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SURFACE SWAB SAMPLING AND RECOVERY OF *ESCHERICHIA COLI*

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Abstract: *The surface that comes into contact with food can be a source of contamination by microorganisms during the production, processing, packaging, and serving of food. The importance and consequences of contamination can be assessed based on the number of microorganisms present or the detection of pathogenic microorganisms.This study was performed on artificial surface contamination of household aluminum foil. For contamination, an overnight suspension of Escherichia coli ATCC 8739 was prepared in Tryptone soy broth. From artificially soiled surfaces measuring 10x10 cm^2, two smaller surface areas were sampled with separated swabs. The first was sampled one swab using a small stainless steel frame (4 cm x 5 cm) for estimation of the total count of aerobic microorganisms. A second swab was used for sampling from another contaminated surface of 20 cm^2 for estimation of the number of Enterobacteriaceae. Plate count agar (PCA) and Violet red bile glucose agar (VRBGA) were used for a total count of microorganisms and Enterobacteriaceae, respectively. Petri dishes were incubated at a temperature of 30 °C. The process was repeated on 16 contaminated surfaces. Compared to the estimated number of Escherichia coli in soiling suspension the recovery of swabbing was less than 60% for the count Escherichia coli on PCA as well as on VRBGA. This finding indicates the effectiveness of swab sampling and that findings of the presence of microorganisms on surfaces that come into contact with food require special attention.*

Keywords: *Escherichia coli, surfaces that come into contact with food, swab*

Introduction

Surfaces that come into contact with food, at any stage of food processing and manipulation, represent a potential source of microorganisms that can contaminate food. During manipulation, food comes into contact with different materials such as plastic, stainless steel, glass, wood, etc. which also have different effects on the survival and reproduction of microorganisms.

In food service establishments, work areas, cutting boards, sinks, and kitchen taps are identified as key surfaces that can cause cross-contamination of food, particularly if these surfaces are contaminated by mesophilic aerobic bacteria and

Enterobacteriaceae [1]. Overall, microbiological contamination in the food industry is highly variable in the food sector and even within a facility at various sampling points and sampling times [2]. According to Schlegelová et al. [3], the work table, cutter, and chicken transportation surfaces in two meat processing facilities sampled after cleaning and disinfection were the most contaminated surfaces, with ca. 6.0 to 7.0 log CFU per sampled surface.

Recently the use of mobile catering is becoming very popular and to reduce the risk of foodborne infections caused by bacteria, it is important to introduce specific requirements for monitoring practices for the hygiene of food contact surfaces, in particular, cutting boards and work surfaces [4].

Hand-contact surfaces in particular are known to be often heavily contaminated and frequently touched before handling ready-to-eat foods [5,6]. Food industries have demonstrated, in various studies, the successful transfer of microorganisms between hands and gloves to food and other surfaces involved in food production, storage, and preparation [7,8]. To maintain satisfy the level of process hygiene, it is necessary to constantly educate workers regarding the sanitary procedures of work surfaces, tools, equipment, and food handlers' hands [9]. Cleaning is essential to minimize microbial build-up and/or the presence of biofilms on food-contact equipment and surfaces as well as the more general environmental areas of food production/preparation premises [10].

Various techniques can be used for surface sampling [11]. Various sampling and monitoring methods, such as plating of swabs, sponges, wipe samples, agar contact plates, and dip slides, can be used in food production facilities to monitor microbiological contamination on surfaces [12].

Microbiological cleanliness test results are often used to verify cleaning and sanitation, as well as to investigate foodborne disease outbreaks [13].

This work aimed at determining the recovery of *Escherichia coli* bacteria sampled from an artificially contaminated surface (Al foil) using different media for the growth of microorganisms.

Material and methods

An overnight suspension of *Escherichia coli* strain ATCC 8739 was prepared in nutrient broth at a temperature of 30±1 ^{o}C for 18 hours. From the bacterial suspension, a logarithmic series of dilutions up to 10^{-8} were prepared in test tubes with 9 ml Buffered peptone water (HiMedia) without adding neutralizers. From dilutions 10^{-7} and 10^{-8}, 1ml each was transferred in Petri dishes in duplicate and covered with Tryptone soy agar (HiMedia). After incubation of the plates at 30±1 ^{o}C for 72±3 hours, the results were used to estimate the total number of bacteria in 1 ml of the bacterial suspension.

