COVID-19 PREVENTION AND TREATMENT ENVIRONMENTAL SANITATION

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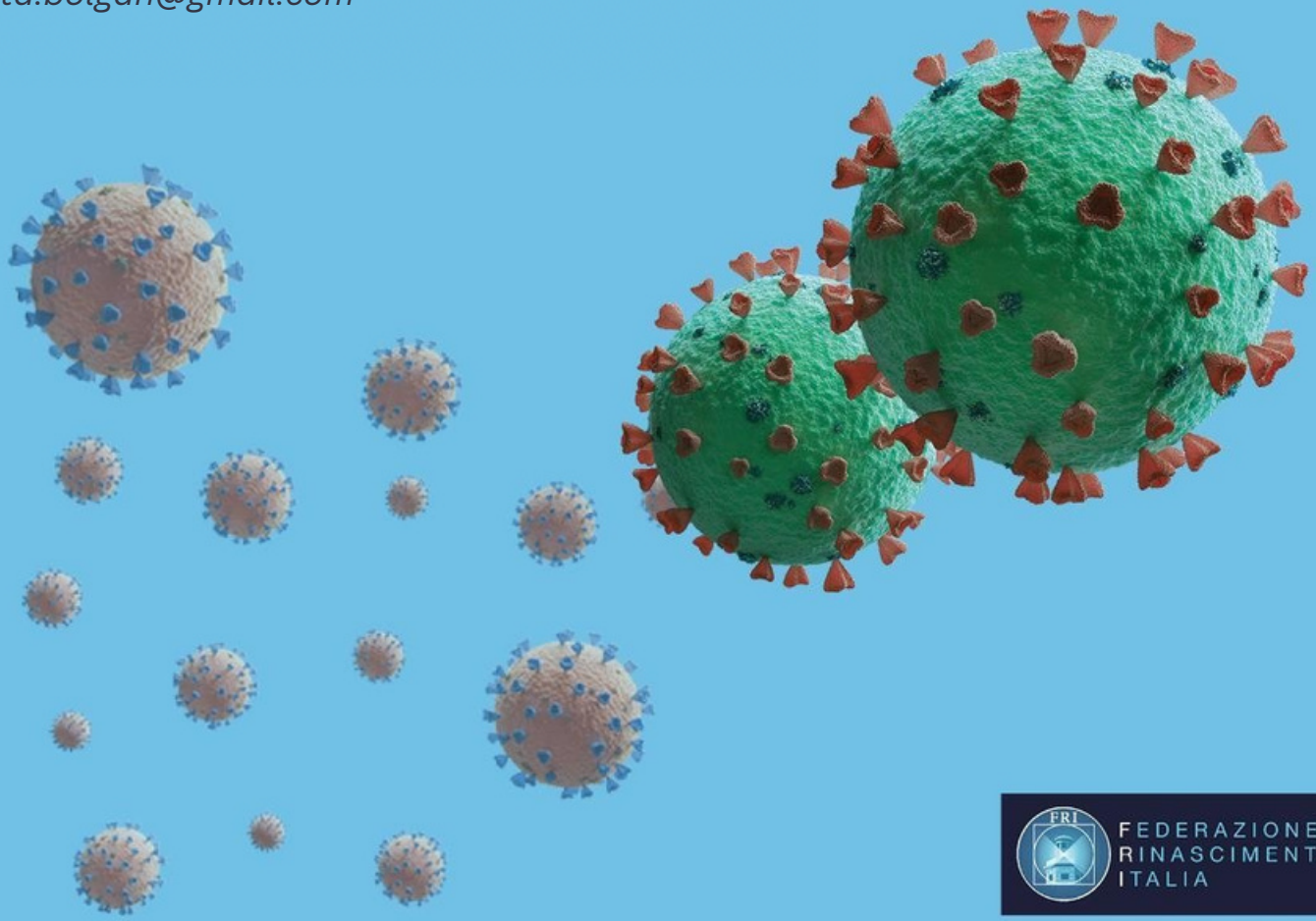

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THE STAGES OF COVID-19 DISEASE

The following phases can be distinguished during the response to SARS-Cov-2 viral infection:

Virus entry:

initiation of viral replication can cause death of infected cells, vascular leakage, and release of pro-inflammatory mediators with activation of an initial wave of inflammatory mediators.

Cytokines are proteins produced by cells in response to infection and are involved in the formation of an antiviral state as the first line of nonspecific defense and a subsequent specific response against the virus.
This process begins through the recognition of viral molecules by PRRs (pattern recognition receptors - receptors of innate immunity), which are present as transmembrane receptors or in different intracellular compartments.
After binding to the virus, the receptor (in the case of SARS-Cov-2 are TLRs 7 and 9) undergoes a structural change that activates a signaling pathway in the cytoplasm, which in turn promotes the expression of several cytokines.
In the process of inflammation, virus-infected cells produce and secrete proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF and IFN, which are involved in the early defense of the body. They can activate cells present at the site of infection and recruit leukocyte cells from the circulatory system.

Depending on the intensity of this initial inflammatory response, the infection can be Asymptomatic or symptomatic.

Symptomatic phase of viral infection:

symptoms are similar to flu-like symptoms and last about 7-10 days.
The innate immune system reacts to block virus replication.
If the person has an efficient immune response, the infection resolves without complications.

The clinical presentation of COVID-19 infection is more consistent with subacute rather than acute viral disease.
Compared with H1N1 influenza infections, in which the median incubation time is 2 days and most intensive care unit admissions occur within 24 to 48 h of admission, patients with COVID-19 infection present to the hospital with a median incubation time of 5 to 7 days and are generally admitted in

hospital for another 3-4 days before requesting admission to the intensive care unit. [1]

It is important to point out that compared with other respiratory viruses, SARS- CoV-2 infection results in a **lower antiviral response** characterized by low levels of Interferon-I (IFN-I) and IFN-III and **hyperinflammation** due to elevated expression of inflammatory mediators and IL-6.

Immunosuppression may occur at this stage**,** due to both depletion (selective elimination of T lymphocytes that have reacted against the virus) and T-cell depletion, and this may contribute to viral persistence and COVID-19 mortality.
Lymphopenia (absolute or relative decrease in the number of lymphocytes in the circulating blood) is the most consistent laboratory abnormality, and it is important to proceed with early and more urgent intervention in the presence of a low T-cell count, as patients are more vulnerable to secondary infections. [2]
This is also accompanied by marked eosinopenia (in this case, absence of eosinophils), which is associated with a poor prognosis. [3]

Thus, in a proportion of infected individuals, SARS-CoV-2 evades recognition by the immune system through suppression of antiviral mechanisms, promoting complication of the disease.

[1] Zhou F, Yu T, Du R, et al.
Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study [published correction appears in Lancet. 2020 Mar 28;395(10229):1038].
Lancet. 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270627/

[2] Front. Immunol. 11:827. (2020) doi: 10.3389/fimmu.2020.00827.
Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19).
Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, Chen L, Li M, Liu Y, Wang G, Yuan Z, Feng Z, Zhang Y, Wu Y and Chen Y
https://www.frontiersin.org/articles/10.3389/fimmu.2020.00827/full

[3] Li Q, Ding X, Xia G, et al.
Eosinopenia and elevated C-reactive protein facilitate triage of COVID-19 patients in fever clinic: A retrospective case-control study.
EClinicalMedicine. 2020;23:100375. Published 2020 May 3. doi:10.1016/j.eclinm.2020.100375
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196382/

Complication of infection:

Onset of pulmonary symptoms. The immune system overreacts to the infection, which was not contained during the first phase, with the production of high amounts of inflammatory mediators. If pharmacological intervention is not taken, the complication can rapidly progress to the most severe stage.
Inflammation of the lung is the main cause of life-threatening airway complications in the severe phase, and as could be shown recently, **diffuse intravascular pulmonary coagulation** is one of the diseases that leads to death if not treated appropriately in the
Initial. [4]

Subsequent to the immunosuppression/hyperinflammation phase, the infected cells undergo cell death and release virus particles along with intracellular components that again trigger the innate inflammatory mechanisms through their recognition by the PRRs present in/on the innate immune cells, resulting in the expression of pro-inflammatory cytokines (including IL-1β, IL-6, TNF-α, etc.), and the activation of adaptive immune cells that are thus involved in host defense.

