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ORIGINAL ARTICLE

Importance of mechanical action in a terminal disinfection process for decontamination of *Clostridium difficile* spores on hospital inert contact surfaces

Mafu Akier Assanta[1,2], Richard Massicotte[3], Gilbert Pichette[3], and Darakhshan Ahmad[3]

1 Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, St-Hyacinthe, QC, Canada

2 Chair of Food Safety and Security, Faculty of veterinary medicine, University of Lubumbashi, Katanga, Democratic Republic of the Congo

3 Laboratoire d'études sur le contrôle des infections nosocomiales, Hôpital du Sacré-Cœur de Montréal, Montréal, QC, Canada

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Abstract

Although the relevance of surface disinfection is increasingly being accepted, there are still a number of issues which remain controversial. In hospital environments in Québec (Canada), a three-step technique of cleaning and disinfection is used following a case of *Clostridium difficile* contamination. This technique brings together three factors: chemical, mechanical and microbiological. The present study investigates the impact of the mechanical action during each of these three-steps on *C. difficile* spore populations. Thus, cleaning and sporicidal products were replaced by water. The reduction rate of *C. difficile* spores was evaluated on four contaminated types of surfaces commonly found in the hospital environment. They included: ceramic, polyvinyl chloride (PVC), melamine and 2-methylprop-2-enoate methyl (PMAM) mattress cover. According to the type of the inert surface, variation in the reduction rate of spores was observed. The mattress cover was the most difficult surface to clean, followed by melamine. In contrast, the ceramic and PVC were the easiest surface to clean. For all the four surfaces, the average rate of spore loss associated with the various steps indicates that the mechanical action in the three-step process is important in reducing the risk associated with the presence of *C. difficile* spores on the hospital contact surfaces.

Keywords: *Clostridium difficile*; Disinfection; Equipment contamination.

Corresponding Author

Dr Mafu Akier Assanta, DVM, MSc, PhD, Postdoc
Food Research and Development Centre, Agriculture and Agri-Food Canada
3600 Casavant Boulevard West, St-Hyacinthe, QC,
CANADA J2S 8E3
Phone: 450-768-7881
Fax: 450-768-7851
E-mail: akierassanta.mafu@agr.gc.ca

Introduction

Clostridium difficile, a spore-forming gram-positive anaerobic bacillus, is commonly associated with diarrhoea and colitis in humans.[1] It was first isolated from faeces and meconium of asymptomatic newborn infants, and was originally named *Bacillus difficile* because of its morphology and the difficulties encountered in its cultivation.[2] In the past 30 years, however, *C. difficile* has been implicated as the principal infectious cause of antibiotic-associated diarrhoea in adult humans and it is now recognized as one of the most important nosocomial human pathogens.[3] Thus, the ability of *C. difficile* to survive within hospital environments remains a great global concern. In Québec's healthcare system, the presence of this microorganism has been largely documented.[4-7]

Since 2008, a strategy of cleaning and disinfection was developed for healthcare facilities following the discharge of patients previously affected by *C. difficile*. This approach is based on a three-step decontamination process.[8] The first step, which entails cleaning with a broad-spectrum cleaner-disinfectant aims to significantly reduce the microbial environmental load.[9] The second step is a rinse with clean water, while the third step involves the use of a recognized sporicide disinfecting product. In these procedures, the physicochemical properties of the products are important as they must be effective against *C. difficile* spores.[8] A common point among all three steps in this procedure is the mechanical action of wiping. Theoretically, as more mechanical action is applied to contaminated surfaces, a greater reduction in the numbers of environmental spores should be achieved. Therefore this mechanical action may be an important element in the reduction of the spores of *C. difficile*, in the three-step terminal disinfection procedure. The frequent presence of spores of this organism in the healthcare institutions strongly suggests that environmental contamination of inert materials may contribute to the transmission of this pathogen when patients come into contact with the contaminated surfaces. Mafu *et al.*[10] showed that attached cells which are intimately associated with the inanimate material may be less susceptible to cleaning processes due to the boundary layer and the secretion of extracellular coatings. The ability of microorganisms to become more resistant to sanitizers and other antimicrobial agents once attached to the surfaces were also documented.[10]

The purpose of this study was to examine the impact of the mechanical action in a three-step cleaning process aimed at the elimination of spores of *C. difficile* on inert surfaces. Here, water was used as the cleaning solution in order to ascertain the effectiveness of the wiping process. Four types of surfaces were included in this study: ceramic, polyvinyl chloride (PVC), melamine and bed liners made of 2-methylprop-2-enoate from methyl (PMAM). No soaps, cleaners or sporicide products were tested.

