Nutrición Hospitalaria

Nutr Hosp. 2015;31(4):1795-1802
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original/*Otros*

Propuesta de valores de referencia ambientales microbiológicos en los servicios de restauración

Carla Soler[1], Jordi Mañes[2], Angelo Savino[3] y José M. Soriano[1]

[1]Unidad Mixta de Investigación en Endocrinología, Nutrición y Dietética Clínica, Universitat de València-Instituto de Investigación Sanitaria La Fe, Valencia, España. [2]Departamento de Medicina Preventiva, Facultat de Farmacia, Universitat de València, España. [3]Department of Medical-Surgical Specialities and Public Health, University of Perugia, Italy.

Resumen

Introducción: **Proponer una nueva referencia microbiológica ambiental en los servicios de restauración.**

Objetivo: **El presente trabajo muestra la determinación y evaluación de la contaminación microbiológica generada en un establecimiento de servicio de alimentos.**

Método: **Se basa en el muestreo microbiológico de superficie utilizando filtros de membrana de ésteres mixtos de celulosa y en el muestreo de aire utilizando placas de Petri.**

Resultados: **Los límites de contaminación se establecieron antes y durante la elaboración de alimentos, mediante el análisis microbiológico de las superficies y del medio ambiente, sistemas de equipos, hasta límites confiables y estableciendo niveles de aceptación en cada punto seleccionado. Por último, se estableció un programa de vigilancia microbiológica del medio ambiente incluyendo la evaluación de todos los parámetros que la componen y están implícitos en la zona, asegurando de esta manera y el apoyo a su continuidad con la documentación y los registros desarrollados para el área de seguridad. Las muestras para el análisis microbiológico se recogieron durante un período durante diez días diferentes, en dos horas diferentes. Se identificaron doce puntos como peligrosos. Además, los alimentos clasificados como de alto riesgo fueron recogidos periódicamente para el análisis microbiológico.**

Conclusiones: **La posibilidad de utilizar de una amplia gama de medios selectivos, muy por encima del número limitado utilizado en este estudio, permite el análisis de muchas especies microbianas individuales.**

(*Nutr Hosp*. 2015;31:1795-1802)

DOI:10.3305/nh.2015.31.4.8287

Palabras clave: *Superficie de muestreo. Muestreo de aire. Servicios de restauración. Contaminación microbiológica.*

Correspondencia: José M. Soriano.
Unidad Mixta de Investigación en Endocrinología,
Nutrición y Dietética Clínica. Universitat de València-
Instituto de Investigación Sanitaria La Fe.
Avenida Fernando Abril Martorell, 106 Torre A. 46026 Valencia.
E-mail: jose.soriano@uv.es

Recibido: 28-X-2014.
Aceptado: 19-XII-2014.

PROPOSAL OF REFERENCE VALUES OF MICROBIOLOGICAL ENVIRONMENT MONITORING IN FOODSERVICE ESTABLISHMENTS

Abstract

Introduction: **To propose a new reference of microbiological environment monitoring in foodservice establishments.**

Objective: **The present work shows the determination and evaluation of the microbiological contamination generated in a foodservice establishment**

Method: **It is based on surface sampling (microbial build-up) using mixed cellulose ester membrane filters and on air sampling (hourly microbial adhesion) using Petri dishes.**

Results: **Limits of contamination are established before and during the food elaboration, by means of the microbiological analysis of the environment, surfaces and equipment systems, until reliable limits and levels of acceptance are established of each selected point. Finally, a program of environmental microbiological monitoring was established including the evaluation of all parameters that compose and are implicit in the area, thus assuring and supporting its continuity with the documentation and registers developed for a safety area. Samples for microbiological examination were collected over a period of one moth on ten different days, at two different times. Twelve selected points having previously been identified as hazardous were monitored. Furthermore, foods though to be of high risk were periodically collected for microbiological analysis.**

Conclusions: **The possibility to use of an ample range of selective media, well over the limited number used in this study, allows the analysis of many single microbial species.**

