Journal of Food: Microbiology, Safety & Hygiene

Gámez, et al., J Food Microbiol Saf Hyg 2016, 1:1
DOI: 10.4172/2476-2059.1000104

Research Article Open Access

Pathogen Persistence in Restaurant Menus: Comparison between Materials

Martín Gámez N, Mengual Lombar M, Carcedo I, Lopez M.A and Álava J.I.*

Basque Culinary Center, Paseo Juan, Abelino Barriola no 101, 20009 Donostia-San Sebastián, Spain

***Corresponding author:** Dr. J.I. Alava, Science Department, Basque Culinary Center, Mondragon University, Paseo Juan Abelino Barriola no 101, 20009 Donostia-San Sebastián, Spain, Telephone:+34678622531; E-mail: ialava@bculinary.com

Received date: January 19, 2016; **Accepted date:** March 15, 2016; **Published date:** March 18, 2016

Abstract

Restaurant menus could represent a source of cross contamination between consumers' hands and food due to the daily manipulation, being a possible vector of certain food borne diseases if not cleaned and disinfected on a regular basis. To the question if those menus are included in daily cleaning protocols, the present study aims to demonstrate the actual bacterial contamination present in their surface. For this purpose, twelve menus from Basque Culinary Center's historical archive made on plastic material and paper/paperboard material are tested in order to find any presence of aerobic microorganisms. In addition, twelve plastic menus from several restaurants in San Sebastian (Spain), which were currently in use, were also sampled to detect presence of aerobic microorganisms, specifically *E.coli* and *S. aureus*. Unable to find paper menus in restaurants, the question of whether plastic material is a really hygienic option arises. Therefore, a follow-up study is designed, consisting inoculation of two different types of menu materials (plastic and paper/paperboard) with a known concentration of E. coli and S. aureus to determine bacterial survival at different times. This second part of the study intends to demonstrate material would be the most appropriate in restaurant menus due to its ability to maintain a minimum level of contamination and bacterial persistence.

Key words:

Restaurant menu; Bacterial persistence; Pathogen persistence; Bacterial contamination; *Escherichia coli*; *Staphylococcus aureus*; Plastic material; Paper material; Cross- contamination

Introduction

Foodborne diseases represent a serious public health problem. A total of 5,196 food-borne outbreaks were reported in 2013 by 24 reporting Member States and 3 non Member States, according to The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. 22.2% of them took place in restaurants, cafes, pubs, bars and hotels [1,2]. 6,043 confirmed cases of Verocytotoxigenic *Escherichia coli* [VTEC] infections were reported in the EU in 2013 of which 13 resulted in death and 73 represented food-borne outbreaks. In the case of *S. aureus* there were 386 food-borne outbreaks caused by staphylococcal toxins, which resulted in 3203 cases, 210 of which ended in hospitalization but there was no case of death. It is estimated that a large percentage of food-borne illnesses are not reported and therefore go unnoticed.

Escherichia coli is a bacterium that is found in the intestines of healthy humans and animals, and which is part of the natural bacterial flora, but some *E. coli* strains can cause serious diseases and lead to infection. VTEC [verocytotoxin-producing *Escherichia coli*] is a group of pathogenic *E. coli* bacteria can cause bloody diarrhea and hemolytic uremic syndrome. (In humans can lead to kidney failure). People get infected with VTEC by handling or consuming contaminated food or water or through contact with infected animals or by person-to-person transmission [3].

Staphylococcus aureus is a common bacteria present on skin and in mucous membranes in 20-30% of healthy people. It may sometimes cause infections in humans, typically local skin and wound infections but occasionally more severe infections in the body. It produces a highly stable enterotoxin, resistant to heat, freeze and irradiation. Ingestion of food contaminated with this toxin courses with symptoms like nausea, vomiting, stomach and abdominal pains. For at risk groups, the disease can lead to more serious problems, such as dehydration, muscle cramps and alteration of blood and coronary pressure [4].

