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Definition of sampling procedures for collective-eating establishments based on the distribution of environmental microbiological contamination on food handlers, utensils and surfaces

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ABSTRACT

Environmental sampling has been identified as an effective procedure to verify correct implementation of food safety control systems in catering establishments. At the same time characterization of microbial distribution of environmental contamination could potentially address effective fit-for-purpose sampling procedures. In this study 1202 environmental samples from three types of food catering establishments located in Madrid, Spain were monitored for presence of mesophilic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus* and *Escherichia coli*. Samples corresponded to food-contact utensils, handlers'-contact utensils and food handlers, using 3M™ Petrifilm™ count plates. Contamination routes were identified through the calculation of Spearman correlation coefficients. Further, characterization of statistical distributions of microbial contamination and suggestion of sampling procedures were also performed. Results showed that 53.0% of the samples were positive for at least one of the bacterial group studied and 328 among those (27.1%) with counts between 1 and 15 CFU/plate. *Enterobacteriaceae* were present in 62.1% of food handlers' samples as well as *E. coli* and *S. aureus* (7.5% and 26.6%, respectively). Contamination routes from food handlers to handlers'-utensils was identified in a bidirectional way, being it subsequently spread to utensils in contact with foods. Finally, it was shown that the selection of the microbial distribution significantly affected significantly the number of samples needed to detect positives above a certain microbial level. As expected, when negative results are present (high zero counts or left censored data), Poisson-log normal distributions can describe properly the distribution of microbial contamination. However, log normal distributions presented better fit for samples with higher microbial counts and right-censored data (mesophilic bacteria) so that they can be used to describe contamination at high levels. The data and results generated in this study could be of high relevance to food safety authorities to appropriately address environmental sampling procedures in catering establishments.

1. Introduction

In recent years, the catering sector has been experiencing an increase in technological innovation in correspondence with changes in consumer habits lifestyles, demographic trends, etc., which have increased consumer preferences for healthy, safe, and convenient foods. Legislation in food hygiene at EU level prioritizes control measures to protect public health, making food operators responsible to assure product safety (EC No. 852/2004, EC No. 178/2002 and EC. No. 2073/2005). Regarding catering establishments, important aspects such as the size of establishments and

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heterogeneity of foods served justify the implementation of prerequisite programs and HACCP systems in food service operations as a part of the food safety management system (Codex Alimentarius Commission, 2003; Jacxsens, Devlieghere, & Uyttendaele, 2009). However, given the complexity of the food chain and variety of menus and meals prepared, simplified and flexible self-control measures must be required in most cases to increase efficiency and homogeneity of such systems. One useful tool that serves to verify that the system is working properly is the establishment of fit-for-purpose sampling procedures throughout the incoming raw materials, intermediate and end products as well as the result of processing environment monitoring programs (Osés et al., 2012; Lahou, Jacxsens, Van Landeghem, & Uyttendaele, 2014).

Additionally, previous studies have highlighted the relevance of considering environmental sampling during processing steps as an effective option to control pathogenic contamination sources (Hedberg et al., 2006; Muhterem-Uyar et al., 2015). It includes evaluation of food handlers, utensils and food-contact surfaces which may help to identify contamination sources (cross contaminations via raw materials or biofilms, hygiene failures, etc.). Such contamination can be an intermediate step in transmission of pathogens from their original habitat in the environment (in biofilms, water and organic soil residues) to food contact surfaces and food under processing (Reij, den Aantrekker, & ILSI Europe RiskAnalysis in Microbiology Task Force, 2004; Da Silva & De Martinis, 2013). The macroscopic visual approach is the common procedure for the evaluation of the efficiency of cleaning (Tebbutt, 1991; Tebbutt et al., 2007), and evaluation of the disinfection methods have been reported in several international organizations' recommendations (Codex Alimentarius, 1993; EC Reg 1441/2007; Hedberg et al., 2006; Rutala et al, 2008; Sagoo, Little, Griffith, & Mitchell, 2003). Nevertheless, in those regulations' definitions only microbiological and hygienic criteria were established, but no limit values or recommendations were indicated. Evaluation of microbial indicators is crucial for determining the food safety of prepared meals and the study and enumeration of microbial indicators in foods represents the major areas of microbiological analysis in food laboratories (Rodríguez-Lázaro & Hernández, 2015). Indeed, some recent studies used environmental monitoring control to search for potential correlations between microbial indicators and the hygienic-sanitary conditions of several food commodities (Milios, Drosinos, & Zoiopoulos, 2014; Tomasevic et al., 2016; Zoellner, Venegas, & Churey, 2016).