*When adaptive immune cells (T lymphocytes play a central role in this phase) become activated, they trigger a **"second wave"** of **inflammation** (cytokine storm syndrome and its subtypes), which can be seen in COVID-19 patients who have rapid deterioration after 7-10 days of infection.*

It should be considered that mast cells are also an important source of these proinflammatory cytokines and bronchoconstrictor mediators[5] and the cause of **mast cell activation syndrome**[6] (virus-activated mast cells induce a storm

[4] Dennis McGonagle, James S O'Donnell, Kassem Sharif, Paul Emery, Charles Bridgewood
Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia
Lancet Rheumatol 2020; 2: e437-45 May 7, 2020 https://doi.org/10.1016/ S2665-9913(20)30121-1
https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30121-1/fulltext

[5] https://www.cebm.net/covid-19/mast-cell-stabilisers-leukotriene-antagonists-and-antihistamines-a-rapid-review-of-effectiveness-in-covid-19/

Mast Cell Stabilizers as a Supportive Therapy Can Contribute to Alleviate Fatal Inflammatory Responses and Severity of Pulmonary Complications in COVID-19 Infection
https://dergipark.org.tr/tr/download/article-file/1061074

[6] Afrin LB, Weinstock LB, Molderings GJ.
Covid-19 Hyperinflammation and Post-Covid-19 Illness May Be Rooted in Mast Cell Activation Syndrome

eicosanoid with massive release of histamine, prostaglandin D2 (PGD2) and leukotriene C_4 (LTC_4) inducing acute bronchoconstriction and lung inflammation), potentially associated with severe COVID-19 forms and increased risk of post-infectious pulmonary fibrosis. [7]

Very severe/fatal phase:

Sudden and rapid clinical deterioration often manifests as an unexpected worsening of symptoms (fever, dyspnea) and is correlated with increased levels of acute phase markers (ESR, PCR, ferritin), coagulopathy (elevated titers of D- dimers, intravascular coagulation) and cell lysis (CK, LDH).

In the most severe patients, clinical and laboratory parameters correlate with increased levels of proinflammatory cytokines (IL-1β, IL-1Ra, IL-6, TNF-α, and sIL2-Rα), suggestive of a cytokine storm. [8]

These manifestations are related to the attack of the body's structures by
Of the immune system.

Cytokine storm can occur due to the combination of a first-line
of defective, or delayed, IFN I-mediated defense, followed by the production of levels

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[published online ahead of print, 2020 Sep 10]. Int J Infect Dis. 2020;S1201-9712(20)30732-3. doi:10.1016/j.ijid.2020.09.016
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7529115/pdf/main.pdf

Theoharides TC, Tsilioni I, Ren H.
Recent advances in our understanding of mast cell activation - or should it be mast cell mediator disorders?
Expert Rev Clin Immunol. 2019;15(6):639-656. doi:10.1080/1744666X.2019.1596800
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7003574/

[7] Panigrahy D, Gilligan MM, Huang S, et al.
Inflammation resolution: a dual-pronged approach to averting cytokine storms in COVID-19?
Cancer Metastasis Rev. 2020;39(2):337-340. doi:10.1007/s10555-020-09889-4
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7207990/pdf/10555_2020_Article_9889.pdf

Sanchez-Gonzalez MA, Moskowitz D, Issuree PD, Yatzkan G, Rizvi SAA, Day K.
A Pathophysiological Perspective on COVID-19's Lethal Complication: From Viremia to Hypersensitivity Pneumonitis-like Immune Dysregulation.
Infect Chemother. 2020;52(3):335-344. doi:10.3947/ic.2020.52.3.335
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7533209/

[8] Clin Immunol. 2020 Apr 27;215:108448. doi: 10.1016/j.clim.2020.108448.
COVID-19: Immunology and treatment options.
Felsenstein S1, Herbert JA2, McNamara PS2, Hedrich CM3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

elevated and persistent cytokines (hypercytokinemia) IL-6, IL-1β, and TNF-α and a response
Dysfunctional T cells (usually of cytotoxicity).
This results in impaired elimination of dead or infected cells, increased viral replication and spread that further activates macrophages, and culminates in the massive release of multiple cytokines and multiorgan damage.

*During the complication, the person may go through **bacterial co-infections** (especially hospital antibiotic-resistant) that further aggravate the clinical picture.*

It should be pointed out that SARS-Cov-2 increases levels in cells of a molecule known as bradykinin causing a phenomenon that can be described as a "**bradykinin storm**."
Bradykinin induces pain and causes expansion of blood vessels and leakage of fluid leading to swelling and inflammation of the surrounding tissue. Fluid leakage into the lungs induced by the bradykinin storm, combined with the excess hyaluronic acid produced, results in the formation of a hydrogel that prevents oxygen uptake and carbon dioxide release in patients' lungs
COVID-19 serious. [9]

Severe SARS-Cov-2 infections appear to cause profound inflammation-induced coagulation abnormalities in combination with severe endothelial cell injury and subsequent massive release of von Willebrand factor and plasminogen activators.
This coagulopathy probably contributes to pulmonary microvascular thrombosis **(COVID-19-associated coagulopathy)**, bronchoalveolar fibrin deposition (which is a hallmark of adult respiratory distress syndrome), and thromboembolic complications. [10]

[9] Garvin MR, Alvarez C, Miller JI, et al.
A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm.
Elife. 2020;9:e59177. Published 2020 Jul 7. doi:10.7554/eLife.59177
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7410499/

[10] Becker RC.
COVID-19 update: Covid-19-associated coagulopathy.
J Thromb Thrombolysis. 2020;50(1):54-67. doi:10.1007/s11239-020-02134-3
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7225095/

Post-infectious phase:

it will be important to monitor patients who have overcome complications, as long-term autoimmune reactions are possible. [11]

Immune evasion strategies of SARS-Cov2

As seen above, in **epithelial cells expressing the ACE2 receptor**, suppression of early pro-inflammatory responses mediated by type I interferons (IFNs) and the cytokines IL-1, IL-6, and TNF-α hinders virus containment.
The induction of endothelial and vascular cell damage and cell death following viral replication result in strong and poorly controlled inflammatory responses, leading to tissue damage and systemic inflammation, both of which contribute to the complication of the disease.

In **tissue monocytes/macrophages,** on the other hand, a process known as antibody-dependent enhancement (**ADE**) occurs in which immune complexes consisting of low-specific antibodies against SARS-Cov-2 and viral particles can be engulfed by macrophages causing their infection. [12]
In infected macrophages, the virus instead of being processed for presentation to other cells of the immune system, on the one hand inhibits type I IFN signaling and on the other hand allows pro-inflammatory expression of IL-1, IL-6, and TNF-α, contributing to the cytokine storm syndrome and fatal potentiation of the disease.