(*Nutr Hosp*. 2015;31:1795-1802)

DOI:10.3305/nh.2015.31.4.8287

Key words: *Surface sampling. Air sampling. Foodservice establishments. Microbiological contamination.*

Introducción

Foodborne illnesses have been described as one of the most widespread problems of the contemporary world, what increase the public concern about the safety of food[1]. This added with the fact that nowadays exists a growing tendency to eat in other places than home, make that modern food services have to confront a difficult equilibrium: consumers demand higher quality food, governments require the guarantee of safety and owners seek increased profits[2-4]. With many people sharing one kitchen the risk of food safety errors is likely to be increased[5,6]. Over the years, food safety concerns have been raised only over the quality of served food, however, recent epidemiological data indicate that a substantial proportion of foodborne disease is attributed to improper food preparation practices in the kitchen questioning that the hygiene standards of food preparation areas, utensils as well as the personal hygiene practices of some of the kitchen personnel[7-10].

The importance of contaminated surfaces in relation to potential transmission of pathogens to food is apparent in food processing and catering environment[11] Exposure of pathogens on working surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles, for what environmental considerations are becoming more important[12]. When bacteria attach to a surface, they can form biofilms which makes more difficult to remove these attached bacteria from films can then cause recontamination of products when bacteria detach from biofilm and end up in the final product[13]. Another contamination of products can occur via air through dust particles or via aerosols. Aerosols are, for instance, formed when contaminated floors or drains are sprayed with high-pressure jets, resulting in the formation of droplets that can be suspended in the air[14]. A number of studies have characterized the prevalence of indicator microorganisms which presence in foodstuffs, food contact surfaces, equipments and utensils provides and relevant measure of hygiene. Total aerobic mesophilic bacteria and Enterobacteriaceae are a useful and most often used means of assessing overall sanitation in the environment of food service establishments[7].

In the recent years, the evaluation of the microbial contamination in places at risk is considered to be a basic step toward prevention. However, there are still problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination because of the number of sampling methods currently available is very large and many of them have already been compared in order to test their effectiveness. In this way, the Department of Hygiene at the University of Perugia developed a method for microbial monitoring of environments at risk, called Microbiological environment monitoring (MEM), principally in hospitals and food industry[15,16]. It is simple, cheap and reliable microbial air and surface monitoring measured with Hourly Microbial Adhesion (HMA) and Microbial build-up (MB), respectively, in any closed workplace at biorisk.

Another problem is that few standards or guidelines have been published on what is an acceptable level of microbial contamination on surfaces. The European Commission[17] laying down rules for regular checks on general hygiene carried out by operators of meat establishments provides that cleaned and disinfected surfaces should have an acceptable range of 0-10 cfu/cm^2 for aerobic counts and 0-1 cfu/cm^2 for Enterobacteriaceae. Another available literature about this topic, < 10 cfu/cm^2 was set as cleanliness criterion on the surfaces at a hospital kitchen[18].

The aim of this study was to determine the microbial environmental monitoring parameter, including levels of the air (with HMA) and surface (with MB) sampling, in food handling places from restaurant. Furthermore, this article carry out a proposal of the maximum acceptable microbial contamination. To date, this is the first article focused in the determination of these parameters in these establishments.

Materials and methods

Tested surfaces

It was carried out microbiologic controls and sampling in different days from the week of the twelve selected points, before and during the preparation of the food in the serial term of one month until completing a total of ten monitored days. The surfaces tested in a University restaurant were (1) the tap, (2) work-tables of meats and fish, (3) work-tables of vegetables, (4) laundry train, (5) grill, (6) cutting board, (7) cutting instruments, (8) fridge, (9) freezer, (10) self-services, (11) bar and (12) canteen, being indicated in the figure 1 with the distribution of the sampling area.

Air sampling with Hourly Microbial Adhesion (HMA) procedure.

