What sampling method to apply for the occupational exposure assessment to bioburden in Portuguese bakeries?

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BOHS The Chartered Society for Worker Health Protection

X2018 Manchester Conference Centre
24 – 26 September 2018

1. Introduction

There are more than 3300 bakeries registered in Portugal.

This is a major industrial activity in Portugal and directly relates with the fact that Portuguese bread is a well-known product that is appreciated both nationally and internationally.

(Guiné et al. 2016; Carbas et al. 2016)

Although without exact numbers, this implies a considerable work force involving many workers in Portugal.

- Flour is a complex organic dust covering one or a mixture of different cereal grains that have been processed or grounded by milling.

(Meo and AL-Dress 2005)

- Flour may contain several contaminants, such as fungi and mycotoxins being the raw materials entering the facilities the principal contamination sources for this occupational environment.

(*Milanowski et al. 2002; Karpinski 2003; Viegas et al. 2016, 2018*)

- Several studies report respiratory health effects in workers exposed both in small and large-scale industries.
- Epidemiological studies have described asthma, conjunctivitis, rhinitis and dermal reactions as the main health effects of flour dust exposure, highlighting baker's asthma as the most severe and frequent expression of occupational allergy.

(*Milanowski et al. 2002; Patouchas et al. 2009; Subbarao et al. 2009; Stobnicka and Górny 2015*).

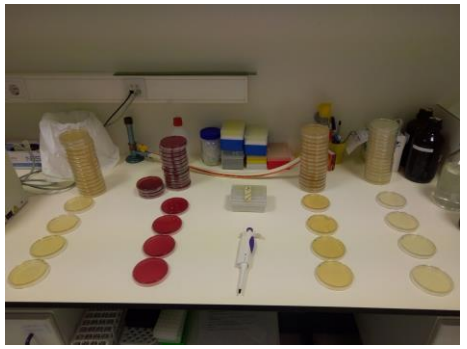
- Bioburden sampling has been mostly achieved through active and stationary samplers where microorganism's exposure can be calculated using the time-weighted average of the airborne concentration in the different sampling sites.

(Reponen et al. 2011)

Culture-based methods will be always crucial as analyses method to follow bioburden sampling, since:

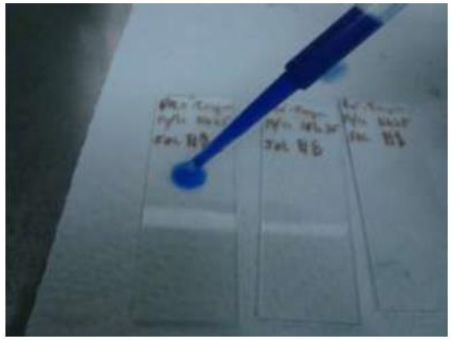
- can provide information about the infection potential of the viable bioburden;
- allows comparison with proposed guidelines.

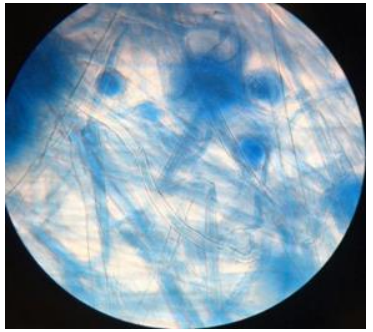
(Hung et al. 2005; Degois et al. 2017; Viegas et al. 2018)



5-7 days

1 week





The study intended to:

Discuss and suggest the best active sampling approach for the occupational **exposure assessment to the bioburden** in bakeries using data collected from impaction and impinger devices and provide new exposure data

JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
2017, VOL. 14, NO. 10, 771–785
https://doi.org/10.1080/15459624.2017.1334901

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Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?