An aluminum foil was spread under the UV lamp in the biosafety cabinet for 15 minutes, which ensured the sterility of the surfaces for conducting artificial

contamination. Stainless steel frames 10 x 10 cm were placed on the aluminum foil. From the 10^{-4} dilution, 300µl was applied and uniformly distributed with a sterile L-shaped hockey stick spreader on the aluminum foil but inside the frame. The contaminated surface was dried for 15 minutes, after which sampling was performed with a swab. An area of 20 cm^2 was sampled from each contaminated surface using a small stainless steel frame (4x5 cm).

Each of the swabs was transferred into test tubes with 10 ml of physiological solution and the swab sticks were broken under sterile conditions. The content of the test tube was intensively homogenized, and then 1 ml was seeded in two petri dishes and poured with Plate count agar (HiMedia) or Violet red bile glucose agar (HiMedia) in a double layer, all in duplicate. Plates with Plate count agar (PCA) were incubated at 30 °C to determine the total number of microorganisms as stated in ISO 4833-1[14]. Plates with Violet red bile glucose agar (VRBGA) for growth of *Enterobacteriaceae* (in this case *Escherichia coli* ATCC 8739) were incubated at 30 °C for the purpose to avoid the influence of temperature on variation or results. The temperature of incubation recommended by ISO 21528-2 [15] for enumeration of *Enterobacteriaceae* as a hygiene indicator is 37 °C, and alternatively, a temperature of 30 °C can be chosen when the detection or enumeration of *Enterobacteriaceae* is conducted for technological purposes and includes psychrotrophic *Enterobacteriaceae*.

Results and discussion

Escherichia coli is a bacteria whose presence on surfaces that come into contact with food indicates a low level of hygiene measures and fecal contamination. Since it belongs to the *Enterobacteriaceae* family, and when there are no official requirements for determining the number of *Escherichia coli* on food contact surfaces, the presence of this bacterium can be indirectly recorded through the number of *Enterobacteriaceae*. Surface contamination and swab sampling were carried out according to the previously described procedure (Figure 1). Checking the presence and number of microorganisms on surfaces can be carried out using different microbiological methods depending on the targeted microorganisms. Sampling from the surfaces can be performed using techniques according to ISO 18593:2018, and most often with the wet swab technique. The ISO 18593 [16] method is a standardized sampling method for testing the microbiological cleanliness of surfaces. In case the surface is dry, the swabs must be moistened with appropriate diluents before use, which was applied, because after 15 minutes of contamination, the surfaces were dry.

