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# Bacterial biogeographical patterns in a cooking center for hospital foodservice

Giuseppina Stellato, Antonietta La Storia, Teresa Cirillo, Danilo Ercolini *

*Division of Microbiology, Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy*

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ABSTRACT

Microbial contamination in foodservice environments plays a fundamental role in food quality and safety. In such environments the composition of the microbiota is influenced by the characteristics of the specific surfaces and by food handling and processing and a resident microbiota may be present in each site. In this study, the bacterial biogeographical patterns in a hospital cooking center was studied by 16S rRNA-based culture-independent high-throughput amplicon sequencing in order to provide a comprehensive mapping of the surfaces and tools that come in contact with foods during preparation. Across all area, surface swab-samples from work surfaces of different zones were taken: food pre-processing rooms (dedicated to fish, vegetables, and red and white meat), storage room and kitchen. The microbiota of environmental swabs was very complex, including more than 500 operational taxonomic units (OTUs) with extremely variable relative abundances (0.02–99%) depending on the species. A core microbiota was found that was common to more than 70% of the samples analyzed and that included microbial species that were common across all areas such as *Acinetobacter*, *Chryseobacterium*, Moraxellaceae, and *Alicyclobacillus*, although their abundances were below 10% of the microbiota. Some surfaces were contaminated by high levels of either *Pseudomonas*, *Psychrobacter*, *Paracoccus*, or *Kocuria*. However, beta diversity analysis showed that, based on the composition of the microbiota, the environmental samples grouped according to the sampling time but not according to the specific area of sampling except for the case of samples from the vegetable pre-processing room that showed a higher level of similarity. The cleaning procedures can have a very strong impact on the spatial distribution of the microbial communities, as the use of the same cleaning tools can be even a possible vector of bacterial diffusion. Most of the microbial taxa found are not those commonly found in food as spoilers or hazardous bacteria, which indicates that food and storage conditions can be very selective in the growth of possible contaminants.

## 1. Introduction

Food safety is a priority for foodservice organizations because inappropriate handling or food spoilage can result in serious problems for both foods and consumers (Cairo et al., 2008; Egan et al., 2006; Oliveira et al., 2013).

Every food has a specific microbiota that is strictly dependent on the nature of the raw materials, the environment in which the food is produced, and the processing, storage and consumption conditions. During their preparation, foods can be contaminated by microorganisms from the processing and storage environments, surfaces, tools, equipment and personnel engaged in handling and production activities (Griffith et al., 2000; Legnani et al., 2003).

Hospital meals have a remarkable associated safety risk because they are prepared for vulnerable people (children, elderly, pregnant women and immuno-compromised people) who are more susceptible to food-borne illness than the rest of the population. Microbiological hazard is one of the most significant food safety hazards because microorganisms cannot be easily detected and they are widespread in the environment and can easily come in contact with humans through foods.

Taking into account the peculiarity of hospital catering systems, which is intended to susceptible consumers, a scrupulous compliance with European food safety regulations is essential (EU Regulation 178/2002, 852/2004, 853/2004). In particular, the regulation (EC) 852/2004 about the hygiene of foodstuffs, lays down the requirements of the premises intended for the production of meals and equipment that come into contact with food and indicates the preventive measures and procedures for sanitizing equipment and kitchen tools.

Colonization of foods by microorganisms depends not only on the ecological conditions that occur in the food itself, but also from the interactions between food and the environment. Mishandling of food in establishments where food is served, such as restaurants, hotels, schools and hospitals can be a remarkable issue for consumers. In the case of

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* Corresponding author at: Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Portici, NA, Italy. Tel.: +39 081 2539449; fax: +39 081 2539407.
*E-mail address:* ercolini@unina.it (D. Ercolini).

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hospital cooking services, safety is a priority at every stage of the production from the arrival of the raw materials to the final delivery of meals to the patient (Cairo et al., 2008). Application of the principles of microbial ecology to food systems is of fundamental importance for both food quality and microbiological safety.

Food contact surfaces are good substrates for the proliferation of microorganisms. Although frequent cleaning and disinfection procedures can generally assure acceptable hygienic conditions in the food processing environments, they may fall short in the elimination of a well-developed microbiota. Such microbial populations tend to settle on sites that are especially difficult to clean due to difficult access, surface irregularities or retention of sticky raw materials. The transfer of environmental microbiota from surfaces or tools to foods, even after their hygienization, is a hazard for food quality and safety (Verran et al., 2008; Shi and Zhu, 2009).

Environment plays an important role in the selection of microorganisms, and optimal conditions for microbial growth can occur in food processing. In such food handling environments, organic residues from food processing can create microenvironments for growth and accumulation of microorganisms and can represent a relevant source of cross-contamination (Brooks and Flint, 2008; McLandsborough et al., 2006; Simões et al., 2010). Operations that can determine cross-contamination during food preparation are trimming, cutting, washing, rinsing, dewatering and packaging, which are all considered as contributing to microbial food safety hazards (Suslow, 2001). Once microbial cells are transferred from the environment or food contact surfaces to food, other ecological factors such as pH, temperature, osmolarity, $O_2$ availability, nutrient composition and presence of other bacteria will determine which microorganisms can develop and eventually cause concerns. Food quality and safety are fundamental in collective foodservice systems. Various bacterial species can reside in the kitchen, preparation rooms and storage facilities and can be a direct source of food contamination. This is a threatening issue especially in foodservices that prepare many meals per day and when the end users are categorized as at risk such as hospital patients. In fact, resident microbiota is not always the cause of disease in healthy individuals, but it may represent a remarkable issue in immunocompromised patients and newborns (Cairo et al., 2008).