Currently there are many studies describing bacterial contamination food contact surfaces in restaurants and bars, and many cleaning protocols describe in detail the proper way of cleaning and disinfect cookware, machinery and surfaces [5-8]. However, restaurant menu cleanliness, due they are not a food contact surface, they could be under-estimated and not covered by those protocols [9-11]. This fact means that restaurant menus could turn into a source of crossed contamination between consumers' hands and food, being a possible vector of foodborne diseases.

To the author's knowledge, there is not much scientific literature to date describing this topic. There is a study from 2013 analyzing presence of aerobic microorganisms in six menu cards taken from two restaurants [12]. Choi J et al. That compared bacterial contamination in regularly cleaned and uncleaned restaurant menus [13]. And there is a thesis analyzing bacterial total count and *S. aureus* in menus from different restaurants [14]. The aim of this study is to determine bacterial presence in restaurant menus, both on stored menus and on menus currently in use.

On the other hand, most of the menus founded were laminated. In view of this fact, the question of whether plastic material is really the most appropriate and whether there is a more hygienic alternative option arises. Neely & Maley [15] ensure that *S. aureus* is able to survive for a period of up to 90 days in different plastic surfaces at ambient temperature [22'9-24'5 ºC]. In a similar study Neely [16]

demonstrates that *E. coli* has the ability to survive on plastic surfaces up to 25 days at room temperature. Another study [17] shows the ability of both *S. aureus* and *E. coli* to survive on paper for more than 7 days at 21-25°C [This study also describes how *E. coli* is able to pass from hand to paper and from paper to hand].

The second part of the study intends to demonstrate which material would be the most hygienic option for using in restaurant menus. Therefore, we have designed a follow-up study consisting of the inoculation of menus of two different types of materials, plastic [laminated] and paper or paperboard, with a known concentration of *E. coli* and *S. aureus*, and determining bacterial survival at 0, 2, 6, 12 and 48 hours.

Materials and Methods

Measure of bacterial persistence in stored menus

We took 6 laminated menus, 4 paper menus and 2 paperboard menus which had been stored for a period longer than one month and less than two years. For sampling we used a 25 cm^2 Rodac TM Contact Plate (Conda Labs, Spain). The two culture Medias used were A.P.H.A. [Standard Method Agar] (Panreac AppliChem, Spain) to determine total viable count and E.M.B. [Eosin Methylene Blue Agar] (Panreac AppliChem, Spain) to determine Gram-negative enteric bacteria, and specific for *E. coli*.

For sampling, the plate's agar surface is directly applied to the menu's surface exerting moderate pressure, as is described by Montes, Lloret & López [18]. The samples were taken from the area with higher probability of contamination, at the bottom of the menu, left or right [13]. Then, samples were incubated for 48 hours at 37°C. We proceed to a plate counting of both media after 48 hours of incubation. Data were represented as means +/- SD of n= 6 samples per group and were analyzed by Student's t test

Measure of bacterial persistence in menus currently in use

In this part of the study 12 laminated and 1 paper restaurant menu were sampled with 25 cm^2 RodacTM Contact Plates (Conda Labs, Spain). The selected culture media were APHA, EMB and Baird-Parker. Baird Parker [Baird-Parker Agar Base], (Cultimed laboratories, Spain) is a selective media for determination of Staphylococci.

The procedure consisted on testing menus of a total of twelve San Sebastian restaurants over a period of 11 days. The menus were tested with 25 cm^2 RodacTM Contact Plates. For this purpose we designed a system, consisting of a hermetically sealed plastic container with three RodacTM Contact Plates (Conda Labs, Spain) [one per each type of culture media], in a thermal bag, keeping them chilled with vacuum-packed ice. The samples were kept under cooling conditions until they were incubated at the laboratory for 48 hours at 37°C. Paper menu was discarded.

Measure of bacterial persistence in plastic and paper menu

Preparation of bacterial inoculum

Two drains of BCC's facilities were sampled with some EMB RodacTM Contact Plates (Conda Labs, Spain) in order to isolate some colonies of *E. coli*. However, *S. aureus* was isolated from the colonies founded in a Baird-Parker RodacTM Contact Plate (Conda Labs, Spain) at sampling one of the restaurants in the previous experiment. Both were incubated for more than 72 hours at 37°C until the experiment was performed.

