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# Impact of lockdown on the microbiological status of the hospital water network during COVID-19 pandemic

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ABSTRACT

The COVID-19 pandemic started in China in early December 2019, and quickly spread around the world. The epidemic gradually started in Italy at the end of February 2020, and by May 31, 2020, 232,664 cases and 33,340 deaths were confirmed. As a result of this pandemic, the Italian Ministerial Decree issued on March 11, 2020, enforced lockdown; therefore, many social, recreational, and cultural centers remained closed for months. In Apulia (southern Italy), all non-urgent hospital activities were suspended, and some wards were closed, with a consequent reduction in the use of the water network and the formation of stagnant water. This situation could enhance the risk of exposure of people to waterborne diseases, including legionellosis.

The purpose of this study was to monitor the microbiological quality of the water network (coliforms, *E. coli*, Enterococci, *P. aeruginosa,* and *Legionella*) in three wards (A, B and C) of a large COVID-19 regional hospital, closed for three months due to the COVID-19 emergency. Our study revealed that all three wards' water network showed higher contamination by *Legionella pneumophila* sg 1 and sg 6 at T1 (after lockdown) compared to the period before the lockdown (T0). In particular, ward A at T1 showed a median value = 5600 CFU/L (range 0–91,000 CFU/L) vs T0, median value = 75 CFU/L (range 0–5000 CFU/L) (*p-value* = 0.014); ward B at T1 showed a median value = 200 CFU/L (range 0–4200 CFU/L) vs T0, median value = 0 CFU/L (range 0–300 CFU/L) (*p-value* = 0.016) and ward C at T1 showed a median value = 175 CFU/L (range 0–22,000 CFU/L) vs T0, median value = 0 CFU/L (range 0–340 CFU/L) (*p-value* < 0.001).

In addition, a statistically significant difference was detected in ward B between the number of positive water samples at T0 vs T1 for *L. pneumophila* sg 1 and sg 6 (24% vs 80% *p-value* < 0.001) and for coliforms (0% vs 64% *p-value* < 0.001). Moreover, a median value of coliform load resulted 3 CFU/100 ml (range 0–14 CFU/100 ml) at T1, showing a statistically significant increase versus T0 (0 CFU/100 ml) (*p-value* < 0.001).

Our results highlight the need to implement a water safety plan that includes staff training and a more rigorous environmental microbiological surveillance in all hospitals before occupying a closed ward for a longer than one week, according to national and international guidelines.

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## 1. Introduction

The COVID-19 pandemic started in China in early December 2019, and quickly spread around the world, with the number of confirmed cases increasing daily. The epidemic gradually started in Italy at the end of February 2020 (Gagliano et al., 2020), and by May 31, 2020, Italy had 232,664 confirmed cases and 33,340 deaths (WHO, 2019).

The Ministerial Decree issued on March 11, 2020 (DPCM, March 11., 2020) limited the movement of people throughout the nation and stated lockdown: shops, schools, museums, cinemas, theaters, and any other social, recreational, or cultural center remained closed for months (Gallè et al., 2020). Hospitals faced unusual demand for emergency rooms and infectious disease wards, with the urgent need to adapt all the spaces available to place hospital beds (Astley et al., 2015; Capolongo et al., 2020).

In Apulia, Italy, most scheduled hospitalizations, outpatient visits, and non-urgent diagnostic tests were suspended (Disposizione del Dipartimento di Promozione della Salute - 9 marzo 2020), and some pavilions and hospital wards were used in low capacity or even closed, with a consequent reduction in the use of the water network. Although water quality may not seem like a priority during the COVID-19 pandemic, it is important not to neglect the management of water systems because poor water quality can increase the risk of waterborne infections (ESGLI, 2020; WHO, 2011; WHO, 2017). The water supplied by the water system is not free of microorganisms, even if it complies with drinking water standards (Bonadonna et al., 2017). The new Drinking Water Directive (DWD) expected to come into force before December 2020 will introduce a new risk-based approach including an assessment of the possible risks stemming from the domestic distribution systems, including *Legionella* by the Member State ("domestic distribution risk assessment"). Poorly managed water networks may be vulnerable to microorganisms, including *Legionella* and *Pseudomonas aeruginosa*, which can infect hospitalized patients who already have underlying conditions, immunosuppression and use invasive devices (Coppry et al., 2020; Montagna et al., 2016, 2018). Thus, water stagnation in unused buildings (e.g. in water storage system, pipes) as a result of the lockdown implemented due to the pandemic could lead to hazardous conditions enhancing the risk of exposure of people to potentially infectious bacteria.