The index of microbial air contamination were measured with settle plates[18]. Petri dishes (55 mm of diameter) containing a solid nutrient media are left open to air for a one hour. Microorganisms carried by inerter particles fall onto the surface of the nutrient. After the incubation they grow colonies in a number proportional to the level of microbial contamination of the air. The result was expressed as cfu/cm^2.

Surface sampling with Microbial build-up (MB) procedure.

Sampling with the mixed cellulose ester membrane filters (MFs) (Scharlau, Barcelona, Spain) was carried

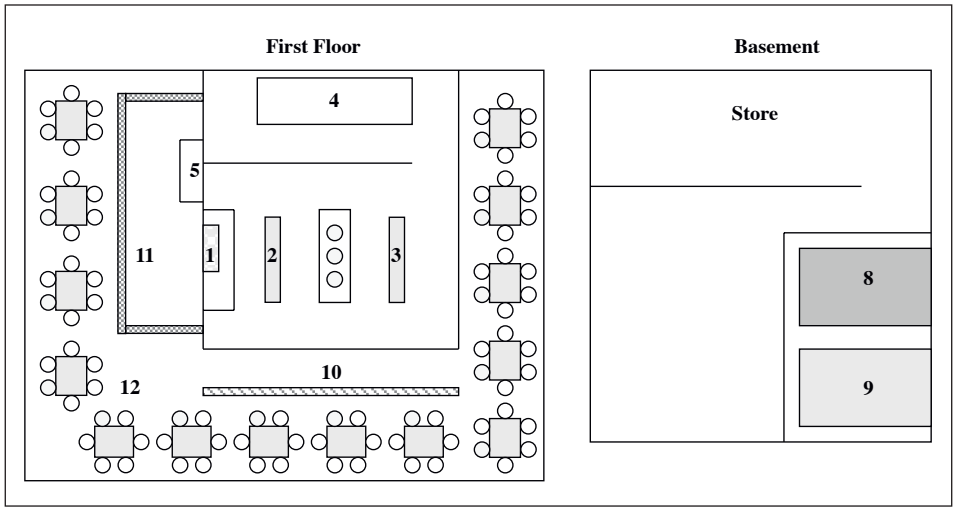

Fig. 1.—Some of tested surfaces in the studied food service establishment (not to scale).

out by manually pressing the membrane itself on the surface for 15 seconds, by fingertips covered with sterile disposable glove. Then the membrane was moved onto the Petri dishes of 55 mm of diameter (Insulab, Valencia, Spain) with the selective media: plate count agar (PCA) standard (Insulab) for the aerobic plate counts (APC) and violet red bile glucose (VRBG) agar (Insulab) for the determination of Enterobacteriaceae. After 72h (PCA) or 48h (VRBG) incubation at 30ºC (PCA) or 37ºC (VRBG), the colony forming unit (cfu) were counted. The results were recorded as cfu/cm^2.

Food microbiological methods.

Food samples were taken at the end stage of processing and preparation each day of the sampling environmental collection. The samples were taken then placed in sterilized plastic containers, refrigerated immediately and carried to the laboratory (at temperatures between 0 ºC and 4 ºC in an icebox) and cultured within 2 h of collection. A portion of the homogenate (25 g) were weighed into sterile stomacher bags, diluted with 225 ml buffered peptone water (BPW) (Scharlau) and homogenized in a Stomacher (Classic, IUL, Barcelona, Spain). Four 10-fold dilutions were made with each sample, 1 ml of each step was inoculated in duplicate plate count agar standard (PCA) (Oxoid) at 30ºC for 72 hours, according to the ISO 4833 reference method[19] to determine the number of aerobic plate counts (APC).

According to the ISO 21528-2[20], Enterobacteriaceae were determined using duplicate pour plates with 1 ml of each dilution in violet red bile glucose (VRBG) agar (Oxoid) over-layered with a further 10-15 ml of VRBG agar. The plates were incubated at 37ºC for 24 h. Typical colonies were counted on all plates having not more than 150 typical colonies and were recorded as "presumptive" Enterobacteriaceae.