Then they were diluted with a sterile loophole in 10 ml of peptone water. Actual bacteria concentration in each inoculum was determined by serially diluting until 10-4 and plating the samples onto sterile Petri dishes, following the method described by Montes, Lloret & López [18]. The obtained concentrations were 6 x 10^3cfu/ml for *E. coli* inoculum, and 81 x 10^4cfu/ml for *S. aureus* inoculum. The difference of inoculum concentration selected for both microorganism is due at previous data about microorganism survival in surfaces [19].

Preparation of plastic and paper materials

We prepared a bench work in a remote area of the laboratory. Its surface was cleaned with Assert Lemon (Ecolab, Spain), and dried with paper hand towels. Then, 4 laminated menus and 4 new paper menus [2 made of paper and 2 made of paperboard] were collected from 8 restaurants. Laminated menus were cleaned and dried following the same procedure. After that, laminated and paper menus were allocated into a designated area on the bench work, and two 10x10 cm plastic templates were placed on each one, corresponding one per *E. coli* and one per *S. aureus*. Menus were stored at room temperature [21-22°C] until the end of the experiment.

Bacterial inoculation of laminated and paper menus

One ml of each bacterial inoculum was placed on each menu card using sterile pipettes [hence, 1ml of *E. coli* (*E. coli* J53-R (Lac +) from Reading University Collection) inoculum in one of the templates and one ml of *S. aureus* (*Staphylococcus aureus subsp. aureus* Rosenbach (ATCC® 29213™) from quality control strain for API), inoculum in the second template] and spread with sterile cotton swabs.

Sampling of menus

Immediately thereafter, a sample of each template was taken with a sterile swab. The procedure consisted in brushing 10 times horizontally and 10 times vertically [5 from left to right and 5 from right to left]. Then, swabs were streaked onto the surface of the media contained in Petri dishes, as the protocol described by JS Baker [20]. Culture media was EMB for *E. coli* and Baird-Parker for *S. Aureus* (Cultimed laboratories, Spain). This part of the study corresponded to Time 0. The same procedure was performed at 2, 6, 12 and 24 hours. Petri dishes were stored 48 hours at 37°C. We proceed to a plate counting of both media after 48 hours of incubation for each time (0, 2, 6, 12 and 24 hours). Data were represented as means +/- SD of n= 4 samples per group (80 samples in total) and were analyzed by two way ANOVA followed by Bonferroni test.

Results

Stored menus

At plate counting, we obtained negative results in all plates with EMB media. On the other hand, we obtained positive results in all plates with APHA media. Figure 1 shows the comparison of total plate count in APHA media in plastic material and paper material. The results show no significant differences between both types of materials. Results are expressed in cfu/cm^2.

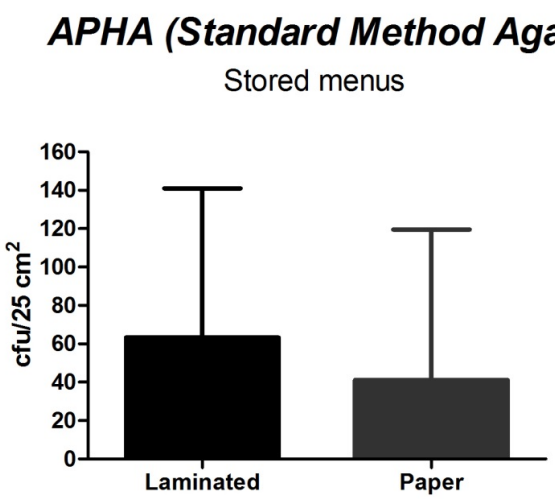

Figure 1: Comparison of means and standard deviation of the total plate count obtained in APHA media at sampling of laminated and paper/paperboard menus. Data are means +/- SD of n= 6 samples per group and are analyzed by Student's t test ($p<0.05$).

