Evaluation of bacterial survival on inert surfaces in a hyperbaric environment

Laetitia Hendier[1], Hervé Soule[2], Mohamed Abbas[2], Didier Pittet[2], Rodrigue Pignel[3], Sylvain Boet[3]

[1] *Internal Medicine Department, University of Geneva Hospitals, Switzerland*
[2] *Infection Control Programme and WHO Collaborating Centre on Antimicrobial Resistance and Infection Prevention and Control, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland*
[3] *Diving and Hyperbaric Medicine Unit, Division of Emergency Medicine, Department of Anaesthesiology, Clinical Pharmacology, Intensive Care and Emergency Medicine, Geneva University Hospitals and Faculty of Medicine, University of Geneva, Geneva, Switzerland*

Corresponding author: *Dr Laetitia Hendier, Internal Medicine Department, The University of Geneva Hospitals, rue Gabrielle-Perret-Gentil 4, 1202 Geneva, Switzerland*
ORCiD: *0009-0000-2276-4712*
laetitia.hendier@hcuge.ch

Keywords
Bacteria; Hyperbaric oxygen; Infection control; Microbiology; Pressure chamber; Safety

Abstract

(Hendier L, Soule H, Abbas M, Pittet D, Pignel R, Boet S. Evaluation of bacterial survival on inert surfaces in a hyperbaric environment. Diving and Hyperbaric Medicine. 2025 30 September;55(3):231–235. doi: 10.28920/dhm55.3.231-235. PMID: 40986918.)

Introduction: Surface cleaning and hand hygiene within hyperbaric chambers are challenging because of the risk of fire with currently used products containing alcohol or glycerine. This study aimed to investigate if hyperbaric conditions could have inhibitory effects on bacteria present on inert materials.

Methods: We deposited *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* on inert materials in an experimental chamber (Comex1200Alu) and compressed the chamber environment with air (253 kPa, 95 minutes) (referred to as indoor). The control was contaminated materials placed outside the chamber (referred to as outdoor). We chose inert materials including plastic, metal, and seat upholstery (imitation leather). We measured bacterial growth and survival and compared the groups using a Student's *t*-test.

Results: Regardless of the surface types tested, there were no significant differences in bacterial reduction between indoor and outdoor conditions for either *E. coli* or *S. aureus* and any of the materials ($P > 0.05$).

Conclusions: We found that pressurised air (253 kPa for 95 minutes) has neither proliferative nor bactericidal action on *S. aureus* and *E. coli* colonies deposited on inert surfaces compared to those present outside a hyperbaric chamber in normobaric air conditions.

Introduction

Hyperbaric oxygen therapy (HBOT) is treatment approved for multiple elective and urgent indications, including treating some infectious processes. To attain a hyperbaric condition, special compression chambers are used. There are two kinds of chambers: monoplace chambers that allow treatment of a single patient at a given time, and multiplace chamber that allow treatment of several patients simultaneously in the same chamber. Multiplace chambers are compressed with air while monoplace chambers are often compressed with oxygen.

For infection control, disinfection must be ensured between sessions for different patients to avoid nosocomial spread. In this regard, it is important to know if an environment has inherent bactericidal or bacteriostatic effect. The most important measures to reduce transmission of bacteria and the risk of healthcare associated infections are environment cleaning and hand hygiene with alcohol-based hand rubs.[1] However, many products recommended for disinfection such as alcohol-based hand rubs and soaps containing glycerine are flammable in the hyperbaric environment or are corrosive for the structure. Cleaning measures have therefore been put in place, avoiding any product likely to catch fire in the hyperbaric chamber, such as ultraviolet-C (UV-C). UV-C has germicidal properties as it is absorbed by nucleic acids in microorganisms, resulting in irreversible cell damage, and rapid cell death. There is a significant reduction in bioburden following adjunctive UV-C disinfection than with standard cleaning alone. However, this technique may fail to disinfect shadowed surfaces, and may be damaging to acrylic components of monoplace or other chambers.[2]