*Legionella* grows in free-living amoebas, replicates between 25 °C and 45 °C (Garrity et al., 2005), but can survive at temperatures ranging from 5.7 °C to 63 °C, especially if the water is stagnant (Fliermans, 1996) with an optimal growth temperature of 35 ± 2 °C (Katz and Hammel, 1987).These bacteria can cause a serious form of pneumonia, known as Legionnaires' disease, or a flu-like illness, the Pontiac fever, which is normally acquired by inhaling aerosols produced by sources of contaminated water (De Giglio et al., 2019; Montagna et al., 2018; Napoli et al., 2010; Orkis et al., 2018). In Italy, 2964 cases of legionellosis were reported in 2018 (incidence rate = 48.9 cases per 1 million inhabitants), 3.4% of which was of nosocomial origin (ISS, 2019).

In Apulia (southern Italy), the large University Hospital (Azienda Ospedaliero-Universitaria, AOU) of Bari was among the COVID-19 regional hospitals. During the pandemic, some wards that had a predominantly outpatient or day hospital activities were closed, and the healthcare personnel were transferred to support the needs of the COVID-19 units.

The aim of this study was to monitor the microbiological water quality in the water network in three wards of the AOU of Bari after a period of closure due to COVID-19 emergency. To our knowledge, this is the first study evaluating the impact of the lockdown due to the COVID-19 pandemic on the microbiological status of the hospital water network.

## 2. Materials and methods

### 2.1. Study design

This study was conducted from April to May 2020 in the AOU, which has 1400 beds in 33 separate buildings. Three wards (hereinafter, referred as A, B and C) closed following the nosocomial reorganization for the COVID-19 emergency were selected. The ward A has 22 beds with a total area of 700 $m^2$; the ward B has 20 beds with a total area of 1929 $m^2$ and the ward C has 20 beds with a total area of 617 $m^2$. According to the water monitoring plan provided by the AOU, the 50% of total points of water network (taps and showers) evenly distributed in each ward was subjected to microbiological control every six months. The last pre-lockdown control was carried out in February 2020 (T0): in particular, 10 (nine taps and 1 shower) points of the water network were analyzed in ward A, 25 (all taps) in ward B and 18 (13 taps and five showers) in ward C. In March 2020, the activities were suspended because the staff was transferred to the COVID wards. At the end of May 2020 (T1), before returning to normal activities, sampling was repeated on the same points of the water network for the three wards.

Microbiological parameters (coliforms, *E. coli*, Enterococci, *P. aeruginosa*) and *Legionella* were analyzed using the methods described in sections 2.2.

### 2.2. Microbiological investigation

Sampling and processing were performed according to the Legislative Decree No. 31/01 (Decreto Legislativo 2 Febbraio, 2001, n. 31) for *Escherichia coli*, coliforms, Enterococci, and *Pseudomonas aeruginosa*. Cold water samples of 1 L were collected in sterile bottles, transported to the laboratory at 4 °C, and analyzed within 4 h. Specific aliquots of each sample were filtered through a cellulose ester membrane with a diameter of 47 mm and a pore size of 0.45 μm (Millipore, Milan, Italy).

For *E. coli* and coliform investigation, 100 mL of each water sample was filtered. Subsequently, the membrane was placed on chromogenic coliform agar (Biolife Italiana Srl, Milan, Italy) and incubated at 36 ± 2 °C for 24 ± 2 h. The blue-violet colonies were identified as *E. coli*, and the salmon pink, oxidase negative ones were identified as coliforms (UNI EN ISO 9308-1, 2017).