Menus currently in use

It proceeds to a quantitative count of the cfu in all plates of the three media at 48 hours. As in the previous experiment, all results in APHA media were positive. Nevertheless in this part of the study we surprisingly found two positives of *E. coli* in EMB media, and unusual number positives in Baird-Parker media corresponding to *S. aureus*. Table 1 shows the results obtained at plate counting of the three types of culture media in all laminated restaurant menus tested. The table shows positive and negative results and the rates of positives. Results are expressed in cfu/25 cm^2.

Measure of bacterial persistence in plastic and paper menus

We proceed to a plate counting of both media after 48 hours of incubation for each time [0, 2, 6, 12 and 24 hours]. *E. coli* survives in menus in both types of material for a period longer than 12 hours and less than 24 hours, while in laminated menus there is a high rate of bacterial growth until 6 hours, in paper bacterial growth is significantly decreased at 2 hours. On the other hand, *S. aureus* has the ability to survive beyond 24 hours in both types of material, while in laminated menus it is able to survive without barely perceptible variations in its growth, in paper bacterial growth decreases progressively from the beginning. Figure 2 and Figure 3 show bacterial survival of *E. coli* and *S. aureus* respectively at 0, 2, 6, 12 and 24 hours comparing plastic and paper material. Results are expressed in cfu/plate/100 cm^2.

Culture Media	Sampled	Positives	Negatives	Rate of positives
APHA (Total viable count)	12	12	0	100%
EMB (*E. coli* determination)	12	2	10	16,67%
Baird-Parker (*S. aureus* determination)	12	9	3	75%

Table 1: Comparison of results achieved at plate counting of the three types of culture media obtained at plastic menus testing.

Discussion

At a microbiological analysis in stored menus no significant differences between both types of materials are observed, which may indicate that the existing residual bacterial contamination may be due to the environmental contamination. Also no presence of *S.aureus* has been observed in stored menus (data not shown), obviously due to long storage time.

Furthermore, in restaurant menus that are currently in use, two positive *E. coli* and a significant amount of *S. aureus* have been observed. Poor hygiene habits of handlers and guests could be one of the possible reasons. A study by the University of Michigan of 3,749 people [21] ensures that only 5% of the people going to the toilet wash their hands long enough to kill disease-causing organisms. It also ensures that 33% do not use soap, and 10% do not even wash them. On the other hand, this lack of hygiene may be due to the non-inclusion of restaurant menus in cleaning protocols and its corresponding storage together, in dirty and wet conditions [22]. Similarly, in official restaurant inspections, microbiological evaluation is not performed as part of the inspection process; this is usually done in a visual way and therefore insufficiently.

The present study demonstrates that *E. coli* has the ability to survive in menus for more than 12 hours and *S. aureus* for more than 24 hours [12], demonstrated that microorganisms can be transferred from wet menus to fingertips for more than 24 hours. Due to the intense manipulation menus suffer from restaurant traffic, lack of hygiene in menus could turn them into a reservoir of bacteria. Handlers and guests could be responsible for cross contamination between surfaces, menus, hands and food, leading to a foodborne illness.

The problem could become especially acute for high-risk groups [immunocompromised population, people over 65 and children under 5 years], which could suffer major complications in case of foodborne disease and may end up in hospitalization or even in death [23].

Moreover, according to data obtained in the present study, paper material seems to support a lower bacterial contamination compared to plastic material. *E. coli* is able to survive in both types of material for a period longer than 12 hours, but in paper bacterial growth is significantly decreased at 2 hours in comparison with plastic where there is a high rate of bacterial growth until 6 hours. *S. aureus* has the ability to survive beyond 24 hours in both types of material, but while in laminated menus it is able to survive without any perceptible variations in its growth, in paper bacterial growth decreases progressively from the beginning.