Materials and Methods

Type of surfaces

Test surfaces with standardized dimensions were used in this study (225 cm^2; 15 cm x 15 cm). The sample surfaces, representing four different types of materials included: melamine (5.6 mm, #992, Ébénisterie Prestige Inc., Joliette, Quebec, Canada), ceramic (3.8 mm, polished, BC, Rona, Joliette, Québec, Canada), "used" mattress covers (Hôpital Sacré-Cœur de Montréal, Montréal, Québec, Canada) and PVC (1 mm, doubles matte finish, blue white, Princeville, Vision Comfort, Rawdon, Québec, Canada).

Surface sterilization procedures

The ceramic tiles and bed liners were sterilized in an autoclave using a cycle gravity (121°C, 30 min with a drying time of 10 min). The melamine and the PVC were irradiated at the Food Research and Development Centre (Agriculture and Agri-Food Canada, St-Hyacinthe, Québec, Canada) by cobalt 60 ionization with a minimal dose of 15 kGy. All the surfaces were individually placed in Chex-all's autoclave envelopes (Schuman Company, Propper manufacturing Co. Inc., Long Island, New York, USA).

Bacterial strain and culture conditions

A clinical strain of *Clostridium difficile*, was obtained from the American Type Culture Collection (ATCC #BAA1801). Stock cultures of vegetative cells were stored at -80ºC in Brain Heart Infusion broth (Difco Laboratories, Beckton Dickinson, MD, USA) supplemented with 20% (v/v) sterile glycerol as cryoprotectant.

Prior to each experiment, the cells were sub-cultured (1% vol/vol) in a Reinforced Clostridial Medium broth (RCM, Difco Laboratories, Beckton Dickinson, MD, USA) previously reduced in O_2, and incubated anaerobically at 37°C for 48 hours. For spore production, cultures were re-suspended in Wilson sporulation medium (1%),[11] and then incubated at 37°C for 20 days under anaerobic conditions.

Wilson Environment of sporulation (WES)

The environment of sporulation (WES) was created using Wilson sporulation medium containing 90 g of Trypticase peptone (Difco 211921), 5 g of protease peptone no. 3 (Difco 211693), 1 g of $(NH_4)_2SO_4$ (Sigma A4418) and 1.5 g of Trizma base (Sigma T1503) in 1 L of distilled water. The pH of the WES was approximately 7.4 without being adjusted with the NaOH as describing by Wilson *et al.* (1982).[11] The medium was autoclaved at 121°C for 15 minutes and then pre-reduced in an anaerobic environment for 24 hours before being used.

Sporulation

Following anaerobic incubation in Wilson medium the tubes containing the sporulated strain were subjected to thermal shock treatment by placing them in a water bath heated to 80°C for 10 minutes. After heat treatment the tubes were immediately transferred to an ice bath for a few minutes for rapid cooling. Cells were harvested by centrifugation at 4400 rpm for 10 min at 4°C and the pellets were washed in sterile distilled water. The centrifugation and washing steps were twice repeated and the final pellet was suspended in 6 ml of sterile distilled water and stored at 4°C until use. The presence of spores was confirmed by staining the cells with Malachite green and viewing under a microscope. Before each assay, tubes containing spores were centrifuged at 4400 rpm for 7 minutes at 4°C and the pelleted cells suspended in 6 ml of 5% bovine serum.

Malachite green staining

A drop of sample was placed on a microscope slide and allowed to air-dry. Once dried, the smear was heat-fixed and then covered with 5% Malachite green (Sigma-Aldrich, kit * 04551-1KT-F, Schaeffer and Fulton Spore Stain Kit). The slide was heated until the stain began to boil (approximately 30 seconds), at which time excess stain was rinsed away with distilled water. The smear was then flooded with the counter-stain safranine for a contact time of 1 minute before rinsing. The slides were examined under a microscope at 1000 X magnification.