Previous studies have explored the effect of HBOT on bacteria in hyperbaric and hyperoxic environments relevant to monoplace chambers. High tension of oxygen may be bacteriostatic and/or bactericidal.[3] The hypothesised mechanism is that a defect in nucleic acid or energy metabolism, or both, exists in hyperoxic cells.[4] Another possible antibacterial mechanisms of HBOT could be the absence of CO_2 increasing the bactericidal effect of the oxygen alone.[5] Some facultative anaerobic and obligate aerobic bacteria are resistant to hyperoxia but not at higher pressures of oxygen e.g., 100% O_2, 304 kPa (3 atmospheres absolute [atm abs]) over a prolonged exposure (24 hours). The bactericidal activity of hyperbaric oxygen against these species is potentiated by the absence of carbon dioxide.[6] The bactericidal and bacteriostatic effects of hyperbaric oxygen are more evident as the pressure increases and are not always dependent upon which oxygen tension is applied.[7] In biological tissues, HBOT has a bactericidal or bacteriostatic effect depending on the type of bacteria.[8]

While there are several studies concerning hyperbaric hyperoxic conditions applicable to the monoplace chamber environment, there are limited data on hyperbaric air. This is relevant to multiplace chambers compressed with hyperbaric air (not oxygen). The effect of hyperbaric air on bacteria present on inert surfaces of the chamber has been overlooked. It is unclear if hyperbaric air inhibits (or enhances) bacterial growth, and what the implications are for infection control in the hyperbaric environment. This is of clinical importance because multiplace hyperbaric chambers can accommodate several patients at once, in close proximity, which may allow for contact between patients who may be infected or colonised. This poses a risk for patient-to-patient transmission of pathogens, including multidrug resistant bacteria.

This study aimed to evaluate the effect of hyperbaric air on the viability of bacteria on different materials found in multiplace chambers in the absence of specific cleaning measures. We hypothesised that hyperbaric air has an inhibitory effect.

Methods

We evaluated the survival of two facultative anaerobic bacteria deposited on inert surfaces, *Staphylococcus aureus* and *Escherichia coli.* They were exposed to hyperbaric air for 95 minutes (a typical duration of hyperbaric oxygen therapy session).

The method used was inspired by the phase 2 / step 2 application standard NF EN 14561. In this bactericidal standard simulating real conditions, inoculated sheets artificially contaminated with bacteria are exposed to the disinfectant being tested.[9] In this work, the inoculated sheets were not exposed to a disinfectant, but to particular atmospheric conditions in order to study their effect on the survival of the bacteria. Ethics approval was not necessary because we did not use a chamber using for patients and the bacteria came from a collection.

CONTAMINATED TILE

We took three materials often found in clinical hyperbaric chambers: plastic flooring, metal and imitation leather seat coverings. Pieces of about 1 cm^2 were cut and autoclaved. Six to eight sterile Petri dishes, each containing a sprout carrier of each material, were prepared for each series of tests.

PREPARATION OF THE INTERFERING SUBSTANCE SOLUTION

The interfering substance (organic material that may inhibit antibacterial action) used was bovine albumin fraction V for biochemistry (SIGMA). A dilution of albumin with sterile water was performed to obtain a solution at a concentration of 0.6 $g{\cdot}L^{-1}$. This solution was filtered through a 0.45 µm syringe microfilter and stored in the refrigerator until used.

PREPARATION OF CONTAMINATION SUSPENSIONS

The reference strains used to perform the experiment were *E. coli* K12 NCTC 10536 and *S. aureus* NC 10788. Pure cultures of each of the two bacteria were prepared on tryptone-soy agar (TSA) (Biomérieux) for 18–24 h at 36 ± 1°C. Colonies collected on a second subculture were diluted in 100 mL of tryptone-soy broth (TSB) (Biomérieux) and incubated for 18–24 h at 36 ± 1°C. Finally, those cultures were diluted with sterile water to constitute homogeneous bacterial suspensions containing approximately 10^8 colonies forming units (CFU)$\cdot mL^{-1}$.

For each species, the bacterial test suspension used for the contamination of the inert surfaces was prepared by mixing this suspension with the interfering substance solution in equal parts, in order to obtain a final albumin concentration of 0.3 $g{\cdot}L^{-1}$. To accurately estimate bacterial counts within each suspension, serial dilutions were prepared and a 1 mL sample of each of the dilutions was spread over the surface of TSA for *E. coli* and ChromID *S. aureus* (Biomérieux) for *S. aureus*. These were incubated at 36 ± 1°C for 48 h. Bacterial CFU were quantified by visual inspection, adjusted for the corresponding dilution factor, and converted to $\log_{10}$.

The test suspensions were used within one hour of their preparation.

