Bioburden Assessment in Lisbon Groceries

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Abstract *Objective* This study aims to characterise the occupational exposure to the bioburden of groceries workers and to identify the most critical workstations. *Background* To our knowledge, this is the first study performed in Portugal that intends to characterise the microbial contamination in this specific setting. *Method* This study was conducted between November and December of 2020 in fifteen groceries stores (M) located in the municipality of Cascais. Passive sampling methods (Electrostatic dust cloths—EDC) and surface swabs were used at three different locations (checkout, fruits/vegetable and warehouse/dispenser areas). EDC and surface swabs were inoculated in four standard culture media, namely, malt extract agar (MEA), dichloran glycerol (DG18), tryptic soy agar (TSA) and violet red bile agar (VRBA) for further characterisation. *Results* The prevalence of bacterial contamination was higher than fungal contamination in both sampling methods. The highest prevalence was in the fruits/vegetable area regarding fungal and bacterial contamination. *Conclusion* The sampling methods employed effectively identified the most critical workplaces regarding microbial contamination. *Application* This study will be useful for industrial hygienists since it is the first held in small grocery shops focusing in the occupational exposure assessment of microbiological contamination.

Keywords Bioburden · Occupational exposure assessment · Groceries · Critical workplaces

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1 Introduction

The environmental conditions of workspaces are predominant factors for workers' comfort and their daily health and well-being (Cincinelli and Martellini 2017; Tran et al. 2020; Śmielowska et al. 2017). Microbiological contaminants are omnipresent and essential to various forms of life. Their origin alters depending on the type of microorganism and how it was carried (people, animals, soil, plants) (Górny 2020). When deposited on surfaces, microbiological contaminants can maintain their viability for an extended period, representing a threat to workers and all occupants because inhalation or ingestion of microbiological contaminants can be related to numerous adverse health situations. However, their survival decreases when indoor air is deprived of nutrient sources or when there is a variation in humidity and temperature (Górny 2020). Workers are often exposed to microbiological agents as their working environment are generally indoors. However, legal criteria for occupational exposure to microbiologic agents are still unavailable in Portugal (Shan et al. 2019). The workplace microbiological contamination may be essentially due to the presence of bacteria and fungi, which under proper conditions their growth and proliferation can be boosted (Leppänen et al. 2017). The microbiological characterisation of the air, quantified through surface analysis, can identify sources of contamination and thus assess the effectiveness of the cleaning procedure (Viegas et al. 2016).

To adequately evaluate the potential health impacts of occupational exposure to bioburden, comprehensive data on exposure sources and all variables that potentially influence exposure is essential (Viegas et al. 2021a). A multi-approach regarding sampling methods (combining active and passive sampling methods) is useful for revealing a more realistic scenario of bioburden exposure (Viegas et al. 2019, 2020, 2016).

Although the use of only active sampling (air sampling) is the current trend, passive approaches allow for data collection over a longer period time (weeks or several months) (Viegas et al. 2021a). It also provides a valid risk assessment since it permits measurement of the harmful part of the airborne population, which falls onto a critical surface, and it is a readily available, economical, and unobtrusive method (Ribeiro and Faria 2017).

To our knowledge, there has been no research on the exposure of grocery employees to bioburden. The goal was to perform an expanded study to characterise the exposure of grocery employees to microbial contamination identifying the most critical workstations regarding fungi and bacteria in local grocery stores.

2 Material and Methods

This study was conducted between November and December of 2021 in fifteen groceries stores (M) located in the municipality of Cascais.

Passive sampling methods (Electrostatic dust cloths (EDC) and surface swabs were used at three different locations (checkout, fruits/vegetable and warehouse/dispenser areas). EDC and surfaces swabs were inoculated in four standard culture media, namely, malt extract agar (MEA), dichloran glycerol (DG18), tryptic soy agar (TSA) and violet red bile agar (VRBA) for further characterisation (Viegas et al. 2021b). EDC was collected in three different locations (fruits/vegetable, checkout and warehouse/dispenser areas) and extracted in a 50 mL Falcon tube with 20 mL of 0.05% Tween™ 80 saline solution (NaCl 0.9%) for 30 min at 250 rpm on an orbital laboratory shaker (Edmund Bühler SM-30, Hechingen, Germany) (Quendera et al. 2016). After incubation of MEA and DG18 at 27 °C for 7 days for fungi and TSA and VRBA at 30 and 37 °C for 5 and 7 days for mesophilic bacteria and coliforms (Gram-negative bacteria), respectively, fungi and bacteria densities (colony-forming units, CFU m^{-2} g) were calculated.

3 Results

Total bacteria (TSA) presented a higher prevalence in checkout with swabs and on fruits/vegetables with EDC. Regarding Gram—bacteria (RB), fruits/vegetables location presented higher prevalence in both sampling methods (Fig. 1).

The highest prevalence of fungal contamination was in the fruits/vegetables area in both sampling methods and culture media (Fig. 2).