Contamination of the surfaces

The test surfaces were contaminated with *C. difficile* spores by placing 500 µl of spore suspension on the surface and evenly spreading with a sterile applicator. The spore density of the surfaces was 5.82 log CFU/cm^2. Once the suspensions were applied, the surfaces were allowed to dry for 45 minutes.

Enumeration of the bacterial population after cleaning

In order to simulate the wiping action associated with real conditions found in the hospital environment, a piece of cloth was immersed in sterile hard water and the surplus of water was wrung out with sterile gloved hands. The cloth was then used to wipe each test surfaces. Specifically, this involved four in and out movements, beginning at the top-left corner and ending at the left inferior corner, making sure equal and constant pressure was applied. These steps were repeated three times in order to account for the wiping action involved in each stage of the three step cleaning process. A sterile cellulose sponge (B01245WA, Whirl-Pak, Nasco) pre-moistened with D/E neutralizing broth (Oxoid, QBS10DE1)[12] was used to sample each surface by making 3 wiping motions from top to bottom in order to achieve full surface coverage. Each sponge was placed in 90 ml plug D/E neutralizing broth and processed in a stomacher (Stomacher ® 400 Circulator, Seward) for 30 sec at 230 RPM. Viable *C. difficile* was enumerated by plating the samples onto BHI agar supplemented with 0.1% sodium taurocholate. The plates were incubated at 37°C for 24 hours under anaerobic conditions.

Statistical methods

All experiments were conducted in triplicate. Within each experiment a single test surface was replicated three times, with duplicate samples plated for each individual surface. Therefore, the average value of the duplicates was used in the analysis of variance since both values obtained by duplicates did not represent independent assays. The effect of the various factors was tested by analysis of variance (ANOVA), according to

the GLM'S procedure. The normality of the results was verified using Shapiro-Wilks test and the homogeneity of the variances by the Levene test (data not shown).

Results

The mechanical action associated with wiping in each stage of the three step cleaning process impacts the total number of spores removed from the test surfaces. Table I shows the reduction in the number of spores associated with each individual step for each surface. Averaging all surface types, there was an overall increase in spore removal from 0.55 log CFU/cm^2 after step 1 to 1.83 log CFU/cm^2 after the final step, indicating that more than 90% of spores can be eliminated by wiping action alone during the three-step cleaning procedure. The number of cleaning steps significantly impacted the effectiveness of spore removal. The highest level of spore losses for a single cleaning step using water was 0.73 CFU/cm^2 for melamine (Figure 1); whereas, increasing the number of cleaning steps to three resulted in as many as 2.5 log CFU/cm^2 spores removed, as was the case for ceramic. Although the cleaning of ceramic and PVC surfaces appeared to be similarly efficient, results for the bed lining material demonstrated the difficulties associated with removing spores from such surfaces. Despite the application of three cleaning cycles, only 0.74 CFU/cm^2 of spores were removed from the mattress covers. Melamine was intermediate in cleaning efficiency after the three step procedure (2.08 CFU/cm^2).

Table I. Loss of spores associated with cleaning of various surfaces with water and mechanical action. Comparison of the number of steps.

Number of cleaning steps	Loss of spore (log CFU/cm^2 ± standard deviation)				
	Ceramic	PVC	Melamine	Mattress-cover	Average
	Initial population [1]				
	5.14 ± 0.14	5.10 ± 0.33	5.06 ± 0.21	4.73 ± 0.15	5.01
1	0.60 ± 0.50	0.65 ± 0.81	0.73± 0.47	0.28 ± 0.24	0.55
2	1.88± 0.34	2.02 ± 0.53	1.09± 0.36	0.11 ± 0.12	1.27
3	2.25 ± 0.89	2.23 ± 0.47	2.08 ± 0.30	0.74 ± 0.34	1.83
Average	1.58	1.63	1.30	0.35	---