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[11] Talotta R, Robertson E.
Autoimmunity as the comet tail of COVID-19 pandemic.
World J Clin Cases. 2020;8(17):3621-3644. doi:10.12998/wjcc.v8.i17.3621
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7479552/

[12] Wen J, Cheng Y, Ling R, et al.
Antibody-dependent enhancement of Coronavirus
[published online ahead of print, 2020 Sep 10]. Int J Infect Dis. 2020;100:483-489.
doi:10.1016/j.ijid.2020.09.015
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7483033/

Roncati L, Palmieri B.
What about the original antigenic sin of the humans versus SARS-CoV-2?
Med Hypotheses. 2020;142:109824. doi:10.1016/j.mehy.2020.109824
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204740/

Yang L, Liu S, Liu J, et al.
COVID-19: immunopathogenesis and immunotherapeutics.
Signal Transduct Target Ther. 2020;5(1):128. Published 2020 Jul 25. doi:10.1038/s41392-020-00243-2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7381863/

This mechanism occurs when non-neutralizing IgG antibodies are present at the time of infection and in sub-optimal amounts that are formed as a result of previous seasonal coronavirus infections, or following influenza vaccination. [13]

In these cases, the development of acute respiratory disease coincides with antiviral IgG seroconversion.
From the perspective of the mechanism of damage induction, **the severe/fatal complications associated with SARS-Cov-2 infection can be considered a consequence of ADE**.
The ADE explains why the elderly are at greater risk than healthy children and adults, as they possess a greater amount of nonneutralizing antibodies from coronavirus infections or older vaccinations, and have an inefficient immune system to fight infections.

Also susceptible to the enhancement of the disease are pregnant women and the Infants under one year of age, in case of reinfection.
Hyperimmune serum and IVIGs are effective in treating COVID-19 patients because the transfused antibodies are able to block viral immune complexes from entering immune system cells. [14]

ADE is a major risk factor for vaccination against COVID-19[15] *and influenza*[16] *, because of the high variability of viruses that may predispose to the production of nonneutralizing antibodies.*

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[13] Cegolon L, Pichierri J, Mastrangelo G, et al.
Hypothesis to explain the severe form of COVID-19 in Northern Italy.
BMJ Glob Health. 2020;5(6):e002564. doi:10.1136/bmjgh-2020-002564
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7295427/

[14] Rojas M, et al.
Convalescent plasma in Covid-19: Possible mechanisms of action
Autoimmun Rev. 2020;102554. doi:10.1016/j.autrev.2020.102554
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/

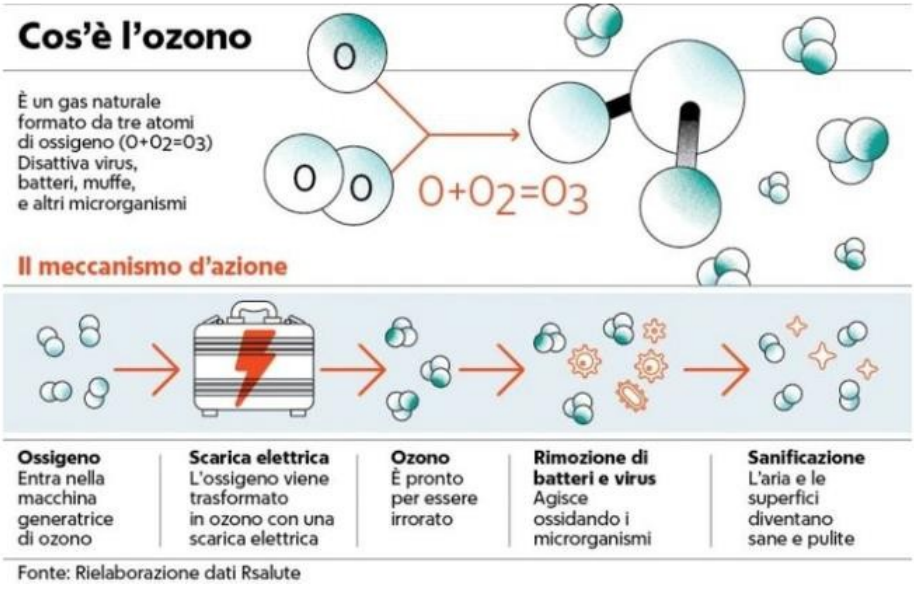
[15] Lee WS, Wheatley AK, Kent SJ, DeKosky BJ.
Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies.
Nat Microbiol. 2020 Oct;5(10):1185-1191. doi: 10.1038/s41564-020-00789-5. Epub 2020 Sep 9. PMID: 32908214.
https://www.nature.com/articles/s41564-020-00789-5

[16] Rajão DS, Chen H, Perez DR, Sandbulte MR, Gauger PC, Loving CL, Shanks GD, Vincent A.
Vaccine-associated enhanced respiratory disease is influenced by haemagglutinin and neuraminidase in whole inactivated influenza virus vaccines.

Room sanitation

With regard to sanitization, ozonation and the use of
Of fermentative microorganisms.

OZONATION



For the use of ozone for sanitizing environments, please refer to the material kindly made available by Dr. Vincenzo Simonetti, an expert in ozone therapy and AMPAS member[17] and to documents compiled by other bodies regarding its use in non-health care facilities such as

-the "ISS COVID-19 Report - No. 25/2020 Interim Recommendations on the Sanitation of non-sanitary facilities in the current COVID-19 emergency: surfaces,

J Gen Virol. 2016 Jul;97(7):1489-1499. doi: 10.1099/jgv.0.000468. Epub 2016 Mar 31. PMID: 27031847.
https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.000468#tab2

[17] CV Dr. Vincenzo Simonetti
https://www.promosalus.it/vincenzo-simonetti-curriculum/

https://rinascimentoitalia.it/wp-content/uploads/2020/10/Ozonoterapia_Dr-Simonetti.pdf

indoor environments and clothing ISS Biocides Working Group COVID-19 Version of May 15, 2020" and "Focus on: professional use of ozone also with reference to COVID-19 Version of July 23, 2020 ISS-INAIL Working Group" [18],

- Regulatory and technical considerations on sanitation services using ozone during pandemic Sars-Cov-2 (Covid19). ANID 25.05.2020 [19]

It should be noted that EPA has not listed ozone as a useful product for sanitation[20], and that ISS does not suggest it for domestic use.

The following is the scientific and technical evaluation (p.12-14 ISS Covid-19 Report) prepared in May 2020:

"The virucidal activity of ozone is rapidly expressed upon ozonation. As with many other products used in disinfection, there is no specific information on its efficacy against SARS COV- 2. In contrast, several studies are available that support its virucidal (Norovirus) efficacy in healthcare and non-healthcare settings.
Even at low concentrations, with high humidity, ozone has a high action
Virucidal disinfectant in air.
The *International* Ozone *Association* (www.iao-pag.org) confirms the effectiveness of ozone for the inactivation of many viruses although it is not aware of specific research on SARS-CoV-2.
At the industrial level, ozone is generated *in situ* using ozonators, which must be adapted from time to time in relation to spaces (size, materials involved) and *targets*.
Ozone generators must comply with the Low Voltage (Directive 2014/35/EC), Electromagnetic Compatibility (Directive 2014/30/EC) and Restriction of Hazardous Substances (RoHS) Directive 2011/65/EC.
Ozone is an unstable gas and decays spontaneously to oxygen. The time it takes for ozone to decay, depends on temperature, humidity, and chemical and biological contamination, and is always a function of usage concentrations.