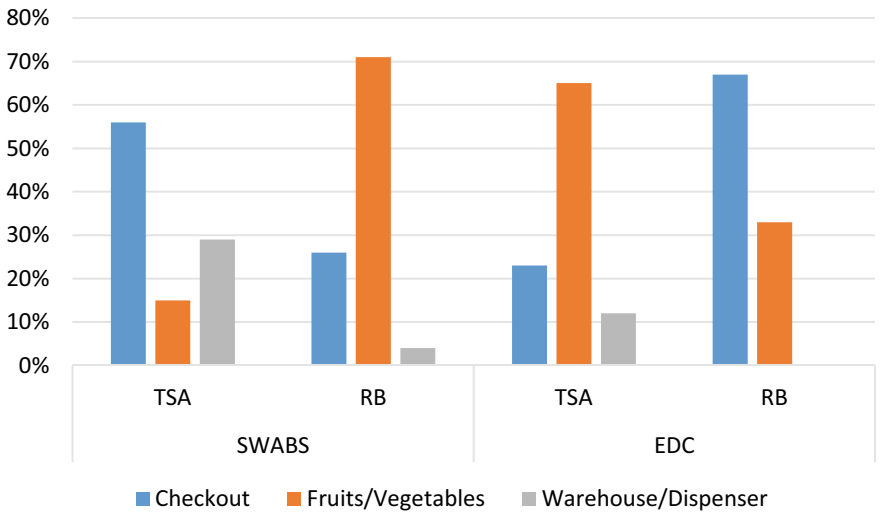

Fig. 1 Bacterial distribution in both environmental samples

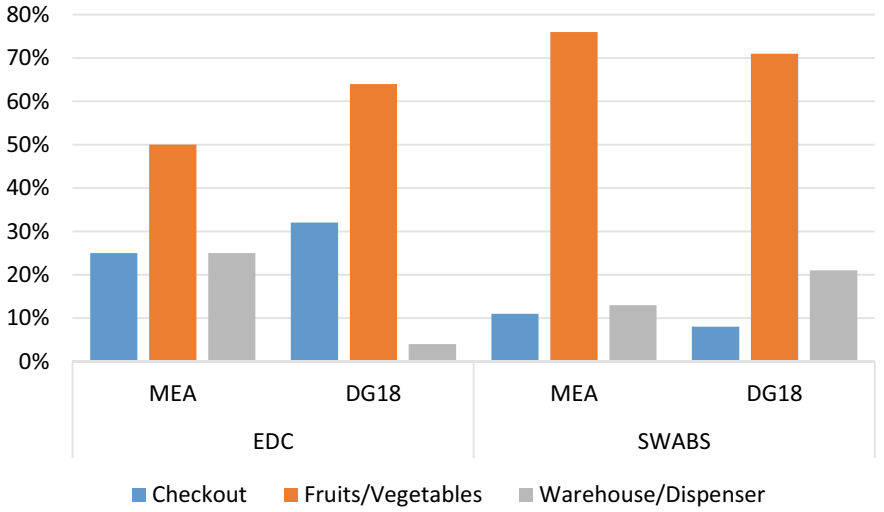

Fig. 2 Fungal distribution in both environmental samples

4 Discussion

The sampling approach applied was useful to screen the most critical workstations in what concerns bacterial and fungal contamination occupational exposure. In fact, EDC presents several advantages already reported in different studies, such as overcoming the limitation of overloaded plates through extracts dilution before inoculation and selecting the best culture media depending on the microorganisms to be assessed (Viegas et al. 2018; Badyda et al. 2016). However, to place EDC in locations where the sampling devices are not disturbed or damaged during working activities (e.g. elevated, surfaces) can lead to a collection of particles that may never contribute to human exposure through inhalation (Viegas et al. 2018; Madsen et al. 2012). Surface swabs are used to direct sample critical points and identify potential sources of contamination (ISO 18593:2018; Viegas et al. 2017, 2020). The use of selective culture media allowed one to understand the growth of the existing microbial contamination and identify potential contamination sources. Despite concerns about bacterial contamination of food in supermarkets (Lakicevic et al. 2015; Quendera et al. 2016; Reynolds et al. 2005; Calle et al. 2020), there has been little research published on the surveillance of specific food contact surfaces at the retail level (Calle et al. 2020). Bacterial contamination was higher in checkout and that may have several reasons such as handling and hygiene behaviors that may increase the risk of disease transmission (Paulin et al. 2017). It is well recognized that contaminated hands can spread infections (Paulin et al. 2017; Chung et al. 2008), since tasting and handling vegetables could raise the possibility for foodborne illness. Bacteria on a customer's hand can be transferred to and remain on the product, whether it's chosen for personal use or returned to the shelf for the next customer. Additionally, numerous customers

may be touching and returning the same or multiple pieces of the product (Paulin et al. 2017). Other reason that can justify both bacterial and fungal high contamination in checkout is the fact that many fuits/vegetables may get contaminated at any stage of production and supply chain, through direct contact with fecal waste during farming, such as wastewater irrigation and the use of biosolids or animal manure as fertilizer (Chee-Sanford et al. 2009; He et al. 2020; Rahman et al. 2022). Aditionally, it is important to highlight that fruits and vegetables, particularly raw leafy greens, are rapidly becoming recognized as key vehicles for the spread of human infections previously associated with animal-derived diets (Rahman et al. 2022). In fact, the fruits and vegetables can be indoor contamination sources, since fungi are commonly associated with the spoilage of these food commodoties (Moss 2008).

5 Limitations

Since the EDC must remain intact for 30 days, the choice of locations for placing the sampling devices may not be the most effective for the collection of particles that reflect exposure through inhalation.

6 Conclusions

This study emphasizes the need for the surveillance of microbial contamination in this occupational environment. Since it is the first held in small grocery shops it will be useful to raise awareness among employers, workers and other stakeholders. The passive sampling methods were effective in identifying the most critical workstations. However, further studies should combine active and passive sampling methods and ensure species identification to ensure a better risk characterization and management.

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