The microbial quality of studied samples were compared with the Council Directive 93/43/EEC[21] and Commission Regulation No. 2073/2005[22].

Results and discussion

Microbiological profiles of APC and Enterobacteriaceae from the twelve surfaces in the studied restaurant at the seventh day (as an example) are shown in table I (air sampling) and table II (surface sampling). The surfaces were sampled before starting the working day, during the food processing and after the food process subjected to the cleanliness. The first and last sampling moment (before and after), only indicated the effectiveness of the cleanliness after the working day where all the facilities and instruments are carefully cleaned and before the work this environment is still clean, not being representative of the microbial contamination index. For this reason, although in the 10 days that this study was developed, the sampling was carried out at these three different moments to evaluate the hygienic conditions, only the values obtained during the process are presented, since they indicate the hazard of cross-contamination.

The results obtained from the evaluation of HMA are given in the figure 2A and 2B for APC and Enterobacteriaceae, respectively, during the food preparation. In two cases, the highest counts were obtained from the fifth to eighth days of sampling. These results are related to the fact that these days were the exams period, increasing in a big number the transit of students and the number of menus. Moreover, these elevated values were obtained in the (2) work-tables of meats and fish, (3) work-tables of vegetables, (6) cutting board, and some times the (7) cutting instruments, what is logical since these places have the potential to be contaminated during the food preparation because there are much employed in this situation. The bleach is always near to the (1) tap, for what is very difficult

Tabla I
Microbiological profiles for air samples before, during and after the food process in the studied restaurant

Sampling site number	Aerobic mesophils (CFU/cm²)			Enterobacteriaceae (CFU/cm²)		
	Before	During	After	Before	During	After
1	<0.04	2.18	0.04	0.04	0.17	0.04
2	0.13	6.95	0.04	0.17	0.25	0.13
3	0.08	6.82	<0.04	<0.04	0.21	0.08
4	0.08	0.93	0.04	0.13	0.08	<0.04
5	<0.04	1.18	<0.04	0.04	0.04	0.04
6	0.04	3.79	0.04	0.13	0.25	0.08
7	0.17	2.57	0.13	0.08	0.25	0.04
8	0.13	0.21	0.08	<0.04	<0.04	0.04
9	<0.04	0.08	<0.04	<0.04	<0.04	<0.04
10	0.04	0.63	<0.04	0.21	0.21	<0.04
11	<0.04	0.72	0.04	0.04	0.08	<0.04
12	0.08	0.67	<0.04	0.04	0.15	<0.04

Tabla II
Microbiological profiles for surface samples before, during and after the food process in the studied restaurant

Sampling site number	Aerobic mesophils (CFU/cm²)			Enterobacteriaceae (CFU/cm²)		
	Before	During	After	Before	During	After
1	0.04	2.02	0.04	<0.04	0.59	0.04
2	0.13	4.98	0.08	0.17	7.9	0.08
3	0.08	2.61	<0.04	0.08	5.49	0.13
4	<0.04	0.55	0.04	0.04	0.29	0.08
5	<0.04	0.38	<0.04	<0.04	<0.04	<0.04
6	0.04	9.15	<0.04	0.04	6.7	0.13
7	0.08	6.62	0.04	0.13	6.5	0.21
8	0.13	0.21	0.13	0.08	0.17	0.13
9	<0.04	0.04	<0.04	<0.04	<0.04	<0.04
10	0.08	3.16	0.13	0.04	0.13	0.04
11	0.04	0.29	0.08	<0.04	0.04	0.04
12	<0.04	0.13	0.04	<0.04	0.34	<0.04

to detect microorganisms. As well, owing to the high or low temperatures, is very unusual the microbial contamination in the (4) laundry train, (5) grill, (8) fridge and (9) freezer. According to the Pitzurra et al.[16], HMA method is a sterile, economical and readily available with reproducible and reliable results. Friberg et al.[23] suggested <3.5 10-2cfu/cm² a standard for this method in operating rooms with ultra clean laminar air flows.