[18] https://www.iss.it/documents/20126/0/Rappporto+ISS+COVID-19+n.+25_2020.pdf/90decdd1-7c29-29e4-6663-b992e1773c98?t=1589836083759
https://www.iss.it/documents/20126/0/Rapporto+ISS+COVID-19+56_2020.pdf/6be24ac7-d084-2f51-feb0-7ea0957e5781?t=1595858146906

[19] https://www.ciip-consulta.it/attachments/article/1166/anid%20ozono%20Covid19.pdf

[20] https://cfpub.epa.gov/giwiz/disinfectants/index.cfm https://www.epa.gov/coronavirus/will-ozone-generator-protect-me-and-my-family-covid-19

Under actual conditions, the natural decay time required to make the premises accessible is at least 2 hours. If possible, *it is preferable to carry out treatments at night so that when work resumes, the amount of ambient ozone is within sanitary safety limits.*
Avoid removing residual ozone by resorting to forced ventilation to convey it to the outdoor environment: the DL.vo 155/2010 also sets limit values and quality objectives for ambient air concentrations of ozone.

Based on the CLP and REACH regulations, registrants have self-classified ozone as: a substance that can cause or aggravate a fire; lethal if inhaled, causes severe skin burns and serious eye injury, causes organ damage upon prolonged or repeated inhalation exposure, very toxic to the aquatic environment with long-lasting effects.
Some notifiers identify ozone as a suspected mutagen. German competent authorities in 2016 indicated to ECHA their intention to propose harmonized classification and labeling for ozone also as a category 2 mutagen and category 2 carcinogen. [1]

The environmental risk as a result of using ozone for surface treatment appears negligible at present, given the high percentage of ozone normally present in the atmosphere.
In accordance with the HACCP standards[2] and DL.vo 81/2008, in the absence of values adopted in the Italian regulatory framework, operators must comply with the TLV -TWAs of the ACGIH[3] below, in relation to workload and cumulative duration of exposure:

- TLV - TWA (8 hours), 0.05 ppm (0.1 mg/m^3), *heavy work;*
- TLV - TWA (8 hours), 0.08 ppm (0.16 mg/ m^3), *moderate work;*
- TLV - TWA (8 hours), 0.10 ppm (0.2 $mg/m3$), *light work;*
- TLV - TWA (≤ 2 hours), 0.2 ppm (0.39 mg/m^3), *light, moderate or heavy work fractions.*

Considering that at concentrations below 2 ppm, ozone has a pleasant characteristic odor, which becomes pungent and irritating at higher levels, and which is recognizable even at very low concentrations (0.02 and 0.05 ppm), potentially exposed individuals are forewarned about reaching high and potentially health-damaging concentrations. Odor is not, however, a reliable index of the concentration present in the air due to habituation phenomena.
The WHO Guidelines for *Outdoor* Air Quality (2005) recommend a limit
daily of 100 µg/m^3 (approx. 0.05 ppm).

The *National Institute for Occupational and Safety Health* (NIOSH) indicates an IDLH (concentration immediately dangerous to life or health) value of 5 ppm (10 mg/m^3) for ozone, and concentration levels similar to the IDLH value or higher are in fact achieved under conditions of use.
In general, the practice of re-entering treated areas after a
certain period of time from the end of ozonation.
The use of ozone should be in unoccupied and properly confined environments.
To reduce the risk, visual devices can be provided at each access point of the rooms being treated, and similarly, free-access markers can be provided. Therefore, before resorting to the use of this substance for the treatment of premises, it is necessary to assess the risk of exposure of both the sanitization operators and the personnel using the sanitized premises. Operators must be trained and experienced and provided with appropriate personal protective equipment (PPE).
In light of the above, it is therefore not suitable for domestic use.

The absence of specific data with the tests provided to demonstrate efficacy as a biocidal disinfectant is common to several disinfectants on the market today, both as PMCs and as Biocides, for which no data are available on efficacy against SARS- CoV-2.
A single study to date has evaluated the effect of some disinfectants specifically on SARS-CoV-2 virus in suspension, noting that all of those assayed - household bleach (sodium hypochlorite) at various dilutions, 70 percent ethanol, 7.5 percent povidone iodine, 0.05 percent chlorhexidine, and 0.1 percent benzalkonium chloride - rendered the virus undetectable after 5 minutes of incubation. [21]

[1] The Register of Intentions (RoI), maintained by ECHA and publicly available on the Agency's website, contains information from parties wishing to submit a dossier to the Agency for harmonization of classification and labeling
(https://www.echa.europa.eu/it/web/guest/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e180dfd06a)

[2] HACCP (*Hazard Analysis and Critical Control Points*) is a system for implementing self-control
To ensure food safety at all stages of the food chain

[3] TLV - TWA (*Threshold Limit Value - Time Weighted Average*): Limit Value for prolonged exposures over time, also called Time Weighted Limit Value. It represents the time-weighted average concentration of pollutants in the air of workplaces over the entire work shift and indicates the level of exposure at which it is assumed that, in the state of current scientific knowledge, the worker

[21] Chin AWH, Chu JTS, Perera MRA, et al.
Stability of SARS-CoV-2 under different environmental conditions.
Lancet Microbe. 2020;1(1):e10. doi:10.1016/S2666-5247(20)30003-3
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7214863/

can be exposed 8 hours a day, 5 days a week, for the entire working life, without suffering from harmful health effects.

There is evidence that enveloped viruses such as coronaviruses are more susceptible than envelope-less viruses due to the interaction of ozone with the lipid layer.
A high concentration of 27.73 ppm ozone has been reported to inactivate SARS-CoV-1 in 4 min. Medium (17.82 ppm) and low (4.86 ppm) concentrations could also inactivate SARS-Cov-1 with different speed and effectiveness.
In another study, the maximum antiviral efficacy of ozone required a short period of high humidity (> 90% relative humidity) after the maximum ozone concentration (20-25 ppm) was reached. Mouse coronavirus (MCoV) on different surfaces (glass, plastic, and stainless steel) and in the presence of biological fluids was inactivated by ozone by at least 3 $\log_{10}$ in laboratory and simulated field trials. [22]

Regarding SARS-Cov-2, an important study published by Nara Medical University (Japan) should be noted. [23] The research team showed that at a 55-minute exposure for an ozone concentration of 6 ppm, inactivation of the virus in culture from 1/1000 to 1/10,000 occurred, and with a 60-minute exposure for an ozone concentration of 1 ppm, inactivation of the virus from 1/10 to 1/100 occurred.
For some years already, environmental ozonation at low concentrations, in human presence, has been used in 2 schools in Bergamo, in the offices of Multiossigen in Gorle (BG), and in several medical offices.

It follows that ozonation can also be considered an effective technique for SARS- Cov-2 with the necessary precautions described above for use. Although ISS advises against its domestic use, it should be mentioned that there are several[24] gaseous ozone and ozonated water generators available on the market. [25] Great care should be taken when evaluating the

[22] Dev Kumar G, Mishra A, Dunn L, et al.
Biocides and Novel Antimicrobial Agents for the Mitigation of Coronaviruses.
Front Microbiol. 2020;11:1351. Published 2020 Jun 23. doi:10.3389/fmicb.2020.01351
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7324725/

[23] https://www.vastfast.it/it/news/confermata-inattivazione-del-nuovo-coronavirus-tramite-ozono
[24] https://www.domoticafull.it/miglior-generatore-di-ozono/
https://www.articoitaly.com/wp-content/uploads/schedetecniche/ArticOzono.pdf

[25] https://www.sanitysystem.it/benefici-dell-acqua-ozonizzata/ http://www.abioconsulting.it/acqua-ozonizzata-cosa-e-chi-e-come-si-puo-utilizzare/#page-content

quality when choosing equipment for purchase because the pandemic has greatly increased the demands and uncontrolled products risky to health are also on the market. Please refer to the links mentioned above for technical specifications.