[1] Spore population attached to the surface before cleaning/disinfection

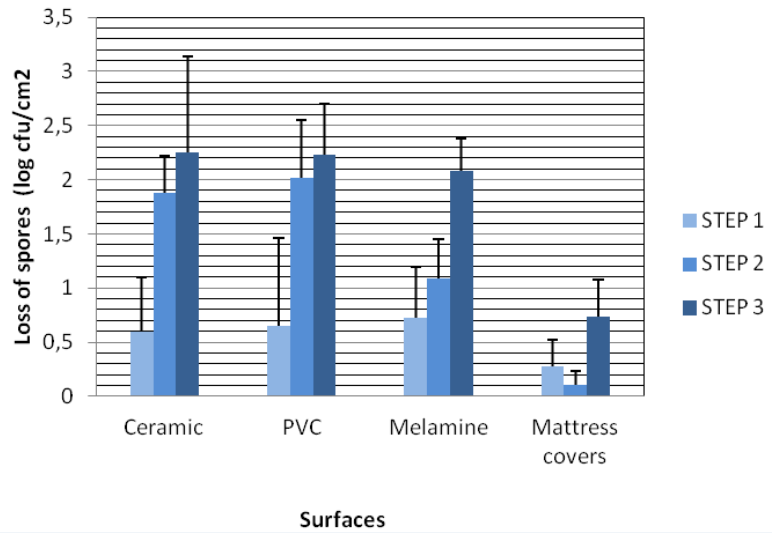

Figure 1. Loss of spores associated with a cleaning of various surfaces by using mechanical action and water (method of cleaning varying from 1 to 3 steps).

Table II. Results of the analysis of variance for comparison of the methods of cleaning with some water in 1 to 3 steps

Source	p-value
Surface	<0.0001
Cleaner	<0.0001
Surface x method	0.15

Table III. Results of the test of multiple comparison of Duncan for the surface factor

Duncan group *	Loss of spore Average (log CFU/cm²)	Surface
A	1.63	PVC
A	1.58	Ceramic
A	1.30	Melamine
B	0.35	Mattress-Covers

* The averages associated with the same letter are not significantly different.

Table IV. Results of the test of multiple comparison of Duncan for the method factor

Duncan group*	Loss of spore Average (log CFU/cm²)	Method
A	1.83	3 steps
B	1.27	2 steps
C	0.55	1 step

[1] The averages associated with the same letter are not significantly different.

Statistical analysis

Table II presents the results of ANOVA allowing comparison of the use of water as the cleaning agent with the implementation of various cleaning steps (i.e. 1 step versus 2 and 3 steps). It is possible to conclude that the type of surface and number of cleaning steps significantly influence the reduction of spores ($P < 0.0001$).

Table III presents the results of the Duncan multiple comparisons test for the surface type factor only. The spore reduction values on the PVC (1.63 CFU/ cm²), ceramic (1.58 CFU/ cm²) and melamine (1.30 CFU/ cm²) surfaces were significantly greater than those found in mattress covers (0.35 CFU/ cm²). On the other hand, the statistical analysis does not show significant differences between the surfaces of PVC, ceramic and melamine when water is used as cleaning agent. This was probably due to the small sample size in this present statistical analysis.

The factor method of the Duncan multiple comparisons test is presented in Table IV. All three steps resulted in significant reductions in spore numbers. Accordingly, the three-step procedure resulted in the greatest reduction of spores (1.83 CFU/ cm²), while the single step method resulted in the smallest reduction (0.55 CFU/ cm²). This analysis clearly demonstrates the effect of the mechanical cleaning action in the elimination of spores of *C. difficile*. These data demonstrated that it is possible to get rid of more than 90% of contaminants by strongly rubbing the surfaces.

Discussion

The use of a three-step cleaning-disinfection procedure for disinfection following a case of *Clostridium difficile* brings together three types of factors: chemical, mechanical and microbiological.[8] The present study was focussed on the impact of the mechanical action of wiping on the removal of spores of *C. difficile* on four types of surfaces commonly found in the hospital environment. Therefore, for the purpose of our studies, the disinfectants and sporicide products normally implemented in the first and last steps of the three-step procedure were omitted and replaced with sterile water.

