listeria* spp determination the main advantage of cyclonic method is in liquid medium used as normal sampling procedure, what is not the case using impaction method where the solid medium is more convenient. Presumably the selective liquid medium was more suitable for *L. monocytogenes* isolation.

Nevertheless, the percentage of airborne *Listeria* in samples determined by both methods is alarmingly high. Since airborne bacteria likewise *Listeria* are often bound with dust and detritus and form bioaerosols, which easily contaminate food during the meat processing or recontaminate cleaned and disinfected surfaces, it is particularly worrying about large number of positive samples on airborne pathogenic *L. monocytogenes* (McEvoy et al., 2006; Posh et al., 2006). In the present experiment even higher number of positive samples on airborne *L. monocytogenes* was determined than *L. innocua* which presence also indirectly indicates to the possible presence of *L. monocytogenes* as well. Anyway, almost 41% of samples on airborne *Listeria* spp and among them 24% of samples positive on *L. monocytogenes* is an alarming figure considering that samples were taken in poultry slaughterhouses which already meets Camylobacter contamination. The investigations on determining the main sources of airborne *Listeria* spp should be emphasized in the future with the great possibility that any positive *Listeria* findings in meat processing plants indicate to the presence of pathogenic L. monocytogenes.

Conclusions

Due to *Listeria* spp isolation from the air during the slaughtering and carcasses processing, the risk for airborne contamination of food is significant. Considering the food safety improving, the analysis in the field of microbial aerobic conditions in the food industry must intensify in the future and consequently special measures against airborne contamination need to be implemented.

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Delivered: 20.8.2014. Accepted: 4.9.2014.