To conclude, the biocidal and toxic activity of ozone is summarized below.
When used properly, it is one of the most powerful oxidants in nature: bactericidal, virucidal, antifungal/antifungal (yeasts, molds/fungus, spores) and against microorganisms such as mites, with an oxidation potential (2.07) far greater than that of chlorine (1.36).

ORGANISMO	CONCENTRAZIONE	TEMPO DI ESPOSIZIONE
BATTERI *(E. Coli, Legionella, Mycobacterium, Fecal Streptococcus)*	0,23 ppm - 2,2 ppm	< 20 minuti
VIRUS *(Poliovirus type-1, Human Rotavirus, Enteric virus)*	0,2 ppm - 4,1 ppm	< 20 minuti
MUFFE *(Aspergillus Niger, vari ceppi di Penicillum, Cladosporium)*	2ppm	60 minuti
FUNGHI *(Candida Parapsilosis, Candida Tropicalis)*	0,02 ppm - 0,26 ppm	< 1,67 minuti
INSETTI *(Acarus Siro, Tyrophagus Casei, Tyrophagus Putrescientiae)*	1,5 - 2 ppm	30 minuti?

Inattivazione di batteri, virus, funghi, muffe ed insetti in seguito ad ozonizzazione

*(**Fonti:** Edelstein et al., 1982; Joret et al., 1982; Farooq and Akhlaque,1983; Harakeh and Butle, 1985; Kawamuram et al. 1986)*

If you stay for a long time in a room with too high a percentage of ozone, you could have serious symptoms and even intoxication.

WARNING: Using ozone generators found online without measuring ozone concentration could be dangerous.

EFFETTI TOSSICI dell'OZONO per l'UOMO

CONCENTRAZIONE (ppm O_3)

di odore acre e pungente (AGLIASTRO) è percepibile già alla concentrazione di **0,02 ppm**

Effetti tossici per l'UOMO

0,02-0,05	Percezione olfattiva
0,4-0,5	Bruciore alle prime vie aeree
0,8 – 1	Lacrimazione, tosse
2	Nausea, cefalea, vomito
5	Broncocostrizione Edema Polmonare
10	Letale per edema polmonare dopo 4 ore di esposizione
50	Letale dopo alcuni minuti di esposizione

PROBIOTIC HYGIENE

Antimicrobial resistance

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, and parasites change over time and no longer respond to drugs, making infections more difficult to treat and increasing the risk of spreading serious illness and death.

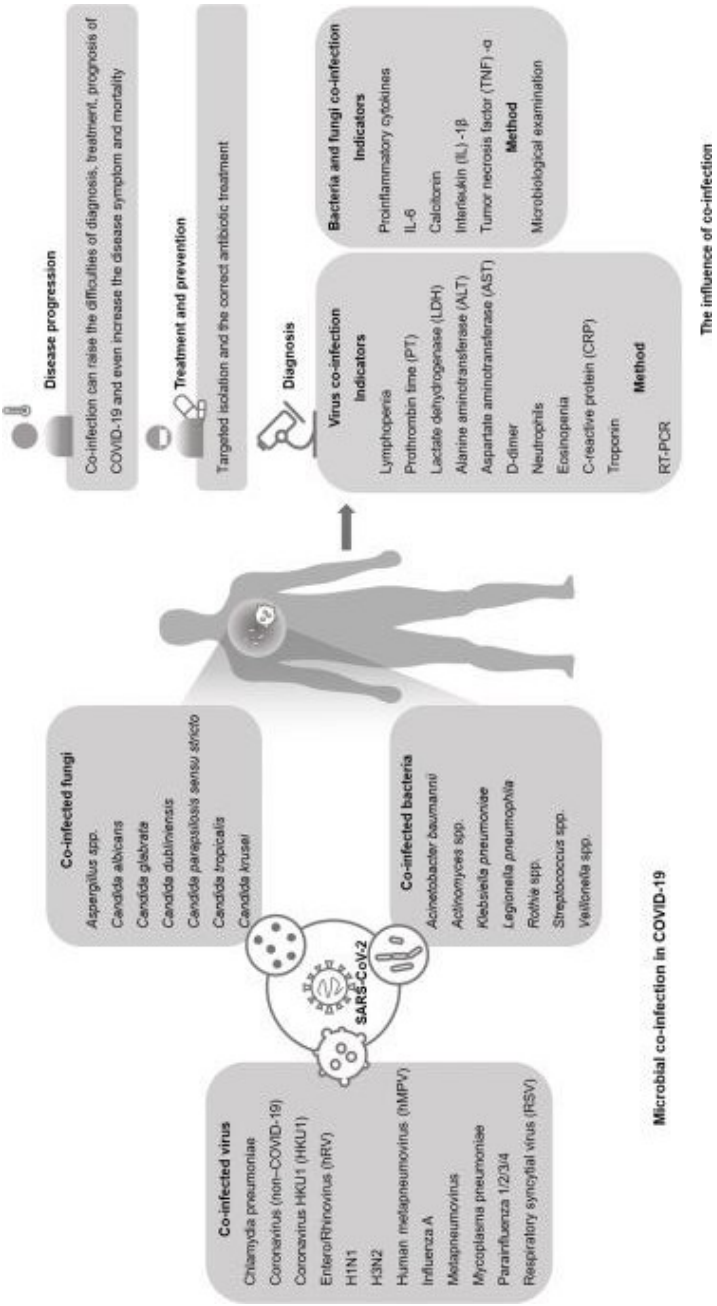
Currently, AMR has become a very serious health emergency, and WHO writes The following: [26]

- Antimicrobial resistance (AMR) is a global threat to health and development. It requires urgent multisectoral action to achieve the Sustainable Development Goals (SDGs).
- WHO has declared that antimicrobial resistance is one of the top 10 global public health threats facing humanity.
- Misuse and abuse of antimicrobials are major drivers in the development of drug-resistant pathogens.
- Lack of clean water and sanitation and inadequate infection prevention and control promote the spread of microbes, some of which may be resistant to antimicrobial treatment.
- The cost of AMR to the economy is significant. In addition to death and disability, prolonged illnesses result in longer hospital stays, the need for more expensive medications, and financial challenges for those affected.
- Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, is most at risk.

Bacterial infections treated unsuccessfully due to antimicrobial resistance cause at least 700,000 lives a year worldwide and are expected to be associated with the deaths of 10 million people a year by 2050. [27]

[26] https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

[27] https://amr-review.org/sites/default/files/AMR Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf


Disease progression
Co-infection can raise the difficulties of diagnosis, treatment, prognosis of COVID-19 and even increase the disease symptom and mortality
Treatment and prevention
Targeted isolation and the correct antibiotic treatment
Diagnosis
Virus co-infection
Indicators
Lymphopenia
Prothrombin time (PT)
Lactate dehydrogenase (LDH)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
D-dimer
Neutrophils
Eosinopenia
C-reactive protein (CRP)
Troponin
Method
RT-PCR
Bacteria and fungi co-infection
Indicators
Proinflammatory cytokines
IL-6
Calcitonin
Interleukin (IL) -1β
Tumor necrosis factor (TNF) -α
Method
Microbiological examination
The influence of co-infection
Co-infected fungi
Aspergillus spp.
Candida albicans
Candida glabrata
Candida dubliniensis
Candida parapsilosis sensu stricto
Candida tropicalis
Candida krusei
Co-infected bacteria
Acinetobacter baumannii
Actinomyces spp.
Klebsiella pneumoniae
Legionella pneumophila
Rothia spp.
Streptococcus spp.
Veillonella spp.
SARS-CoV-2
Co-infected virus
Chlamydia pneumoniae
Coronavirus (non-COVID-19)
Coronavirus HKU1 (HKU1)
EnteroRhinovirus (hRV)
H1N1
H3N2
Human metapneumovirus (hMPV)
Influenza A
Metapneumovirus
Mycoplasma pneumoniae
Parainfluenza 1/2/3/4
Respiratory syncytial virus (RSV)
Microbial co-infection in COVID-19