The results obtained from the evaluation of MB are given in the figure 3A and 3B for APC and Enterobacteriaceae, respectively, during the food preparation. According to the previous suggestions, the results are very similar to those obtained in the HMA. However, the values of Enterobacteriaceae were higher than HMA due probably to that the surface is directly measure with the nitrocellulose membrane that obviously it is not the same that to evaluate the microorganisms precipitated. In this case, in the second sampling day there were high values, while in the sixth day the values were not so elevated being the sixth day, the

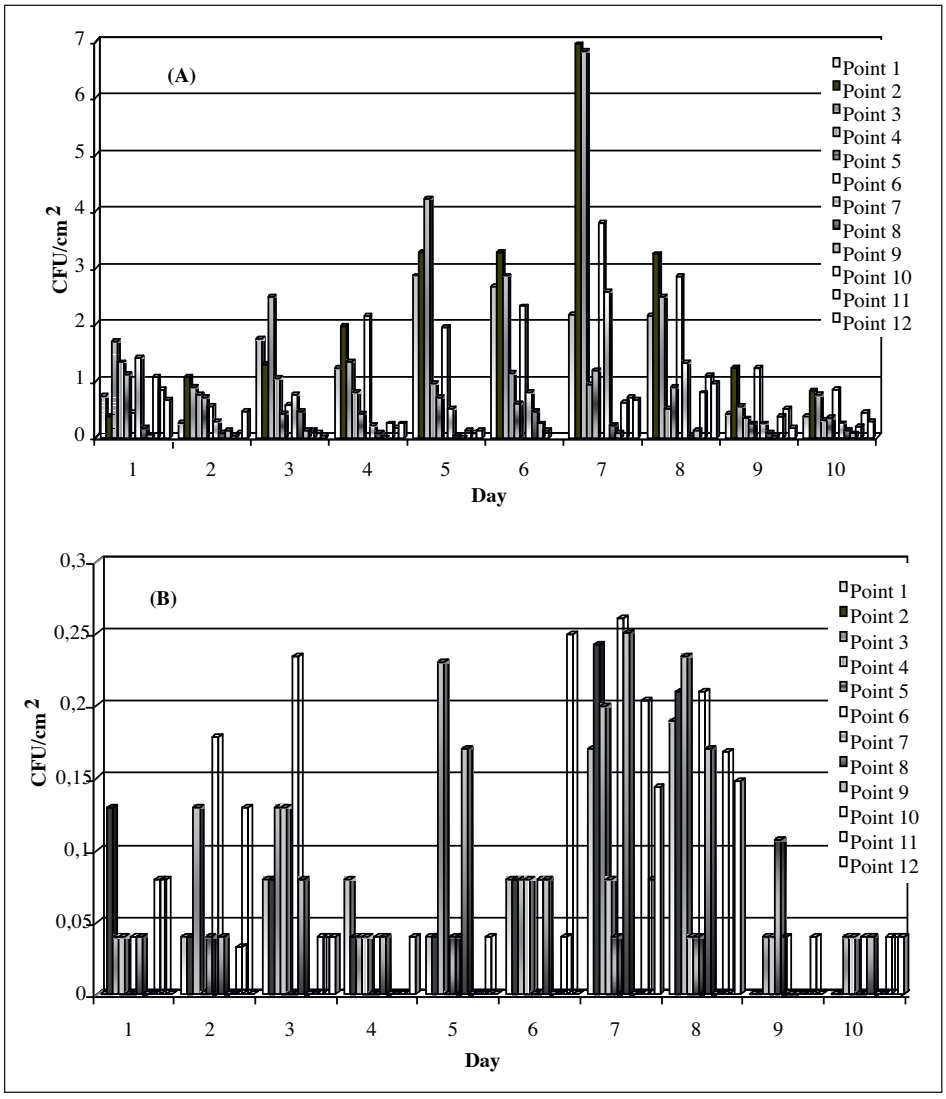

Fig. 2.—Media values (n=3) of HMA measurements expressed as number of colony forming units for (A) APC and (B) Enterobacteriaceae per cm² in 12 sampling sites during food processing.

exams period that increase the student population and, maybe increase the risk of the air contamination but not the surface contamination. The second day one staff started to work in this kitchen, that can explained these values. This mistake was rectified as soon as the person was informed about the observed results.