This may be due to the adsorption of water by paper and the remaining water activity on the surface thereof. As plastic is unable to absorb water, water activity on its surface would be 100% during the

time it takes to evaporate, which would facilitate bacterial growth. In contrast, paper, as an absorbent material, favors reduction of water activity on its surface in a short time, so bacterial growth is faster diminished. Another possible factor in the antibacterial properties of paper could be ink presence. Cummings & Stewart [24] showed that the presence of ink on paper reduces bacterial growth on the surface thereof, by coating the cellulose fibers and reducing bacterial adhesion to the substrate.

We must also take into consideration that menus are more frequently replaced when it comes to paper menus, since dirt is more easily detectable, and shows an unpleasant aspect for guests.

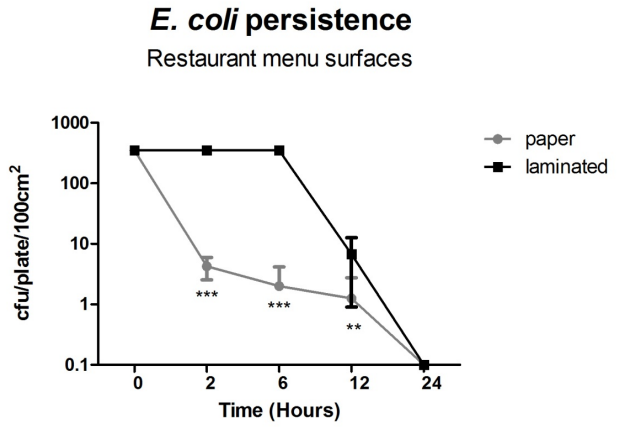

Figure 2: Survival of *E. coli* at 0, 2, 6, 12 and 24 hours, comparing Plastic material and Paper material. Measure of bacterial persistence in plastic and paper menus: Data are means +/- SD of n= 4 samples per group and are analyzed by two way ANOVA followed by Bonferroni test.(**p<0.01, ***p<0.001).

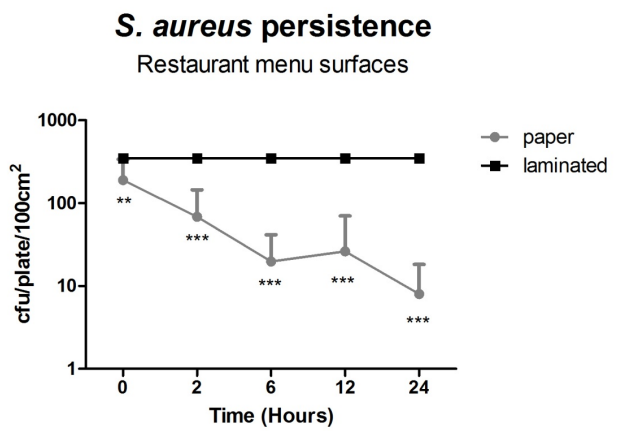

Figure 3: Survival of *S. aureus* at 0, 2, 6, 12 and 24 hours, comparing Plastic material and Paper material. Measure of bacterial persistence in plastic and paper menus: Data are means +/- SD of n= 4 samples per group and are analyzed by two way ANOVA followed by Bonferroni test, (**p<0.01, ***p<0.001).

Conclusion

The present study demonstrates that there is a significant bacterial population [*E. coli* and *S. aureus*] in restaurant menus, either due to a lack of hygiene by the staff [lack in personal hygiene or due to a non-inclusion of restaurant menus in daily cleaning protocols] or due to deficiencies in guests' hygiene [25]. These bacteria's have also the ability to survive in restaurant menus for more than 12 hours, so besides to the unfavorable image of businesses because of the perception of dirt by guests, these menus could be a source of cross contamination and cause foodborne illnesses. It must be corrected with proper education of the population in handwashing, proper cleaning and disinfection of all elements in the restaurant, not only food contact surfaces. As an alternative to the daily cleaning of plastic menus, this study proposes its replacement with paper menus. Paper represents a more hygienic alternative, not only because of clear evidence of dirt detection and ease of daily replacement, but also due to the lower bacterial growth on its surface compared with plastic.