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7417782/

Studies in Italian hospitals show an incidence of 5-10% with a mortality rate of up to 20-30%. [28]

Although antibiotics are ineffective for the treatment of COVID-19, they are prescribed to patients with suspected or documented COVID-19 for a variety of reasons. This includes the difficulty in ruling out bacterial coinfection at presentation, but also the possibility of secondary bacterial infection during the course of the disease.
A recent meta-analysis showed that in patients with COVID-19, coinfection was found in 3.5% of cases (95% CI: 0.4-6.7%) and secondary infection in 14.3% (95% CI: 9.6-18.9%). Overall, reported bacterial infection was 6.9% (95% CI 4.3-9.5%) but varied slightly by patient population, ranging from 5.9% in hospitalized patients to 8.1% in critically ill patients. [29] Superinfections of antibiotic-resistant bacteria occur in 1.3% of ICU patients and 0% in non-ICU patients. [30]

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[28] http://www.salute.gov.it/imgs/C_17_pubblicazioni_2791_allegato.pdf

Cassini A, Högberg LD, Plachouras D, et al.
Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis.
Lancet Infect Dis. 2019;19(1):56-66. doi:10.1016/S1473-3099(18)30605-4
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6300481/

[29] Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, Soucy JR, Daneman N.
Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis.
Clin Microbiol Infect. 2020 Jul 22:S1198-743X(20)30423-7. doi: 10.1016/j.cmi.2020.07.016. epub ahead of print. PMID: 32711058.
https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(20)30423-7/fulltext

Chen X, Liao B, Cheng L, et al.
The microbial coinfection in COVID-19.
Appl Microbiol Biotechnol. 2020;104(18):7777-7785. doi:10.1007/s00253-020-10814-6
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7417782/

[30] Fattorini L, Creti R, Palma C, Pantosti A;
Unit of Antibiotic Resistance and Special Pathogens; Unit of Antibiotic Resistance and Special Pathogens of the Department of Infectious Diseases, Istituto Superiore di Sanità, Rome.
Bacterial coinfections in COVID-19: an underestimated adversary.
Ann Ist Super Sanita. 2020 Jul-Sep;56(3):359-364. doi: 10.4415/ANN_20_03_14. PMID: 32959802.
https://pubmed.ncbi.nlm.nih.gov/32959802/

A study done in Wuhan reported secondary infection in 50% of the deceased and only 1% of the survivors. [31]
A more recent study carried out on 19 patients admitted to the ICU for about 15 days, treated by invasive ventilation, with a mean age of about 67 years, and with multiple diseases, reported more serious results: all patients were found positive for antibiotic-resistant bacterial infections (90% *Acinetobacter baumannii* and 10% *Staphylococcus aureus)*, and of these patients 95% died. [32] It should be remembered that one of the causes of patient deaths in ICUs is ventilation-associated pneumonia, which has also been described for COVID-19 cases, and infection with drug-resistant pathogens increases the incidence of mortality. [33]

It follows that despite an overall low rate of bacterial infections (except for patients ventilated in ICUs for a long period), more than 70% of patients received antibiotics, with the majority consisting of broad-spectrum agents such as fluoroquinolones and third-generation cephalosporins, and this approach raises concerns about overuse of antibiotics and the subsequent harm associated with bacterial resistance. [27]

In addition, it is well known that hospital surfaces are contaminated with many microorganisms that can contribute to the transmission of care-related infections (HAIs), and that control of surface contamination is achieved by conventional chemical-based sanitization, which, however, is limited by the fact that it cannot prevent recontamination (for most disinfectants within 60 min)[34] , has a high environmental impact, and can contribute to the

[31] Zhou F, Yu T, Du R, et al.
Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study
[published correction appears in Lancet. 2020 Mar 28;395(10229):1038] [published correction appears in Lancet. 2020 Mar 28;395(10229):1038]. Lancet. 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270627/

[32] Sharifipour E, Shams S, Esmkhani M, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis. 2020;20(1):646. Published 2020 Sep 1. doi:10.1186/s12879-020-05374-z
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7461753/

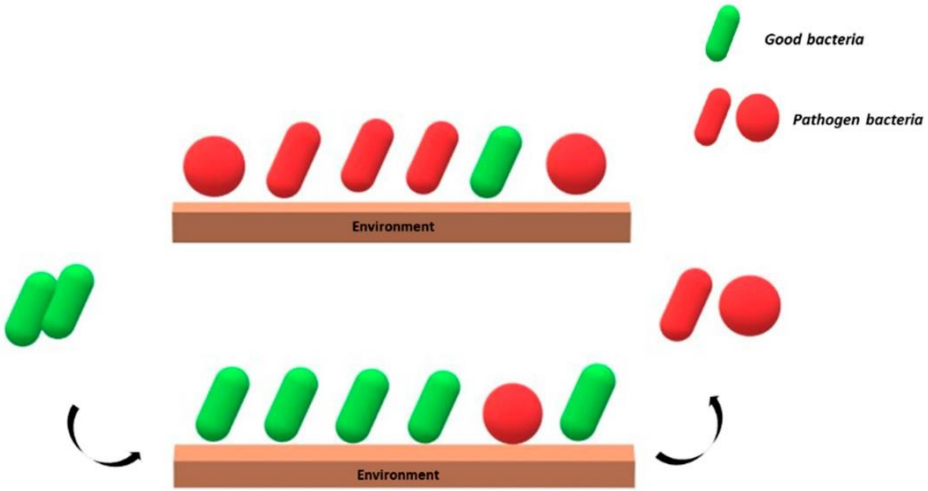
[33] Póvoa HCC, Chianca GC, Iorio NLPP. COVID-19: An Alert to Ventilator-Associated Bacterial Pneumonia. Infect Dis Ther. 2020;9(3):417-420. doi:10.1007/s40121-020-00306-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7260454/

[34] Hota B.

selection of pathogens resistant to the same disinfectants[35] and antibiotics and thus also to the occurrence of drug-resistant pathogens associated with HAIs. [36]

The Probiotic Cleaning Hygiene System (Probiotic Hygiene).

Recently, the concept of the "health" of hospital surfaces has been rethought using the approach for the health of the human body, where it is known that rather than eliminating all microbes, it is more effective for the maintenance of health to use beneficial microbes that can displace and replace pathogenic ones, by the well-known mechanism of competitive antagonism.



Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection?
Clin Infect Dis. 2004;39(8):1182-1189. doi:10.1086/424667
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7107941/

[35] Wand ME, Bock LJ, Bonney LC, Sutton JM.
Mechanisms of Increased Resistance to Chlorhexidine and Cross-Resistance to Colistin following Exposure of Klebsiella pneumoniae Clinical Isolates to Chlorhexidine.
Antimicrob Agents Chemother. 2016;61(1):e01162-16. Published 2016 Dec 27. doi:10.1128/AAC.01162-16
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5192135/

[36] Neubeiser A, Bonsignore M, Tafelski S, Alefelder C, Schwegmann K, Rüden H, Geffers C, Nachtigall I.
Mortality attributable to hospital acquired infections with multidrug-resistant bacteria in a large group of German hospitals.
J Infect Public Health. 2020 Feb;13(2):204-210. doi: 10.1016/j.jiph.2019.07.025. epub 2019 Aug 13. PMID: 31420314.
https://www.sciencedirect.com/science/article/pii/S1876034119302606?via%3Dihub
https://www.mdpi.com/1422-0067/20/7/1535/htm

Schematic representation of the "Bygiene" principle (two-way hygiene). The good (green) bacteria introduced from the outside counter the colonization of the environment by potential pathogens (red).