In our viewpoint, MB technique is more representative than HMA values are obtained (besides from its advantages as easy-use, inexpensive and rapid), since this measurement include both the microbial contamination of the surfaces with the membranes and the microbial air contamination that precipitate on the surface. This fact suggests the possibility to know the environmental contamination, only by this technique, making two measurements with one hour of difference. The difference between the results will be the bacteria that come from the air.

In the other hand and according to previous results, the aim of this study is to establish the maximum acceptable contamination levels. For this purpose, the microbiological quality of the food prepared under these conditions was evaluated. All the results, which are demonstrated in tables III and IV, are compared with the European values concerning the ready-to-eat food[21,22]. Limit of total aerobic mesophilic bacteria are exceeded in the first, fifth, seventh and eighth day. Respect to the Enterobacteriaceae results, this microorganism was exceeded in the seventh and eighth day. The alarming situation occurred the seventh and eighth day, when almost all the samples exceeded these limits involving a great hazard for the consumers. The last step of this study was to set the limits between the safety and the risk of the food processing. For this purpose, the values of air, surface and food sampling were compared.

According to our results, the proposed limits are summarized in Table V. The highest limits (designated as hazard owing to the real danger for the consumers) were set out regarding the food samples that exceeded the values established for the Council Directive 93/43/EEC[21] and Commission Regulation No. 2073/2005[22],