Firstly, the results obtained show that the mechanical action of the cleaning has variable effects according to the type of surface. The ceramic and PVC surfaces appear to be similar in terms of ease of cleaning and spores could be effectively removed. In contrast, the mattress cover surface was found to be difficult to clean, while spore reductions on melamine were somewhat intermediate. This variation in the ease of cleaning between surfaces can be explained by the interaction of various factors specific to each material.[13] The physico-chemical properties of surfaces play an important role on microbial adhesion and cleaning efficiency.[14-18] Microbial attachment to inert surface is the result of a succession of steps which involves van der Waals, Lewis' acid-basis and electrostatic interaction forces.[19-20] There are also physical characteristics of the surfaces such as the roughness, the nature of the components, as well as the environmental conditions which can influence bacterial adhesion.[21-23] These factors can modulate the hydrophobic or hydrophilic nature of a surface which is an important parameter to consider in microbial adhesion. The hydrophobic or hydrophilic characteristic of the inert materials can apply as much to the surfaces of the solids as to that of the bacteria.[24-25]

Depending on the bacteria, this hydrophobic/hydrophilic characteristic varies according to the composition of the secretions of exopolymers specific to each bacterial species.[21,26-28] These exopolymers are important participants in the attachment phenomenon. According to Mafu *et al*.[24] as the hydrophilic character of a bacterium increases, it will tend to become less attached to a solid surface. Thus, it is a parameter which can modulate, in this particular case, the effect of the mechanical action on the removal of spores of *C. difficile* on a surface.

For the spores of *C. difficile,* as well as those of *Bacillus cereus,* an exopolymer consisting of glycoproteins creates an additional layer around the spore called exosporium.[29-30] It is reasonable to consider that the absence or the presence of an exosporium could influence the adhesion of a bacterium on surfaces within hospital environments in the same manner as in the food processing industry.[31] According to Koshikawa,[27] the exosporium would generally be more hydrophobic than the envelope of the spore. This correlation between the relative hydrophobicity of the spore and the capacity of adhesion on a stainless steel surface has been reported in the literature.[32-34] Bernardes *et al*.[35] considered stainless steel as hydrophobic. It is generally accepted that the hydrophobicity of spores facilitates the adhesion on hydrophobic surfaces.[32-33] On the other hand, with increasing hydrophilicity of bacteria, cells will tend to become less attached to inert material.[24] Furthermore, it is also interesting to note that the attachment can be variable according to the nature of the strain. Lovleen *et al*.[32] observed that a clinical strain of *C. difficile* could have superior adhesion capacities to surfaces compared with a laboratory strain.

It is important to point out that once bacteria have attached to a surface, they become more resistant to sanitizing agents compared with bacteria in suspension.[24] In order to obtain optimum activity of chemicals, it is thus necessary to remove and re-suspend surface associated bacteria and spores. This re-suspension in medium requires the use of a mechanical action which will distort the energy connections which intervene in the adhesion between a solid surface and bacterium. However, the mechanical action can have variable effects on the efficiency of removal of microorganisms according to the surface and the species/strain.[36] A study carried out by Foschino *et al*.[18] shows that a stainless steel surface contaminated with *Escherichia coli* cleaned with distilled water achieved a 98% reduction in cell numbers; and only 34% with *Aspergillus niger*. The hydrophobic surfaces would be more difficult to clean according to Faille *et al*.[37]

It should be noted that the mechanical action of wiping with or without a cleaning product does not kill the spores of C. *difficile*. It simply allows a reduction in the number of spores on a surface.[38] Once removed from the surface, it is the activity of the sporacidal agent in the three-step process that actually kills the spores. Nevertheless, the effectiveness of the sporacide is highly dependent on the dislodgement of spores by mechanical action. Data shown in Figure 1 demonstrates that with a single step of mechanical action, the spore removal varies according to the nature of the surface. Moreover, the effectiveness of a single step was low as the average reduction of spore numbers was 0.55 log CFU/cm^2 for all surfaces combined. Besides the difficulty in breaking the links between two associated

surfaces, including aggregates of spores, there is a possibility that a percentage of spores removed during the mechanical action can simply be moved from one spot to another.[39] Therefore, it is possible that spores can reattach to another location on the surface.[33-37] This phenomenon increases the difficulty in achieving spore-free surfaces during cleaning. According to Sylla *et al.*[40] the strategies for cleaning should take into account this re-attachment phenomenon to limit the risk of re-contaminating the surfaces.