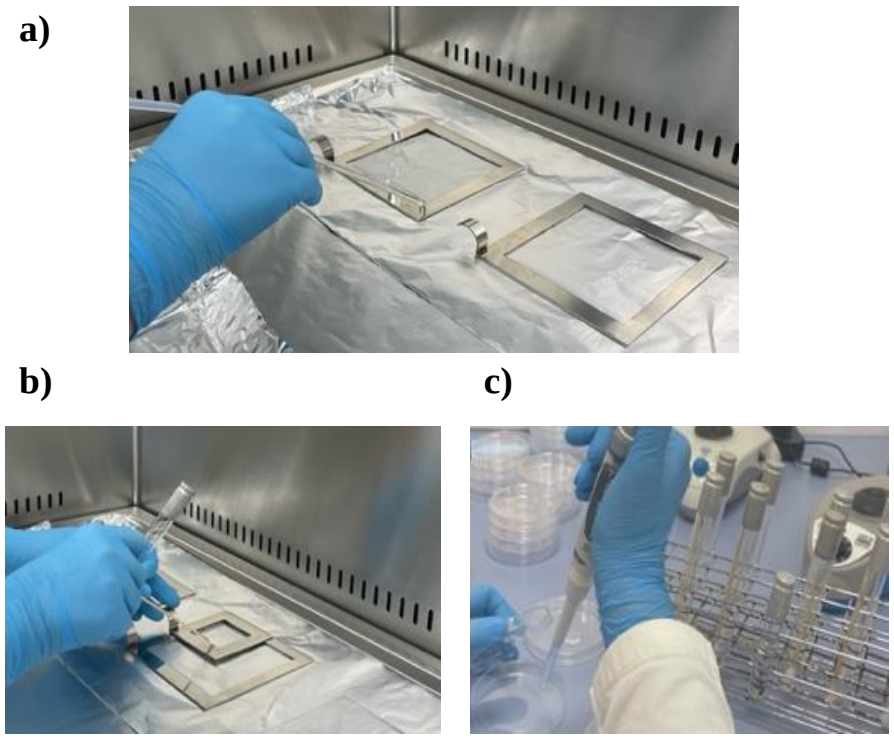

Figure 1. Performed experimental design
A) contamination of Aluminium foil; B) swab sampling; C) plating

Swabbing on a dry surface decreased the efficiency of all swab types to 30%. For recovery from bacterial biofilms, the swab efficiency was 40% lower than those of wet surfaces. Keeratipibul et al. 2017 reported [17] that the swab type and surface condition can affect the swab efficiency, and choosing the appropriate type of swab for the surface condition will increase the swab efficiency.

Estimated cell numbers in overnight bacterial suspension is presented in Table 1 and theoretically expected results after performed experimental design described in Material and methods is 74.4 CFU/cm^2.

Table 1. Cell number of *Escherichia coli* in overnight suspension

Dilution	Number of colony-forming units (CFU)	
	Petri Plate 1	Petri Plate 2
10^{-7}	129	118
10^{-8}	12	14
Estimated numbers of *Escherichia coli* ATCC 8739 in overnight suspension: 1.24 x 10^9 CFU/ml		

Experimental results show a higher colony count on PCA agar than VRBGA, 41.8±12.2 CFU/cm^{2,} and 38±14.2 CFU/cm^2, respectively (Table 2). A very high standard deviation indicates a large spread of results. The coefficients of variation of

growth on PCA and VRBGA are 29.3% and 37.2%, respectively. Compared to theoretically expected results, recovery is less than 60%. The explanation can be in the loss through spreading bacterial suspension with an L-shaped hockey stick, but also including other circumstances. The reasons for low recovery can be different and also related to the type of swab, the type of microorganisms being tested and whether a biofilm has formed, as well as the type of surface being testedCarpentier [18] and More et Griffith [19] indicated that a small number of microorganisms are picked up from the surface during sampling, especially from dry surfaces. Also, in some way, they remain trapped on the tip of the swab stick, which is why intensive vortexing is necessary to transfer the microorganisms into the liquid medium from which they will be seeded.

Table 2. Results of enumeration of *Escherichia coli* after swabbing

No.	Colony count of *Escherichia coli* per analyzed surface (CFU/cm^2)	
	Plate count agar	Violet red bile lactose agar
1	30	32
2	45	39
3	62	38.5
4	22	61
5	24.5	44
6	43	26.5
7	53	27
8	22.5	28.5
9	36	58
10	42.5	18.6
11	46	21.5
12	49	48.4
13	56.5	44
14	48	17
15	36	56
16	52	48
Average±standard deviation	41.8±12.2	38±14.2

The shape of the swab head and the material it is made of can affect the effectiveness of swab sampling. Some authors such as Dalmaso et al. [20] managed to raise the recovery level from 20% to 60%. Also, the innovative approach of sampling from surfaces is often simulated by findings in the field of medicine and biomaterials [21,22]. Low reproducibility was observed when using swabs, which may be related to the differential pressure applied to the surface when sampling multiple individuals [23].

The aluminum foil that was used to simulate the food contact surface has multiple purposes.

Aluminum foil is an important material in laminates and has wide applications in food packaging. Its barrier function against the migration of moisture, oxygen, and other gases, and volatile aroma, as well as against the impact of light is generally higher than any plastic laminate material [24].

A great amount of knowledge about microbial composition in packaging products is available. It is known that packaging material has a certain microbial load after production, ranging from 10^1 to 10^5 CFU/g [25,26]. There are already studies that have investigated and compared the microbial transfer of different spiked packaging materials (mainly cardboard and plastics) to packaged fruit. These studies revealed that the use of cardboard, compared to plastic, can significantly reduce the potential for the cross-contamination of packaged food [27,28]. A study by Maitz et al. [29] clarified that the bacterial load of the samples did not influence the number of transferred microorganisms. The study strongly indicated that the properties of the packaging material itself profoundly influence the transfer. The study included six samples belonging to fiber-based food packaging materials (FCM) produced by different factories in Europe.