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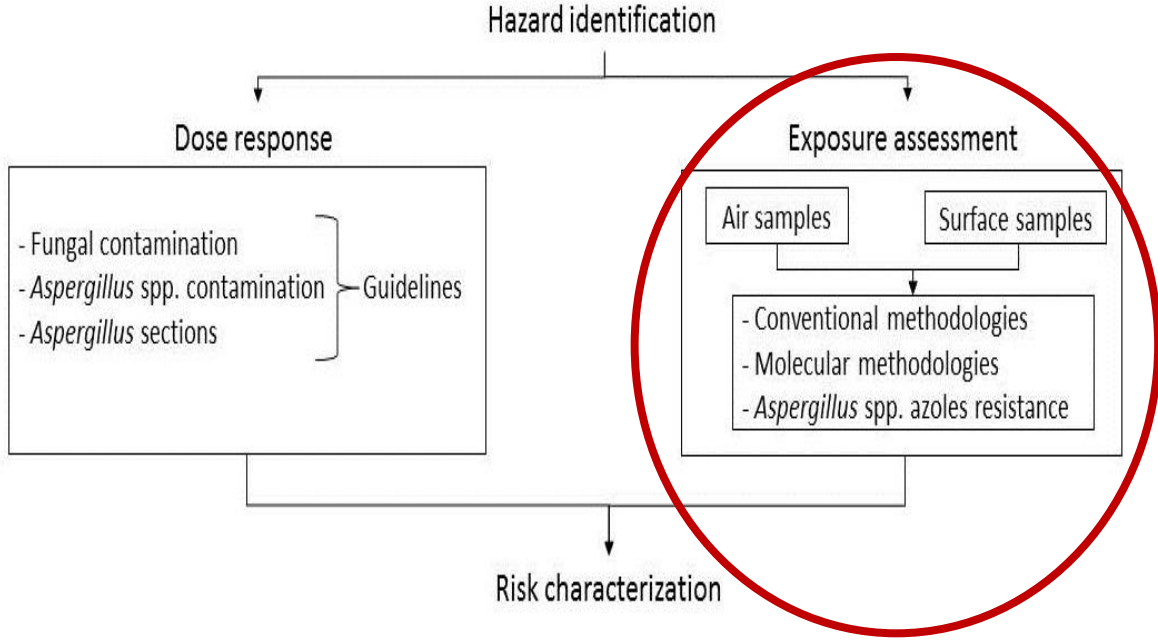
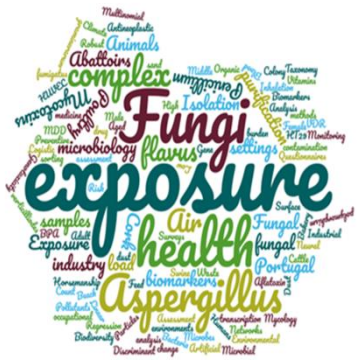




2. Materials and methods

13 Portuguese bakeries located in the Lisbon district

Financial support from the Portuguese Authority for Working Conditions

Three different areas were assessed:

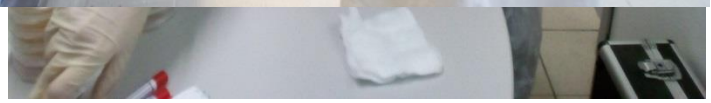
- Production—where kneading machines and ovens were located and where dough is prepared and shaped;
- Raw material warehouse—where workers have to go several times to collected the raw materials for dough preparation;
- Store—where final product is sold (bread or pastry).

Bakery	Facilities	Sampling approaches (samples number)				
		Indoor air sampling impaction MEA and DG18	Indoors air sampling impinger	Surfaces swabs	Settled Dust	EDC
1 (22-11)	Enlarged company	3	3	3	-	2
2 (6-12)	Enlarged company	5	5	5	-	3
3 (10-01)	Enlarged company	4	4	4	1	2
4 (19-01)	Enlarged company	4	4	4	1	2
5 (24-01)	Enlarged company	5	5	5	1	3
6 (31-01)	Enlarged company	4+	4	4	1	3
7 (8-2)	Enlarged company	5	5	5	1	3
8 (15-2)	Enlarged company	4	4	4	1	3
9(12-4)	Supermarket	4	4	4	1	3
10(26-4)	Supermarket	4	4	5	1	3
11(18-5)	Supermarket	4	4	4	1	3
12(23-5)	Supermarket	3	3	3	1	3
13 (7-6)	Supermarket	4	4	4	1	3
Total		**53**	**53**	**58**	**11**	**36**

- **8 bakeries** - enlarged company producing for selling in their own stores and also for different school canteens and vending machines.
- **5 bakeries** - supermarket facilities and belonged to the supermarket holder

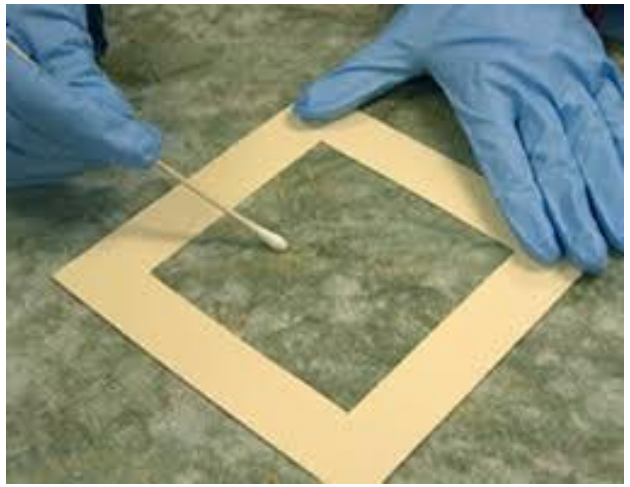
Multi-approach sampling strategy – Active methods