The characterization of the environmental microbiota and the understanding of the correlation between ecological factors and the microbiota of food are of crucial importance for the control of food quality and safety.

Since characteristics of specific surfaces, environmental conditions and food processing can affect the structure of the microbiota it is expected that a specific resident microbiota can occur in each site of a foodservice facility. Although several studies have reported on the microbial contamination in mass catering establishments, and have indicated environment, equipment and personnel as key sources of meal contamination (Cairo et al., 2008; Egan et al., 2006; Legnani et al., 2003; Oliveira et al., 2013; Matyjek et al., 2012), no studies have explored in depth the structure of the microbiota in foodservice environments to highlight the possible sources of contamination of the prepared meals.

Recent advances in microbial ecology have provided sensitive culture-independent tools for rapid and effective evaluation of microbial contamination in many sorts of environments (Ercolini, 2013). In the specific case of foodservice kitchens, a rapid mapping of the contamination of surfaces and equipment that come in contact with food can be of great interest in order to identify possible sources of food contamination and to evaluate the effectiveness of cleaning procedures.

In this study, the environmental microbiota in a hospital foodservice was investigated over two separate days of production by using a culture-independent amplicon sequencing approach in order to describe the potential sources of contamination during food handling and preparation.

## 2. Material and methods

### 2.1. Sampling and DNA extraction

Two separate environmental sampling experiments (December 2013 and two months after) in the same hospital cooking center were performed. Across all area, surface swab-samples from work surfaces of different zones were taken: food pre-processing rooms (dedicated to fish, vegetables, and red and white meat), storage room and kitchen (Fig. 1). The sampling took place on cleaned surfaces, 2 h after the routine cleaning and more precisely at the time when they were ready to come in contact with food for the processing. The sampling was performed after the cleaning in order to avoid an overestimation of the microbial diversity that could come in contact with food during preparation. In addition, the sampling experiments were performed in days when all the pre-processing rooms had been previously used for food preparation. Surfaces were sampled with sterile cotton-tipped swab that was moistened with sterile PBS and rubbed vertically, horizontally and diagonally across the sampling site (100 $cm^2$) delineated by a template, rotating the swab to ensure full contact of all parts of the swab tip and surface (Bokulich et al., 2013).

After collection, swabs were placed into a sterile 10 ml polyethylene tube containing 1 ml of sterile PBS, cooled at 4 °C for the necessary time of transport to laboratory and analyzed within 3 h. Prior to DNA extraction, tubes were vigorously stirred in a vortex to transfer the cells from the swab to solution. Total DNA extraction from the swab samples was carried out by using a Biostic Bacteremia DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). The extraction protocol was applied to the pellet (12,000 ×*g*) obtained from 2 ml of suspension.

### 2.2. 16S rRNA gene amplicon library preparation and sequencing

The microbial diversity was studied by pyrosequencing of the amplified V1–V3 region of the 16S rRNA gene amplifying a fragment of 520 bp by using primers Gray28F 59-TTTGATCNTGGCTCAG and Gray519r 59-GTNTTACNGCGGCKGCTG (Ercolini et al., 2012). 454-Adaptors were included in the forward primer followed by a 10 bp sample-specific Multiplex Identifier (MID). Each PCR mixture (final volume, 50 ml) contained 60 ng of template DNA, 0.4 mM of each primer, 0.50 mmol l21 of each deoxynucleoside triphosphate, 2.5 mmol l21 MgCl2, 5 ml of 10 PCR buffer and 2.5 U of native Taq polymerase (Invitrogen, Milano, Italy). The following PCR conditions were used: 94 °C for 2 min, 35 cycles of 95 °C for 20 s, 56 °C for 45 s and 72 °C for 5 min, and a final extension at 72 °C for 7 min. After agarose gel electrophoresis, PCR products were purified twice by an Agencourt AMPure kit (Beckman Coulter, Milano, Italy), quantified using the QuantiFluor™ (Promega, Milano, Italy) and an equimolar pool was obtained prior to further processing. The amplicon pool was used for pyrosequencing on a GS Junior platform (454 Life Sciences, Roche, Italy) according to the manufacturer's instructions by using a Titanium chemistry (Ercolini et al., 2012).

### 2.3. Bioinformatics and data analysis

In order to remove sequences of poor quality, 16S rRNA raw reads were first filtered according to the 454 processing pipeline. Sequences were then analyzed and further filtered by using QIIME 1.8.0 software (Caporaso et al., 2010) and a pipeline previously described (De Filippis et al., 2014). Briefly, raw reads were demultiplexed and further filtered through the split_library.py script of QIIME. To guarantee a higher level of accuracy in terms of Operational Taxonomic Unit (OTU) detection, the reads were excluded from the analysis if they had an average quality score of lower than 25, if there were ambiguous base calls, if there were primer mismatches and if they were shorter than 300 bp. Sequences