CONTAMINATION OF THE INOCULATED SHEETS

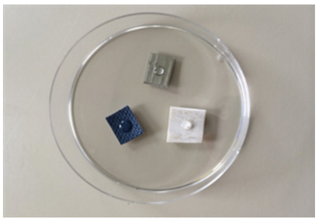
For each test, one of the two bacteria tested was used. Each of the three inoculated sheets was contaminated with 20 µl of the contamination suspensions, corresponding to approximately 10^6 CFU (Figure 1). They were allowed to dry at room temperature for 90 minutes before the actual test.

HYPERBARIC CHAMBER

The experimental hyperbaric chamber used was a Comex 1200Alu which is 1 m long and has a diameter of 1.2 m. It is

Figure 1

Three germ-supports: imitation leather metal, plastic, placed in a sterile Petri dish



equipped with pressure, temperature, and humidity sensors. Although not a clinical chamber, the tests reproduced typical pressurised air conditions of a multiplace HBOT session.

TEST METHOD

Three different inoculated sheets previously contaminated with one of the two contamination suspensions were placed in two sterile dishes. One dish was introduced inside the hyperbaric chamber, and the other one was left outside, close to the chamber.

The inoculated sheets were exposed for 95 minutes to hyperbaric air. Specifically, the hyperbaric exposure involved compressing the chamber with air to 253 kPa over 15 minutes, maintenance at 253 kPa for 65 minutes, followed by decompression to surface pressure over 15 minutes. Outside the chamber, the inoculated sheets remained for 95 minutes in normobaric air (101 kPa). At pressure, the internal chamber temperature was 24°C, 2°C higher than the external temperature. The humidity was 12% inside compared to 40% outside. A single compression cycle was performed for each bacteria tested. Once the cycle was completed, each inoculated sheet was immediately introduced into a vial containing 100 mL of tryptone soy mixture (Biomérieux) using sterile forceps. Each test was repeated ten times with each bacteria.

ENUMERATION OF RESIDUAL BACTERIA AFTER THE TEST (FINAL VALUE)

The vials were stored in a refrigerator. The analysis began immediately. Each vial was vigorously shaken for three minutes to remove the residual bacteria from their inoculated sheets. Three agar plates (TSA for tests with *E. coli*, and ChromID *S. aureus* for tests with *S. aureus)* were inoculated on the surface from each vial: one with 1 mL of undiluted liquid and the other two with 10^{-1} and 10^{-2} dilutions respectively. The agar plates were incubated at 36 ± 1°C. After 48 h, bacterial CFU were quantified by visual inspection, adjusted for the corresponding dilution factor and the total initial volume of broth used to recover bacteria from the inoculated sheets, and converted to $\log_{10}$.

STATISTICAL CALCULATION

For each inoculated sheet, the difference between the initial contamination, close to 10^6 CFU, and the final value was calculated. This is the logarithmic reduction expressed in $\log_{10}$. The average logarithmic reductions between inside and outside the chamber were compared, for each surface material and each bacteria. This difference was assessed using Student's *t*-test to evaluate whether the difference was statistically significant. A *P*-value of less than 0.05 was considered statistically significant.

Results

For each of the three surfaces (metal, seat coverings and plastic floor covering) and for each of the two bacteria tested, we obtained ten results under indoor conditions (i.e., within the hyperbaric chamber) and ten results under outdoor conditions (i.e., outside the chamber). The results are summarised in Table 1. Although the average initial logarithmic contamination of *E. coli* (6.46 CFU·mL^{-1}) was higher than for *S. aureus* (6.12 CFU·mL^{-1}) this was of no functional relevance to the experiment.

Both bacteria on all surfaces showed a reduction in CFU over the course of both inside and outside exposures, but there were no significant differences for either bacterial species between inside (hyperbaric) and outside (normobaric) environments. We showed a lower logarithmic reduction for *S. aureus* than for *E. coli* both inside and outside the chamber.