In the current study, the application of a second mechanical action resulted in a significantly greater reduction in spores found on the test surfaces. The average of reduction for all combined surfaces was more than double (1.27 log CFU/cm^2) that obtained with the single step procedure (Figure 1). Implementation of the third mechanical step, even without the use of a sporicide, further decreased the number of spores on the four surfaces; however, it should be noted that the presence of residual spores on the surfaces after all three cleaning steps remains problematic. This is especially true with the mattress cover surface where a reduction average of only 0.74 log CFU/cm^2 was obtained after the three step procedure. As mentioned previously, the unique characteristics of this surface could explain this observation as this type of surface is similar to woven synthetic fibres.

Conclusion

The results obtained in this study show that various surfaces used in the hospital environment can impact the adhesion of spores of *C. difficile* and consequence the cleaning efficiency. As an example, the mattress covering material examined here was found to be the surface most difficult to clean using only the mechanical action of wiping. The importance of these observations from a practical perspective comes to light when "more difficult to clean" surfaces are part of the furniture in places of increased risk for the clientele of the hospital. The use of such surfaces as well as the cleaning-disinfection protocols in this environment require particular attention.

The results obtained from this study confirmed that the mechanical action of wiping in a three-step cleaning strategy aimed at terminal disinfection of *C. difficile* is an important consideration in reducing the risk of contamination. Nevertheless, irrespective of the important role this mechanical action of wiping plays in a three-step process, that alone cannot achieve the necessary margin of safety required to mitigate the risk of contamination as residual spores of *C. difficile* will remain on surfaces. The use of products containing surfactants and/or sporacidal agents would be complementary to the mechanical action and would so allow a maximization of this safety margin.