In this direction, a sanitization approach based on detergents containing spores of probiotic bacteria of the genus Bacillus (Probiotic Cleaning Hygiene System, PCHS) has been studied, showing that it is safe for hospitalized patients and can stably decrease resistant surface pathogens by up to 99.9% compared with conventional disinfectants without increasing sanitization costs. [37]

The University of Ferrara has conducted studies in multiple hospitals to test the efficacy of PCHS, and the study published in *Infect Drug Resist in* 2019 carried out in the internal medicine wards of 6 Italian public hospitals for 18 months demonstrated that probiotic bacilli can compete with and replace pre-existing pathogens, limiting the colonization and spread of new potentially pathogenic and drug-resistant microorganisms. In particular, PCHS was able to reduce and remodel environmental contamination by competitive antagonism, inducing a significant decrease (-83%) in the overall surface pathogen load and resistance genes present in the surface microbiota (up to 2 Log). [38]

[37] https://pchs.it/video/
https://www.camera.it/application/xmanager/projects/leg18/attachments/upload_file_doc_acquisiti/pdfs/000/002/000/Professoressa_Caselli_04.07.2019_.docx

[38] https://pchs.it/en/our-researches/publications/

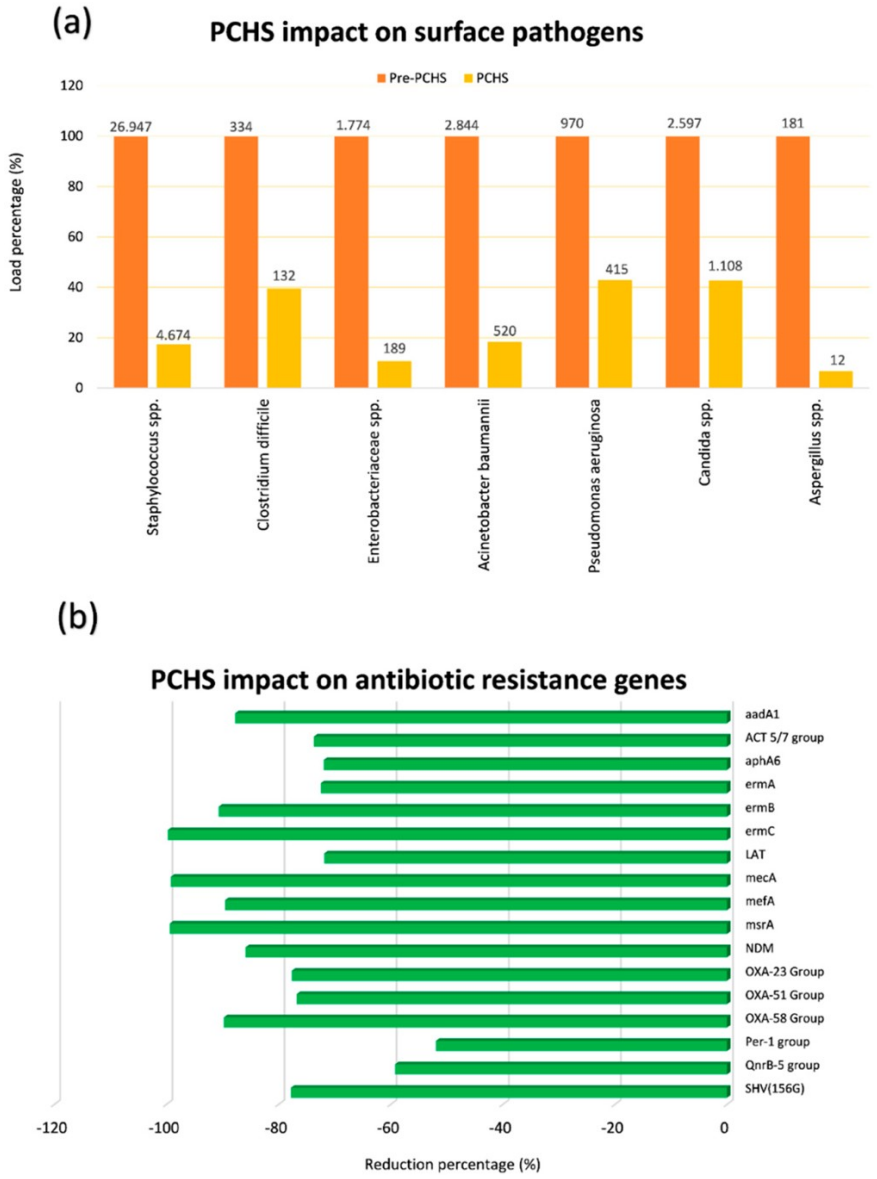
Caselli E, Brusaferro S, Coccagna M, et al.
Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: A multicenter, prospective, intervention study.
PLoS One. 2018;13(7):e0199616. Published 2018 Jul 12. doi:10.1371/journal.pone.0199616
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6042698/
REDUCING THE INCIDENCE OF CARE-RELATED INFECTIONS USING A PROBIOTIC-BASED SANITATION SYSTEM: RESULTS OF A MULTICENTER PROSPECTIVE INTERVENTION STUDY
https://pchs.it/wp-content/uploads/2020/08/2019_PubbicazioneScientifica_PlosOne_CIAS.pdf

Caselli E, Arnoldo L, Rognoni C, et al.
Impact of a probiotic-based hospital sanitation on antimicrobial resistance and HAI-associated antimicrobial consumption and costs: a multicenter study. Infect Drug Resist. 2019;12:501-510. Published 2019 Feb 27. doi:10.2147/IDR.S194670
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6398408/

Tarricone R, Rognoni C, Arnoldo L, Mazzacane S, Caselli E.
A Probiotic-Based Sanitation System for the Reduction of Healthcare Associated Infections and Antimicrobial Resistances: A Budget Impact Analysis.

Pathogens. 2020;9(6):502. Published 2020 Jun 23. doi:10.3390/pathogens9060502
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7350316/

D'Accolti M, Soffritti I, Mazzacane S, Caselli E.
Fighting AMR in the Healthcare Environment: Microbiome-Based Sanitation Approaches and Monitoring Tools.
Int J Mol Sci. 2019;20(7):1535. Published 2019 Mar 27. doi:10.3390/ijms20071535
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6479322/



https://www.mdpi.com/1422-0067/20/7/1535/htm

Effect of Probiotic Cleaning Hygiene System (PCHS) on microbial contamination and its antimicrobial resistance (AMR) characteristics on hospital surfaces. (a) Six pathogens associated with health care-associated infections (HAIs) were measured by Colony Forming Unit (CFU) counts on hospital surfaces in five Italian hospitals before and after the introduction of PCHS; the load

microbial is expressed as a percentage for each individual pathogen analyzed (average values per m^2) are also displayed; (b) the resistome of the contaminating microbial population was analyzed by qPCR microarray before and after the introduction of PCHS; the most prevalent antibiotic resistance genes are reported and expressed as a percentage reduction of genes during the PCHS phase compared with the pre-PCHS phase.