For the isolation of Enterococci, 100 mL of the sample was filtered, and the membrane was placed on a Slanetz and Bartley agar medium (Biolife Italiana Srl, Milan, Italy). When dark pink-red colonies developed after 48 h at 36 ± 1 °C, the membrane was transferred to Bile Esculin Azide agar (Biolife Italiana Srl, Milan, Italy) and incubated at 44 °C for 2 h; brown colonies with brown-black halos were identified as Enterococci (UNI EN ISO 7899-2, 2003).

*P. aeruginosa* was investigated in samples of 250 mL. After the sample was filtered, the membrane was placed on a plate containing *Pseudomonas* selective agar supplemented with cetrimide (0.20 g) and nalidixic acid (15 mg) (Microbiol, Cagliari, Italy) and incubated at 36 ± 2 °C for 44 ± 4 h. Blue-green pyocyanin producing colonies were directly confirmed to be *P. aeruginosa* (UNI EN ISO 16266, 2008).

The samples were considered compliant to Legislative Decree 31/01 when *E. coli*, coliforms, and Enterococci were absent in 100 mL of each sample, and *P. aeruginosa* was absent in 250 mL samples.

For investigation of *Legionella*, 1 L water samples were collected in sterile dark glass containers containing sodium thiosulphate pentahydrate (0.01%, w/v) to neutralize chloride present in the water and transported immediately at ambient temperature in isothermal bags so that the analysis could take place within 24 h (Linee Guida per la Prevenzione e il Controllo della Legionellosi, 2015). Each sample was filtered through a 0.2 μm pore-sized Isopore nylon membrane filter with a 47 mm diameter (Millipore Corporation, Bedford, MA, USA). The membranes were then suspended in 10 mL of the same water sample and vortexed, after which 200 μL of each sample was seeded on plates containing a glycine vancomycin polymyxin cycloheximide medium

(GVPC, Liofilchem Srl, Teramo, Italy). The plates were incubated at 37 ± 1 °C for 10 d in a humid environment under 2.5% $CO_2$ and examined after 2, 4, and 10 d of incubation. Suspect colonies were subcultured on buffered charcoal yeast extract (BCYE) agar (BioMérieux, Marcy l'Etoile, France) with and without L-cysteine. Colonies that grew only on BCYE agar plates with cysteine were identified as *Legionella* and confirmed by using a latex agglutination test with polyvalent (Biolife Italiana Srl, Milan, Italy) and monovalent antisera (Biogenetics Srl, Tokyo, Japan). Water samples containing <50 colony-forming units per liter (CFU/L) were considered negative for *Legionella* (hereinafter, referred to as 0 CFU/L) (UNICHIM 1037:14); this concentration falls within the threshold below which no intervention is required in healthcare facilities (Linee Guida per la Prevenzione e il Controllo della Legionellosi, 2015).

### 2.3. Statistical analysis

For all wards, Fisher's exact test was used to compare the microbiological parameters in the different periods. Wilcoxon signed-rank test with continuity correction for paired data were used to compare the load of microbiological parameters and *Legionella* at T0 (before lockdown) and T1 periods (after lockdown).

R software version 3.5.1 was used for statistical analysis, and results with $p < 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Ward A

Ten water samples (nine taps and 1 shower) were collected at T0 (pre-lockdown) and T1 (post-lockdown). None of non-*Legionella* microrganisms (coliforms, *E. coli*, Enterococci, *P. aeruginosa*) were detected in the samples. There were no statistically significant differences between the number of positive samples of *L. pneumophila* sg 1 and sg 6 at T0 and T1 (50% and 80%, respectively, Fisher's F test *p-value* = 0.350). The median value of the load was 75 CFU/L (range 0–5000 CFU/L) at T0 and 5600 CFU/L (range 0–91,000 CFU/L) at T1, showing a statistically significant increase (Wilcoxon signed-rank test with continuity correction for paired data V = 0, *p-value* = 0.014) (Fig. 1 A).