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Reference

1. Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. *N Engl J Med* 1994; **330:** 257-262. http://dx.doi.org/10.1056/NEJM199401273300406
2. Hall IC, O'Toole E. Intestinal flora in newborn infants with a description of a new pathogenic anaerobe, *Bacillus difficilis*. *Am J Dis Child* 1935; **49:** 390–402. http://dx.doi.org/10.1001/archpedi.1935.01970020105010
3. Conly JM. *Clostridium difficile*-associated diarrhea - The new scourge of the health care facility. Can J Infect Dis 2000; **11(1):** 25–27.
4. Institut national de santé publique du Québec (INSQ), Prévention et contrôle de la diarrhée nosocomiale associée au *Clostridium difficile* au Québec, Comité sur les infections nosocomiales du Québec, Direction des risques biologiques environnementaux et occupationnels, 2005: 87 p.
5. Ministère de la Santé et des Services sociaux du Québec. Diarrhées associées au *Clostridium difficile* (DACD) - Surveillance périodique, Rapport sur les données provenant du système de surveillance des diarrhées associées à *Clostridium difficile* du laboratoire de santé publique du Québec, 2006: 18 p.
6. Direction régionale de santé publique de la Capitale-Nationale. *Maladies à déclaration obligatoire, Intoxication, infections et autres événements sous surveillance*, Rapport annuel 2009, 2010: 132 p.
7. Ministère de la Santé et des Services sociaux. La Direction de la protection de la santé publique rattachée à la Direction générale de la santé, Le Plan d'action sur la prévention et le contrôle des infections nosocomiales au Québec 2010-2015, 2011: 90 p.
8. Groupe de travail Hygiène et salubrité au regard de la lutte aux infections nosocomiales, Mesures d'hygiène et de salubrité au regard du *Clostridium difficile*: Lignes directrices, Direction des communications du ministère de la Santé et des Services Sociaux du Québec, 2008: 24 p.
9. Ministère de la Santé et des Services sociaux, Gouvernement du Québec, Massicotte R, Désinfectants et désinfection en hygiène et salubrité: principes fondamentaux. 2009: 73 pages. (http://msssa4.msss.gouv.qc.ca/fr/document/publication.nsf/4b1768b3f8495519c852568fd0061480d/ecc5fa387ee1319b852575e700462a41)
10. Mafu AA, Roy D, Goulet J, Savoie L, Roy R. Efficiency of sanitizing agents for destroying *Listeria monocytogenes* on contaminated surfaces. *J Dairy Sc* 1990; **73(12):** 3428-3432. http://dx.doi.org/10.3168/jds.S0022-0302(90)79040-6
11. Wilson KH, Kennedy MJ, et Fekety R. Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. *Journal of Clinical Microbiology* 1982; **15(3):** 443-446.
12. Engley Jr FB, Dey BP. A universal neutralizing medium for antimicrobial chemicals. In: Prindle RF ed. Proceedings of the 56th Mid-year Meeting of the Chemical Specialties Manufacturers Association. New York, NY: Chemical Specialties Manufacturer's Association Inc, 1970; 100-106.
13. Boulané-Petermann L. Processes of bioadhesion on stainless steel surfaces and cleanability: A review with special reference to the food industry. *Biofouling* 1996; **104:** 275-300. http://dx.doi.org/10.1080/08927019609386287
14. Hamadi F, Latrache H, Mliji E, Mallouki B, Mabrouki M, Ellouali M. Adhésion de *Staphylococcus aureus* au verre et au téflon. *Rev Microbiol Indust Sanit Environ* 2009; **3:** 1–16.
15. Mafu AA, Roy D, Goulet J, Magny P. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene and rubber surfaces after short contact time. *J Food Prot* 1990; **53:** 742-746.
16. Whitehead KA, Verran J. The Effect of Substratum Properties on the Survival of Attached Microorganisms on Inert Surfaces. Springer Series on Biofilms,2008; http://dx.doi.org/10.1007/7142_2008.
17. Jullien C, Bénézech T, Carpentier B, Lebret V, Faille C. Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry. *Journal of Food Engineering* 2003; **56(1):** 77–87. http://dx.doi.org/10.1016/S0260-8774(02)00150-4
18. Foschino R, Picozzi C, Civardi A, Bandini M, Faroldi P, Comparison of surface sampling methods and cleanability assessment of stainless steel surfaces subjected or not to shot peening. *J Food Eng* 2003; **60(4):** 375-381. http://dx.doi.org/10.1016/S0260-8774(03)00060-8
19. Rubio C. Compréhension des mécanismes d'adhésion des biofilms en milieu marin en vue de la conception de nouveaux moyens de prévention, Thèse de doctorat de l'Université Paris, 2002; no 6, p. 216.
20. Briandet R, Herry J, Bellon-Fontaine MN. Determination of the Van der Waals, electron donor and electron acceptor surface tension components of static Gram positive microbial biofilms. *Colloids Surf B Biointerfaces* 2001; **21:** 299-310. http://dx.doi.org/10.1016/S0927-7765(00)00213-7
21. Flint SH, Brooks JD, Bremer PJ. The influence of cell surface properties of thermophilic *streptococci* on attachment to stainless steel. *Journal Applied Microbiol* 1997; **83:** 508–517. http://dx.doi.org/10.1046/j.1365-2672.1997.00264.x
22. Scheuerman TR, Camper AK, Hamilton MA. Effects of substratum topography on bacterial adhesion. *Journal colloid interface Science* 1998; **208:** 23-33. http://dx.doi.org/10.1006/jcis.1998.5717
23. Faille C, Membré JM, Tissier JP, *et al*. Influence of physicochemical properties on the hygienic status of stainless steel with various finishes. *Biofouling* 2000; **15**: 261–274. http://dx.doi.org/10.1080/08927010009386317
24. Mafu AA, Plumety C, Deschênes L, Goulet J. Adhesion of pathogenic bacteria to food contact surfaces: influence of pH of culture. *Int J of Microbiol* 2011; **2011:** 972494. http://dx.doi.org/10.1155/2011/972494
25. Hüsmark U, Rönner U. The influence of hydrophobic, electrostatic and morphologic properties on the adhesion of *Bacillus* spores. *Biofouling* 1992; **5:** 335–344. http://dx.doi.org/10.1080/08927019209378253
26. Dufrêne YF, Boonaert CPJ, Rouxhet PE. Adhesion of *Azospirillum brasilense*, role of proteins at the cell-support interface. *Colloids Surf B Biointerfaces* 1996; **7:** 113–128. http://dx.doi.org/10.1016/0927-7765(96)01288-X
27. Koshikawa T, Yamazaky M, Yoshimi M, *et al.* Surface hydrophobicity of spores of *Bacillus* spp. *J Gen Microbiol* 1989; **135:** 2717-2722.
28. Oliveira R, Azeredo J, Teixeira P, Fonseca AP. The role of hydrophobicity in bacterial adhesion. BioLine 2001; 11-22.
29. Lequette Y, Garénaux E, Tauveron G, *et al*. Role played by exosporium glycoproteins in the surface properties of *Bacillus cereus* spores and in their adhesion to stainless steel. *Appl Environ Microbiol* 2011; **77(14):** 4905–4911. http://dx.doi.org/10.1128/AEM.02872-10
30. Henriques AO, Moran CP. Structure, assembly, and function of the spore surface layers. *Annu Rev Microbiol* 2007; **61:** 555–588. http://dx.doi.org/10.1146/annurev.micro.61.080706.093224
31. Faille C, Tauveron G, Le Gentil-Lelièvre C, Slomianny C. Occurrence of *Bacillus cereus* spores with a damaged exosporium: Consequences on the spore adhesion on surfaces of food processing lines. *J Food Prot* 2007; **70:** 2346–2353.