- Air samples of 100 liters (impaction method)
- 600 liters (impinger method)



Multi-approach sampling strategy – Passive methods

- Surface samples
- Settled dust samples
- Electrostatic dust cloths (EDC)



Multi-approach analyses strategy

- Quantification and morphological identification by culture-based methods from; Bacteria quantification
- MEA, DG18, TSA and RB
- Molecular detection of the toxigenic *Aspergillus* sections *Flavi*, *Fumigati*, *Circumdati* and *Versicolores*.
- 36 Mycotoxins in the air and settled dust samples were analyzed by LC-MS/MS system.

3. Results

Fungal and bacterial load distribution

	Minimum (CFU.m-3)	Maximum (CFU.m-3)	Median (CFU.m-3)	Interquartil Range 25 – 75 (CFU.m-3)	Counts of cases in which no load was detected (%)
Impaction Fungal Load MEA	10.0	5140.0	235.0	110.0 – 1210.0	7 (9.6%)
Impinger Fungal Load MEA	3.0	2620.0	85.0	13.0 - 310.0	18 (26.5%)
Impaction Fungal Load DG18	3.0	10310.0	140.0	17.0 – 740.0	9 (12.3%)
Impinger Fungal Load DG18	3.0	1670.0	10.0	7.0 – 80.0	33 (48.5%)
Impaction Total Bacteria Load TSA	10.0	4120.0	235.0	150.0 – 475.0	1 (1.4%)
Impinger Total Bacteria Load TSA	3.0	5306.7	7.0	3.3 – 28.5	20 (31.3%)
Impaction Gram- Bacteria Load VRB	4.0	50.0	10.0	10.0 – 10.0	53 (75.7%)
Impinger Gram - Bacteria Load VRB	3.0	3.3	3.2	3.0 – 3.3	69 (94.5%)

Among the two methods - impaction and impinger - statistically significant differences were observed in the following counts:

- fungal on MEA ($z = -2.721$, $p = 0.007$),
- fungal on DG18 ($z = -4.830$, $p = 0.000$),
- total bacteria ($z= -5.435$, $p = 0.000$),
- bacteria Gram – ($z = -3.716$, $p = 0.000$).

Impaction method detects significantly higher concentrations than the impinger method

4. Main findings discussion

- **The impinger method is used in settings with higher microbial loads.**

Allows dilution of the sample prior to plate incubation, also easier the application of molecular tools since a liquid air sample is expected after the sampling.

(Viegas et al. 2015).

However:

Bioburden present in small numbers and as single units may be less represented.

Cannot operate for long periods since liquid evaporation can hamper the fungi and bacteria viability.

(Macher 2001; De Nuntiis et al. 2003)

- **Viable bioburden constitute a small percentage of the total concentration of the microbial load**

A bias should be considered to properly interpret the obtained results.

(Viegas et al. 2018)

The ability of a given airborne microbial population to cultivate on nutrient media is affected by:

- the physiological and physical stress made by the aerosolization process,
- **the sampling methods**
- factors that affect microbial cells during their transport at airborne state

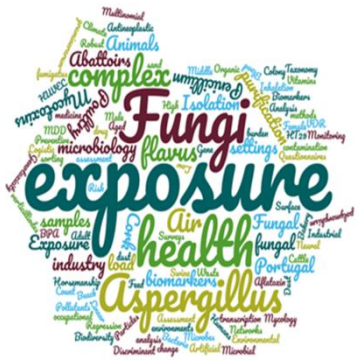
(Heidelberg et al. 1997; Zhen et al. 2013)

- **The following procedure is proposed:**

Apply the **impaction method to obtain information about the viable microbial load** and the impinger method to target for specific microorganisms through molecular tools.

This assessment methodology combining not only the sampling methods but also assays in samples analyses already provided **enriched information about risk characterization to bioburden occupational exposure** .

(Viegas et al. 2015, 2016)



Take home messages:

- The **impaction method is the best active sampling approach** for the occupational exposure assessment to the viable bioburden in this specific occupational environment.

- The use of **more than one different media** for mycobiota and bacteriota assessment can also enrich data for the exposure assessment.

- A **multi-approach in the sampling methods and analyses** applied should be the option to follow, enabling a more refined risk characterization and, consequently, a more suitable risk control measures to reduce workers health outcomes.

The authors are grateful to Portuguese Authority for Working Conditions for funding the Project —Occupational exposure assessment to particulate matter and fungi and health effects of workers from Portuguese Bakeries‖ (005DBB/12) and also to Occupational Health Services from the Bakeries engaged in this study.

Thank you for your attention