### 3.2. Ward B

Twenty-five water samples (all taps) were collected at T0 (pre-lockdown) and T1 (post-lockdown). *E. coli*, Enterococci were never detected in the samples. Statistically significant differences resulted between the number of positive samples of *L. pneumophila* sg 1 and sg 6

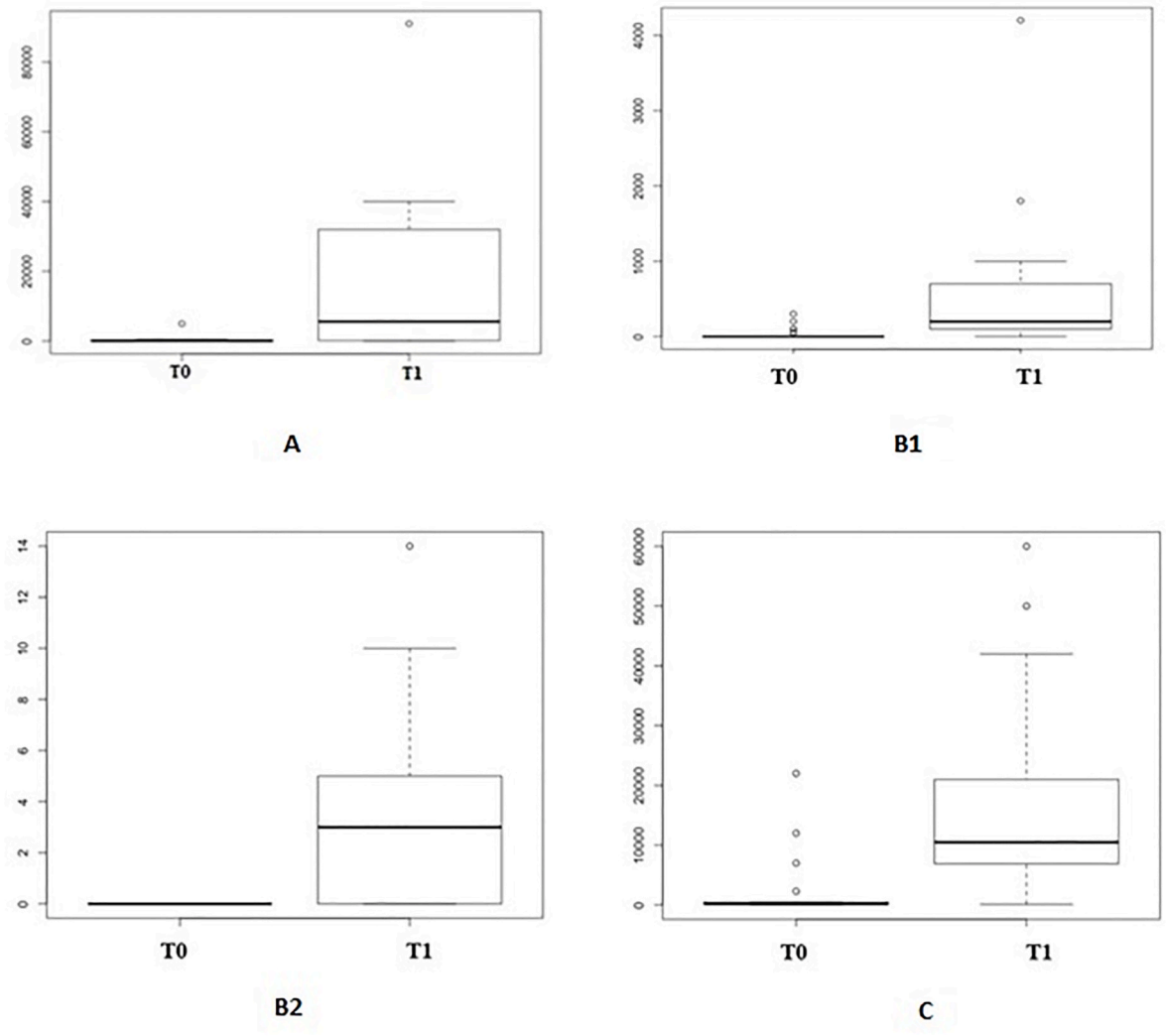


**Fig. 1.** Box plot of *Legionella* load (CFU/L) in wards A (A), B (B1) and C (C), and of coliform load (CFU/100 ml) in ward B (B2) at T0 and T1.