Discussion

This *in vitro* study assessed bacterial survival in hyperbaric and normobaric air environments and found that there was no difference in survival of *E. coli* and *S. aureus*. The hyperbaric air environment did not lead to a bacterial multiplication or bactericidal effect on either of these bacteria colonising inert materials, even though the membrane structure from the bacteria Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) are different. In contrast, a previous study showed that gram-negative bacteria persisted longer than gram-positive bacteria in normobaric air conditions. This may be related to the humidity. Indeed, humid conditions have been showed to improve persistence for most types of bacteria including *E. coli* while *S. aureus* was found to persist longer at low humidity level.[10]

Our study evaluated bacterial survival on inert surfaces in the context of increased pressure. The results obtained are difficult to compare with other studies evaluating the effect of hyperbaric conditions on bacterial survival which focused on the effect of hyperbaric oxygen exposure on bacteria cultured in nutrient media. For example, in the study by Masure et al., the survival of bacteria in a liquid nutrient medium was assessed. The study described a bacteriostatic effect of

Table 1

Mean and standard deviation (SD) initial contamination and mean (SD) reduction inside and outside the hyperbaric chamber reported as $\log_{10}$ colony forming units per mL ($CFU \cdot mL^{-1}$) for different bacteria and germ support; *P*-values are for comparisons between inside the chamber (hyperbaric air) and outside the chamber (normobaric air)

Surface	Inside the chamber		Outside the chamber		*P*-value
	Mean initial contamination ($CFU \cdot mL^{-1} \log_{10}$)	Mean reduction ($CFU \cdot mL^{-1} \log_{10}$)	Mean initial contamination ($CFU \cdot mL^{-1} \log_{10}$)	Mean reduction ($CFU \cdot mL^{-1} \log_{10}$)	
Bacteria 1: *Staphylococcus aureus*					
Metal (aluminium)	6.12 (0.12)	0.57 (0.18)	6.12 (0.12)	0.54 (0.23)	0.37
Seat (Imitation leather)	6.12 (0.12)	1.96 (0.56)	6.12 (0.12)	2.28 (0.37)	0.08
Floor (plastic)	6.12 (0.12)	1.31 (0.48)	6.12 (0.12)	1.39 (0.40)	0.34
Bacteria 2: *Escherichia coli*					
Metal (aluminium)	6.46 (0.11)	1.60 (0.21)	6.46 (0.11)	1.56 (0.19)	0.33
Seat (Imitation leather)	6.46 (0.11)	4.30 (0.27)	6.46 (0.11)	4.22 (0.41)	0.39
Floor (plastic)	6.46 (0.11)	2.46 (0.31)	6.46 (0.11)	2.57 (0.32)	0.22

hyperbaric oxygen for *E. coli* at 304 kPa (3 atm abs) and for *S. aureus* at 507 kPa (5 atm abs) for 24 hours.[8] We did not see this effect on inert surfaces with hyperbaric air exposure.

The average $\log_{10}$ reduction for *E. coli* on the imitation leather (seat) surface was higher inside and outside the chamber (4.30 and 4.22 $CFU \cdot mL^{-1}$ respectively) than on the metal surface (1.60 and 1.56 $CFU \cdot mL^{-1}$ respectively). These results for the different surfaces reveals the hypothesis, in a fortuitous way, that there is a difference in the adhesion of bacteria to materials. Bacteria deposited on the seat cover (imitation leather) seemed to adhere more strongly than those deposited on metal surfaces, regardless of the bacteria tested. Although the agitation time of the inoculated sheets in TSB bottles was the same for each bottle, fewer bacteria were collected from the seat cover than from the metal. Bacteria may have preferential attachment to certain materials such as imitation leather. This suggests that cleaning efforts may need to be adapted depending on the nature of the material.

Cleaning measures have been put in place avoiding any product likely to catch fire in the hyperbaric chamber with the introduction of ultraviolet C (UV-C) devices. A recent study was conducted in the context of the COVID-19 pandemic, focusing on the challenges of disinfecting the chamber and discussing the quarantine of patients or the closure of the hyperbaric centre under these conditions. Ultimately, it was demonstrated that, despite the varying conditions during the different phases of the pandemic, it was possible to ensure at least partial operation of the workplace by integrating UVC radiation into each disinfection procedure.[11] Another study evaluated the effectiveness of two UV-C devices in eradicating multidrug-resistant bacteria (*Clostridioides difficile* and methicillin-resistant *Staphylococcus aureus*). These bacteria were suspended and then inoculated onto sheets, which were placed in the hyperbaric chamber, while control plates were placed outside the chamber during the UV-C disinfection. This technique reduces contamination of high-touch clinical surfaces, but more studies are needed to test the comparative efficacy of UV-C devices in real-world clinical environments.[12]

Conclusions

The results of this *in vitro* study showed that the survival of bacteria *S. aureus* and *E. coli* on inert materials inside (hyperbaric air environment) and outside the chamber (normobaric air environment) were not different. From the point of view of the material used, a more pronounced adherence for the imitation leather (seat coverings) may be a key factor to consider. Therefore, a precautionary approach, particularly through the increased use of personal protective equipment such as gloves when patients are subject to contact isolation precautions for example, appears justified to reduce the risk of cross-contamination in multiplace hyperbaric chambers. Further studies on different bacterial species, the effect of several compressions on bacteria are required to broaden our knowledge of bacterial survival in hyperbaric chambers in order to better prevent infectious risks for patients. Moreover, to apply this study in clinical condition, it would be interesting to test bacterial growth after the disinfection of the chamber with our clinical disinfection protocol.