A problem for food processors is the presence of food spoilage organisms, which can cause off-odors, off-flavors, or deterioration in food texture, resulting in reduced product shelf-life [10].

Garayoa et al. [30] following the application of Hazard Analysis Critical Control Point (HACCP) in the catering service found that 18.3% of the 600 analyzed did not meet the required criteria (≤100 CFU/25cm^2) in the first period, while in the second period, the number of unsatisfactory areas was reduced to 13.6%. De Oliviera et al. [31], when testing surfaces that come into contact with food in schools, found that in 120 schools, 33% of the surfaces showed a high health risk, 64% showed a normal health risk, and only 3% showed a low health risk.

Sampling from surfaces, as part of monitoring, can indicate a trend in the implementation of hygiene measures. Popović et al. [32] reported that over a the-year period, 10,366 swabs were taken from food contact surfaces and the hands of staff in school canteens and the results showed a proper application of good hygiene practices when it comes to surfaces, while the implementation of food employee hygiene practices requires increased supervision.

A prerequisite for usable results is a reliable sampling and testing methodology.

Conclusions

Sampling from surfaces and examination of microbiological cleanliness of surfaces is necessary as a check and confirmation of the implementation of hygiene measures in facilities where food is handled. In addition to various surface sampling techniques, swabbing with a swab stick is the most widely used technique.

After sampling from artificially contaminated aluminum foil and comparing it with the estimated number of *Escherichia coli* in the overnight suspension, the recovery of

Escherichia coli after swab sampling was less than 60% for both PCA and VRBGA. The reasons for the low recovery may be different and related to the type of swab sticks, the type of microorganism being tested and whether a biofilm was formed, as well as the type of surface being tested.

The results of the monitoring of surfaces that come into contact with food are applicable as a guideline for hygiene measures only if they were carried out using a reliable methodology of sampling and testing samples from the surfaces.

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UZORKOVANJE BRISOM SA POVRŠINA I OPORAVAK *ESCHERICHIA COLI*

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Sažetak: *Površine koje dolaze u kontakt sa hranom mogu biti izvor kontaminacije mikroorganizmima tokom proizvodnje, prerade, pakovanja i serviranja hrane. Značaj i posljedice kontaminacije procjenjuju se na osnovu broja prisutnih mikroorganizama ili otkrivanja patogenih mikroorganizama. U ovoj studiji provedena je vještačka kontaminacija površine aluminijumske folije koja se koristi u domaćinstvu. Za kontaminaciju je pripremljena prekonoćna suspenzija Escherichia coli ATCC 8739 u tripton soja bujonu (TSB). Sa veštački kontaminirane površine 10 x 10 cm^2 uzorkovane su dve manje površine pomoću dva zasebna bris štapića. Prvo je uzorkovano jednim brisom koristeći okvir od nerđajućeg čelika (4 cm x 5 cm) za određivanje ukupnog broja aerobnih mikroorganizma. Drugi bris je korišćen za uzorkovanje sa druge kontaminirane površine od 20 cm^2 i određivanje broja Enterobacteriaceae. Podloga za ukupan broj (PCA) je korišćena za određivanje ukupnog broja mikroorganizama, a ljubičasto crveni laktoza žučni agar (VRBGA) je korišćen za određivanje Enterobacteriaceae. Inkubacija petri šolja je bila na 30 ^{o}C. Ovaj proces je ponovljen na 16 kontaminiranih površina. Na osnovu poređenja sa procjenjenim brojem Escherichia coli u suspenziji za kontaminaciju, oporavak Escherichia coli nakon uzorkovanja brisom bio je manji od 60% kako za PCA tako i za VRBGA. Na osnovu dobijenih rezultata, u radu se razmatra eksperimentalni dizajn i efektivnost uzorkovanja brisom sa površina koje dolaze u kontakt sa hranom.*

Ključne riječi: *bris, Escherichia coli, površine koje dolaze u konakt sa hranom*