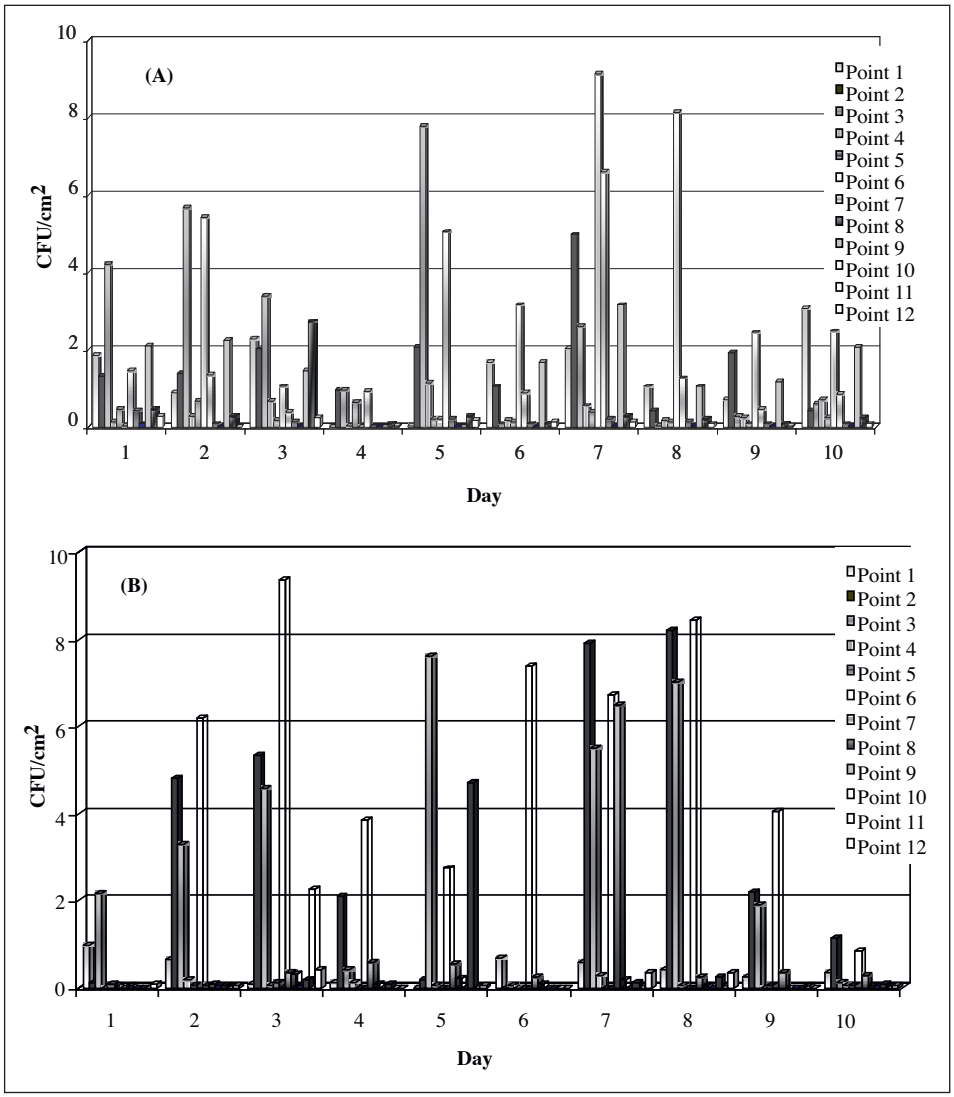

Fig. 3.—Media values (n=3) of MB measurements expressed as number of colony forming units for (A) APC and (B) Enterobacteriaceae per cm² in 12 sampling sites during food processing.

Tabla III
Total aerobic mesophilic bacteria in food samples collected from restaurants

Day	*Number of samples*	*Microbial quality (%)*		
		Acceptable	*Marginal*	*Unacceptable*
1	20	80	10	10
2	20	80	20	0
3	20	60	40	0
4	20	75	25	0
5	20	70	20	10
6	20	70	30	0
7	20	60	10	30
8	20	85	5	10
9	20	100	0	0
10	20	80	20	0

Tabla IV
Enterobacteriaceae in food samples collected from restaurants

Day	*Number of samples*	*Microbial quality (%)*		
		Acceptable	*Marginal*	*Unacceptable*
1	20	100	0	0
2	20	90	10	0
3	20	80	20	0
4	20	100	0	0
5	20	90	10	0
6	20	90	10	0
7	20	60	30	10
8	20	50	40	10
9	20	100	0	0
10	20	100	0	0

Tabla V
Established microbial limits

Aerobic mesophils	*Limits (CFU/cm²)*
HMA	
Safety	0-1.5
Risk	1.5-3.5
Hazard	>3.5
MB	
Safety	0-1
Risk	1-4
Hazard	>4
Enterobacteriaceaes	*Limits (CFU/cm²)*
HMA	
Safety	0-0.04
Risk	0.04-0.15
Hazard	>0.15
MB	
Safety	0-1
Risk	1-2
Hazard	>2

and moreover, were higher than the other values. In the other hand, the lowest limits (designated as safety owing to they did not compromise the health of the consumers) were established checking the food samples that did not present microbial contamination. Moreover, a medium interval was established (designated as risk owing to the possible danger for the consumers). This limit was set up taking into account the food samples that were between m and M.

In conclusion, these results show the possibilities offered by the proposed techniques, being practical and very simple, in the study of the microbial contamination on air and surfaces where there is an infection risk. The possibility to use of an ample range of selective media, well over the limited number used in this study, allows the analysis of many single microbial species.