Acknowledgments

The authors are grateful to the Basque Culinary Center, where the whole study has been developed and has provided the facilities needed to complete it. Thanks, to all the restaurants volunteers to be sampled. Also thanks to MSA and COLVEMA, for giving us a chance to do our internship in Basque Culinary Center.

References

1. European Food Safety Authority (EFSA) Shiga toxin-producing E. coli outbreak(s).
2. European Food Safety Authority, European Center for Disease Prevention and Control (2013) The European Union summary report on trends and sources of zoonoses,, zoonotic agents and food-borne outbreaks in 2013.
3. European Food Safety Authority (EFSA) E. coli.
4. European Food Safety Authority (EFSA) Meticillin-resistant Staphylococcus aureus (MRSA).
5. Ista LK, Fan H, Baca O, López GP (1996) Attachment of bacteria to model solid surfaces: oligo(ethylene glycol) surfaces inhibit bacterial attachment. FEMS Microbiol Lett 142(1): 59-63.
6. An YH, Friedman RJ (1998) Concise review of mechanisms of bacterial adhesion to biomaterials surfaces. J Biomed Mater Res. 43(3):338-348.
7. Sinde E, Carballo J (2000) Attachment of Salmonella spp. and Listeria monocytogenes to stainless steel, ruber and polytetrafluorethylene:the influence of free energy and the effect of commercial sanitizers. 439-447.
8. Katsikogianni M, Missirlis YF (2004) Conscise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. European Cells and Materials . 37-57.
9. Leliévre C, Legentilhomme P, Gaucher C, Legrand J, Faílle C, et al. (2002) Cleaning in place: effect of local wall shear stress variation on bacterial removal from stainless steel equipment. Chemical Engineering Science. 1287-1297.
10. Augustin M, Ali-Vehmas T, Atroshi F (2004) Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. J Pharm Pharm Sci. 55-64.
11. Gibson H, Taylor JH, Hall KE, Holah JT (1999) Effectiveness of cleaning techniques used in the food industry in terms of removal of bacterial biofilms. J Appl Microbiol . 41-48.
12. Sirsat SA, Choi JK, Almanza BA, Neal JA (2013) Persistence of Salmonella and E. Coli on the Surface of Restaurant Menus. J Environ Health. 8-14.
13. Choi J, Almanza B, Nelson D, Neal J, Sirsat S (2014) A strategic cleaning assessment program: menu cleanliness at restaurants. J Environ Health. 18-24.

14. Alsallaiy I (2013) Bacterial recovery, transfer to hands and survival on restaurant menus. Department of Food Science and Human Nutrition, Clemson University.
15. Neely AN, Maley MP (2000) Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic. J Clin Microbiol; 724–726.
16. Neely AN (2000) A survey of gram-negative bacteria survival on hospital fabrics and plastics. J Burn Care Rehabil.523-527.
17. Hübner NO, Hübner C, Kramer A, Assadian O (2011) Survival of bacterial pathogens on paper and bacterial retrieval from paper to hands: preliminary results. American Journal Nursing. 30-34
18. Montes E, Lloret I, López MA (2015) Diseño y Gestión de Cocinas. Manual de higiene alimentaria aplicada al sector de la restauración. Madrid, España. Ediciones Díaz de Santos.
19. Heukelekian H, Heller A (1940) Relation between food concentration and surface for bacterial growth. J Bacteriol. 547-558
20. Baker JS (1984) Comparison of various methods for differentiation of staphylococci and micrococci. J Clin Microbiol. 875–879.
21. Borchgrevink CP, Cha J, Kim S (2013) Hand Washing Practices in a College Town Environment. J Environ Health. 18-24.
22. Sigua G, Lee YH, Lee J, Lee K, Hipp J, et al. (2011) Comparative efficacies of various chemical sanitizers for ware washing operations in restaurants. Food Control. 13-19.
23. Boyce JM (2007) Environmental contamination makes an important contribution to hospital infection. J Hosp Infect. 50-54.
24. Cummings SP, Stewart CS (1994) Newspaper as a substrate for cellulolytic landfill bacteria. Journal of Applied Bacteriology. 196–202.
25. Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 6: 130.