The effective microorganisms

This approach valid for hospital facilities, where the bacterial load is significant, can also be employed for home cleaning and garment sanitization with the use of commercial products readily available on the market, developed by Dr. Teruo Higa, based on fermentative microorganisms also known as Effective Microorganisms or EM, which will be discussed further below in the section on probiotics.

The study of EM began in the 1980s, in Japan, with the production of mixtures of beneficial microorganisms from high-value soils to treat contaminated environments and improve soil fertility.
These mixtures contain a great diversity of probiotic microorganisms, normally found in nature, belonging mainly to the groups of lactobacilli, yeasts and photosynthesis bacteria that coexist in symbiosis in liquid solution.
The great variety present in this mixture makes it extremely versatile and suitable for rebalancing environments by enabling the recovery of biological mechanisms to counteract chemical and biological contamination. [39]
For an in-depth discussion of the composition of EMs and how to use them in agriculture, human and animal health, and sanitation refer to dealers' sites.[40]

Antiviral effects and use to counter SARS-Cov-2 infection

The antiviral effects of EM mixtures were observed as early as 2009 during swine flu, in 2011 on foot-and-mouth disease in Korea and H5N1 avian influenza virus, in 2013 on herpes simplex, and in 2014 positive results were obtained with

[39] https://embio.it/media/wysiwyg/microrganismi-efficaci/Sanificazione_con_EM.pdf

[40] The list of retailers may not be complete
https://www.pulitisenzachimica.com/articoli/approfondimenti-sugli-em/
https://em-ita.com/pages/cosa-sono-gli-em
http://www.italiaem.it/Applicazioni_EM/pulizia_casa.html
http://microrganismi-efficaci.it/media/wysiwyg/microrganismi-efficaci/EM_in_casa.pdf
https://emipiace.it/cose-em-la-scoperta/
https://emagea.it/casa-ambiente-pipes/

EMs in the control of influenza viruses, reported in the proceedings of a conference of the Japanese Society for Virology.[41]

As emerged from the proceedings of the conference "*Actions to Combat ICA and Antibiotic Resistance in the COVID-19 Emergency,*" held at the Catholic University of the Sacred Heart, Rome campus on Sept. 24,[42] for experts the COVID-19 emergency, with the massive use in recent months of antibiotics and sanitizers, will have a devastating impact on the phenomena of antibiotic resistance. According to Prof. Caselli, the use of biological sanitization over 5 years would prevent about 31,000 hospital infections and save at least 14 million euros (11 of which would be for the treatment of antibiotic-resistant infections), and the next frontier, to make this mode of sanitization increasingly efficient, is the use of lytic bacteriophages (Bacteriophages, or phages, are viruses that infect bacteria, commonly causing the death of the host bacterial cell). [43]

This important sanitization approach was used in March 2020 in Korea at the Busan Gijang-Gun Agricultural Technology Center to prevent the spread of new SARS-CoV-2 infections.44 The antiviral efficacy is due to the presence of lactobacilli and citric acid that keep the EM mixture produced by the center at a

[41] Sairenji, Fujii, Higa et al.
Influenza virus inactivation by effective micro-organisms fermented liquid (EM1)
62nd Annual Meeting of the Japanese Society for Virology, Yokohama , 2014.

[42]https://www.policlinicogemelli.it/en/news-events/lotta-alle-infezioni-ospedaliere-e-allantibiotico-resistance-the-recipe-of-the-gems/

https://embio.it/news/video-microrganismi-fagi-igiene-caselli/
https://youtu.be/CPTGYi5I4d0

[43] D'Accolti M, Soffritti I, Piffanelli M, Bisi M, Mazzacane S, Caselli E.
Efficient removal of hospital pathogens from hard surfaces by a combined use of bacteriophages and probiotics: potential as sanitizing agents
[published correction appears in Infect Drug Resist. 2018 Sep 20;11:1521]. Infect Drug Resist. 2018;11:1015-1026. Published 2018 Jul 30. doi:10.2147/IDR.S170071
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6071622/

D'Accolti M, Soffritti I, Lanzoni L, et al.
Effective elimination of Staphylococcal contamination from hospital surfaces by a bacteriophage-probiotic sanitation strategy: a monocentric study.
Microb Biotechnol. 2019;12(4):742-751. doi:10.1111/1751-7915.13415
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6559196/
[44] https://microrganismi-efficaci.it/news/cat/em/post/corea-em-prevenzione-coronavirus/

pH of 3.5. In fact, both the strongly acidic pH 45 and the lactobacilli 46 completely inhibit virus replication.
Gwanak District in Seoul, South Korea, also offered an experimental provision of disinfection in late March to 200 households with cohabitants aged 65 years and older, registered as disabled and located in the quarantined area. Disinfection during quarantine was conducted using alcohol and Effective Microorganisms (EM), certified by the Ministry of Health and Welfare and the Environment Agency (in South Korea). 47
To reinforce the scientific validity of this sanitization approach, studies involving interactions between beneficial microorganisms and viruses have been collected in a folder that can be accessed here.

Home preparation of EMs:

The basis of EM technology is a stable **stock solution** for about a year in which the effective microorganisms do not replicate until they are activated, that is, they are fermented at about 37°C for 7-10 days in the presence of cane sugar or molasses; this produces a solution called EMa (activated effective microorganisms) that normally lasts no more than a month.

EMA = EM-1® activated and multiplied with molasses and water.
Recipe: 3% EM1, 3% sugarcane molasses, 94% water.

Esempi:

per 1 litro di EMA:	**per 5 litri di EMA:**	**per 10 litri di EMA:**
30 ml EM-1®	150 ml EM-1®	300 ml EM-1®
30 ml melassa	150 ml melassa	300 ml melassa
940 ml acqua.	4.7 l acqua.	9.4 l acqua.

[45] Chan KH, Sridhar S, Zhang RR, et al.
Factors affecting stability and infectivity of SARS-CoV-2.
J Hosp Infect. 2020;106(2):226-231. doi:10.1016/j.jhin.2020.07.009
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7343644/

[46] Villena J, Kitazawa H.
The Modulation of Mucosal Antiviral Immunity by Immunobiotics: Could They Offer Any Benefit in the SARS- CoV-2 Pandemic?
Front Physiol. 2020;11:699. Published 2020 Jun 16. doi:10.3389/fphys.2020.00699
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7326040/

[47] https://microrganismi-efficaci.it/news/cat/em/post/disinfezione-em-corea/

Completely dissolve 3% cane molasses in very hot water (no more than 60°) and fill a plastic container with a lid, e.g., a PET bottle, bin or tank. Fill the container with water. The temperature of the liquid should not exceed 38 degrees. To finish, add 3% EM1. The full, closed container should remain for 7-10 days in a place at a temperature of 37°. Reheat the environment if necessary. The liquid should not exceed 38°C. Occasionally open the container slightly to let the gas escape. EMA should have a pH value less than 3.7 and a slightly acidic odor. Fermented EMA should be stored in a dark and cool place, preferably in a cellar. Shelf life: about 2 weeks. Do not reproduce EMA, otherwise the composition of the microorganisms and the quality itself is no longer guaranteed. [48]

see also: Production of EMa[49] Home Manual

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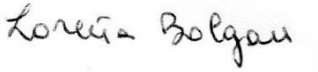

[48] https://www.emipiace.it/documenti/Cat.%20inform%20.%20e%20uso.pdf

[49] http://microrganismi-efficaci.it/media/wysiwyg/microrganismi-efficaci/riproduzione_EMa.pdf