Referencias

1. Creed PG. The potential of foodservice systems for satisfying consumer needs. *Innovat Food Sci Emerg Tech* 2001; 2: 219-27.
2. Green L, Selman C, Banerjee A, Marcus R, Medus C, Angulo FJ, Radke V, Buchanan S, and EHS-Net Working Group. Food service workers' self-reported food preparation practices: an EHS-Net study. *Int J Hyg Environ Health* 2005; 208: 27-35.
3. Griffith C, Worsfold D, Mitchell R. Food preparation, risk communication and the consumer. *Food Control* 1998; 9: 225-32.
4. Rodgers S. Food safety research underpinning food service systems-a review. *FSTC* 2005; 5: 67-76.
5. Sharp K., Walter H. A microbiological survey of communal kitchens used by undergraduate students. *Int J Consum Stud* 2003; 27: 11-6.
6. Soriano JM, Rico H, Moltó JC, Mañes J. Effect of introduction of HACCP on the microbiological quality of some restaurant meals. *Food Control* 2002; 13: 253–61.
7. Moyo DZ, Baudi IA. Bacteriological assessment of the cleaning and disinfection efficacy at the Midlands State University canteen, Zibabwe. *Pak J Biol Sci* 2004; 7: 1996-2001.
8. Neuhaus T. Fabulous: The Culinary Data Base. *Cornell Hotel Restaur Adm Q* 1990; 31: 111-4.
9. Ollinger-Snyder P, Matthews ME. Food safety: review and implications for dietitians and dietetic technicians. *J Am Diet Assoc* 1996; 96: 163-71.
10. Staskel DM, Briley ME, Field LH, Barth SS. Microbial evaluation of foodservice surfaces in Texas child-care centers. *J Am Diet Assoc* 2007; 107*:* 854-9.
11. Chen Y, Jackson KM, Chea FP, Schaffner DW. Quantification and variability analysis of bacterial cross-contamination rates in common food services tasks. *J Food Prot* 2001; 64: 72-80.

12. Kusumaningrum HD, Riboldi G, Hazeleger WC. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to food. *Int J Food Microbiol* 2003; *85:* 227-36.
13. Lee Wong AC. Biofilms in food processing environments. *J Dairy Sci* 1998; 81: 2765-70.
14. Den Aantrekker ED, Boom RM, Zwietering MH, Van Schthorst M. Quantifying recontamination through factory environments-a review. *Int J Food Microbiol* 2003; 80: 117-30.
15. Pasquarella C, Poleti L, Paoletti D. Monitoring of surfaces microbial contamination using nitrocellulose membranes: a quantitative and qualitative study. *Ann Ig* 1998; 11: 95-106.
16. Pitzurra M, Pasquarella C, Savino A. Microbiological environment monitoring (MEM). *Ann Ig* 1997; 9: 439-53.
17. Anonymous. Commission Decision (2001/471/EEC) of 8th june 2001 laying down rules for the checks on the general higiene carried out by operators in establishment according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meta and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. OJEC L165/48-53, 21/6/2001.
18. Aycicek H, Oguz U, Karci K. Comparison of results of ATP bioluminiscence and tradicional higiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *Int J Hyg Environ Health* 2006; 209: 203-6.
19. Anonymous. ISO 4833:2003. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of microorganisms- Colony-count technique at 30º C. Geneva: International Organization for Standardization.
20. Anonymous. ISO 21528-2:2004. Microbiology of food and animal feeding stuffs–Horizontal methods for the detection and enumeration of Enterobacteriaceae–Part 2: Colony count method. Geneva: International Organization for Standardization.
21. European Union. European Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. *Official Journal L175*:1–11.
22. European Union. Commission Regulation (EC) No.2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal L338*:1–26.
23. Friberg B, Friberg S, Burman LG. Inconsistient correlation between aerobic bacterial surface and air counts in operating rooms with ultra clean laminar air flows: proposal of a new bacteriological standard surface contamination. *J Hosp Infect* 1992; 42: 287-93.
24. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. *J Hosp Infect* 2000; 46*:* 241-56.
25. Rayner J, Veeh R, Flood J. Prevalence of microbial biofilms on selected fresh produce and household surfaces. *Int J Food Microbiol* 2004; 95: 29–39.