References

1 Huart D, Henckes A, Cochard G, Zahar JR, Baron R, Saliou P. Infection control in therapeutic hyperbaric chambers: Practical inventory in France. Infect Control Hosp Epidemiol. 2015;36:1375–7. doi: 10.1017/ice.2015.175. PMID: 26189343.

2 Browne K, Wood D, Clezy K, Lehm J, Walsh WR. Reduction of bacterial load with the addition of ultraviolet-C disinfection inside the hyperbaric chamber. Diving Hyperb Med. 2020;50:332–7. doi: 10.28920/dhm50.4.332-337. PMID: 33325012. PMCID: PMC8026220.

3 Mcallister TA, Stark JM, Norman JN, Ross RM. Inhibitory effects of hyperbaric oxygen on bacteria and fungi. Lancet. 1963;282:1040–2. doi: 10.1016/s0140-6736(63)90011-4. PMID: 14062728.

4 Clark JM, Pakman LM. Inhibition of Pseudomonas aeruginosa by hyperbaric oxygen. II. Ultrastructural changes. Infect Immun. 1971;4:488–91. doi: 10.1128/iai.4.4.488-491.1971. PMID: 5005305. PMCID: PMC416336.

5 Bornside GH, Pakman LM, Ordóñez AA. Inhibition of pathogenic enteric bacteria by hyperbaric oxygen: enhanced antibacterial activity in the absence of carbon dioxide. Antimicrob Agents Chemother. 1975;7:682–7. doi: 10.1128/AAC.7.5.682. PMID: 238466. PMCID: PMC429203.

6 Park MK, Myers RA, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. Clin Infect Dis. 1992;14:720–40. doi: 10.1093/clinids/14.3.720. PMID: 1562664.

7 Zanon V, Rossi L, Castellani E, Camporesi EM, Palù G, Bosco G. Oxybiotest project: microorganisms under pressure. Hyperbaric oxygen (HBO) and simple pressure interaction on selected bacteria. Med Gas Res. 2012;2:24. doi: 10.1186/2045-9912-2-24. PMID: 22963601. PMCID: PMC3517458.

8 Masure O, Le Foll C, Barthelemy L, Michaud A, Chastel C. Effets de l’oxygène hyperbare sur le développement in vitro de quatre bactéries pathogènes: E.coli, P.aeruginosa, S.aureus, et C.perfringens. Médecine et Maladies infectieuses; 1977. [cited 2024 May 10]. Available from: https://www.sciencedirect.com/science/article/pii/S0399077X77800607.

9 European Committee for Standardization. European Norm 14561: Chemical disinfectants and antiseptics. Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area. Test method and requirements (phase 2/step 2). Version 2007. [cited 2023 Nov 12]. Available from: https://standards.iteh.ai/catalog/standards/cen/2e84463c-3941-49c5-a664-25f0a76dee20/en-14561-2006.

10 Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 2006;6:130. doi: 10.1186/1471-2334-6-130. PMID: 16914034. PMCID: PMC1564025.

11 Hájek M, Chmelař D, Rozložník M, Lochmanová A, Tlapák J, Vrbová T, et al. COVID-19 infection prevention and control management during hyperbaric oxygen therapy: a best practice implementation project. JBI Evid Implement. 2023;21(S1):S57–S63. doi: 10.1097/XEB.0000000000000400. PMID: 38037449.

12 Warren BG, Masker J, Brown G, Gamez I, Smith B, Anderson DJ, et al. Efficacy of UV-C disinfection in hyperbaric chambers. Infect Control Hosp Epidemiol. 2020;41:1080–3. doi: 10.1017/ice.2020.248. PMID: 32546289.

Conflicts of interest and funding: nil

Submitted: 12 July 2024
Accepted after revision: 26 June 2025