Additionally, to effectively establish environmental monitoring procedures, prior characterization of the distribution of microbial contamination is needed. There are well-known statistical approaches for deriving distributions describing how contamination is distributed in a specific food, in accordance to its composition, nature or contamination level. Statistical distributions can be either continuous (i.e. Log normal), or discrete (i.e. Poisson-log normal, [zero-inflated] Poisson, Poisson-Gamma) being able to reflect microbial concentration in food matrices (Gonzales-Barron & Butler, 2011; Gonzales-Barron et al., 2010; 2012). The use of log normal has been extensively described to deal with homogenous matrices and usually high concentration levels, where bacteria can be described as “continuous” entities. However, in case of censored data (when the observed microbial concentration is only partially known; i.e. concentration values are within a defined range but the true value is unknown), high proportion of negative results or clustering contamination, the use of discrete distributions is more appropriate since log normal distribution does not account for zeros and it can underestimate the proportion of non-defective units in a food lot. The Poisson-log normal distribution considers variability within lots, which is characterized by a Poisson sampling process combined with variability between lots through the assumption that concentration is log-normally distributed (Jongenburger, Bassett, Jackson, Zwietering, & Jewell, 2012).

There are relatively few published data on environmental microbial contamination in food service operations. Characterization of distributions of microbial contamination would help to implement effective sampling procedures which could be used as verification tools of correct implementation of food safety management systems. The present study aimed at evaluating the microbiological contamination on food handlers, food-contact utensils and handlers'-contact utensils during food preparation for collective meals in Spain, as well as to determine contamination routes and their relationships between microbial indicators (aerobic mesophilic bacteria, *Enterobacteriaceae*, *Escherichia coli* and *Staphylococcus aureus*). Further, characterization of statistical distributions of microbial contamination and suggestion of sampling procedures were also performed.

2. Material and methods

2.1. Study design and collection of samples from catering establishments

Seventy-six catering premises were assayed in this study in 31 primary schools, 29 nurseries and 16 nursing homes in Hortaleza Area, Madrid, Spain. Menus were prepared in situ in 51 centres, while food was prepared in a central kitchen and served by a catering company in 25 centres (Supplementary Table 1). Environmental samples were taken in 183 routine official health veterinary inspections, during one-year period, from three type of samples: food handlers (both hands); utensils in contact with food handlers (10 types) and utensils in contact with food (21 types) (Supplementary Table 2).

2.2. Microbiological analyses

Bacterial counts were determined using 3M™ Petrifilm™ count plates (3M-UK, Bracknell, Berkshire, UK): 3M™ Petrifilm™ *E. coli*/Coliform Count Plates *E. coli* counts; 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates for *Enterobacteriaceae*; 3M™ Petrifilm™ Aerobic Count Plates for aerobic mesophilic bacterial counts, and 3M™ Petrifilm™ Staph Express Count Plates for *Staphylococcus aureus*. Plates were prepared following the manufacturer's instructions. Sampling areas corresponded to 20 cm^2 for *Enterobacteriaceae,* mesophilic bacteria, and *E. coli*; and 30 cm^2 for enumeration of *S. aureus*. Briefly, plates were hydrated with 1 mL of 0.1% peptone water, and the top film was carefully lifted avoiding touching the circular growth area. Then the circular gel portion of the top film was put in direct contact with the surface being tested and finally the top and bottom films were re-joined. Plates were individually identified, transported at 4 °C, and incubated at 37 °C during 24 h for enumeration of *Enterobacteriaceae*; or 48 h for mesophilic bacteria, *E. coli* and *S. aureus*. In total 1212 microbiological determinations were done.

2.3. Characterization of statistical distributions for describing microbial contamination in catering establishments

In the present study, microbial contamination was described through statistical distributions. Seven actual datasets corresponding to food-contact utensils (*Enterobacteriaceae*, mesophilic bacteria), handlers'-contact utensils (*Enterobacteriaceae*, mesophilic bacteria) and food handlers' counts (*Enterobacteriaceae*, *E. coli* and *S. aureus*) were considered for the distribution fitting. For the sake of comparison between the evaluated distributions, the results from all premises for each microbial group and type of