32. Lovleen TJ, Phillips DS, Williams CF, Alyousef A, Baillie L. Contribution of spores to the ability of *Clostridium difficile* to adhere to surfaces. *Appl Environ Microbiol* 2012; **78(21):** 7671. http://dx.doi.org/10.1128/AEM.01862-12
33. Rönner U, Hüsmark U, Henriksson A. Adhesion of *Bacillus* spores in relation to hydrophobicity. *J Appl Bacteriol* 1990; **69:** 550–556. http://dx.doi.org/10.1111/j.1365-2672.1990.tb01547.x
34. Tauveron G, Slomianny C, Henry C, Faille C. Variability among *Bacillus cereus* strains in the spore surface properties and influence on their ability to contaminate food surface equipment. *Int J Food Microbiol* 2006; **110:** 254–262. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.04.027
35. Bernardes PC, de Andrade NJ, Ferreira SO, *et al.* Assessment of hydrophobicity and roughness of stainless steel adhered by an isolate of *Bacillus cereus* from a dairy plant, Braz J Microbiol 2010; **41(4):** 984-992. http://dx.doi.org/10.1590/S1517-83822010000400017
36. Faille C, Sylla Y, Le Gentil C, Bénézech T, Slomianny C, Lequette Y. Viability surface properties of spores subjected to a cleaning-in-place procedure: consequences on their ability to contaminate surfaces of equipment. *Food Microbiol* 2010; **27(6):** 769-776. http://dx.doi.org/10.1016/j.fm.2010.04.001
37. Faille C, Julien C, Fontaine F, Bellon-Fontaine M-N, Slomianny C, Benezech T. Adhesion of *Bacillus* spores and *Escherichia coli* cells to inert surfaces: role of surface hydrophobicity. *Can J Microbiol* 2002; **48:** 728–738. http://dx.doi.org/10.1139/w02-063
38. Rutala WA, Gergen MF, Weber DJ. Efficacy of different cleaning and disinfection methods against *Clostridium difficile* spores: importance of physical removal versus sporicidal inactivation. *Infect Control Hosp Epidemiol* 2012; **33(12):** 1255-1258. http://dx.doi.org/10.1086/668434
39. Harsha S, Cooper C, Maillard JY. Efficacy of "sporicidal" wipes against *Clostridium difficile*. *Am J Infect Control* 2011; **39(3):** 212-218. http://dx.doi.org/10.1016/j.ajic.2011.01.006
40. Sylla Y. Nettoyage en place des lignes agro-industrielles: Etude Cinétique d'élimination des biofilms négatifs au sein des installations fermées dans les industries agroalimentaires. Thèse à l'Institut des Sciences et Industries du Vivant et de l'Environnement (AgroParisTech Spécialité): Génie des Procédés. 2011; 191p.