at T0 and T1 (24% and 80%, respectively; Fisher's F test *p-value* <0.001) and coliforms (0% and 64%, respectively; Fisher's F test *p-value* <0.001), while no statistically significant differences resulted between the number of positive samples of *P. aeruginosa* at T0 and T1 (0% vs 8.7%; Fisher's F test *p-value* = 0.490).

The median value of the load of *Legionella* was 0 CFU/L (range 0–300 CFU/L) at T0 and 200 CFU/L (range 0–4200 CFU/L) at T1, showing a statistically significant increase (Wilcoxon signed-rank test with continuity correction for paired data V = 0, *p-value* = 0.016) (Fig. 1 B1).

Coliforms were not detected at T0, while the median value of load was 3 CFU/100 ml (range 0–14 CFU/100 ml) at T1, showing a statistically significant increase (Wilcoxon signed-rank test with continuity correction for paired data V = 0, *p-value*<0.001) (Fig. 1 B2).

*P.aeuruginosa* was not detected at T0, while the median value of load was 0 CFU/250 ml (range 0–9 CFU/250 ml) at T1, without a statistically significant difference (Wilcoxon signed-rank test with continuity correction for paired data V = 3, *p-value* = 0.371).

### 3.3. Ward C

Eighteen water samples (13 taps and five showers) were collected at T0 (pre-lockdown) and T1 (post-lockdown). Coliforms, *E. coli* and Enterococci were never detected.

There were no statistically significant differences between the number of positive samples of *L. pneumophila* sg 1 and sg 6 at T0 and T1 (83.3% and 100%, respectively; Fisher's F test *p-value* = 0.229) and *P. aeruginosa* (0% vs 11.1%; Fisher's F test *p-value* = 0.486).

The median value of the load of *Legionella* was 0 CFU/L (range 0–340 CFU/L) at T0 and 175 CFU/L (range 0–22,000 CFU/L) at T1, showing a statistically significant increase (Wilcoxon signed-rank test with continuity correction for paired data V = 0, *p-value* < 0.001) (Fig. 1C).

*P.aeuruginosa* was not detected at T0, while the median value of load was 0 CFU/250 ml (range 0–340 CFU/250 ml) at T1, showing no statistically significant difference (Wilcoxon signed-rank test with continuity correction for paired data V = 3, *p-value* = 0.371).

## 4. Discussion

Hospital water safety is a major priority and a constant challenge for epidemiologists, safety officers, engineers, and administrators. Due to the COVID-19 emergency, hospital activities were suspended in some wards, and staff were transferred to other COVID-19 units, which increased the risk of waterborne diseases, especially the *Legionella* growth in rarely used water systems (ISS, 2020).

Our study revealed that the water network of examined wards closed for three months because of COVID-19 emergency showed a higher *L. pneumophila* contamination after lockdown period. This phenomenon can be explained by the long period of inactivity. Therefore, closure of hospitals or parts of them, the renovation of old wards and the limited use of the water system can cause malfunctions in the water system; vibrations or significant changes in water pressure can detach the biofilm present on the internal surface of the water network, releasing microorganisms into the water. Thus, accommodations, if not managed properly during the COVID-19 pandemic, can pose a potential danger to patients and healthcare professionals. Some studies have shown that COVID-19 patients had *L. pneumophila* co-infection (Arashiro et al., 2020).

In Europe and in Italy, approximately 10% of community-acquired cases of Legionnaires' disease die; however, for hospital-acquired cases, the death rate can rise to 50% (ESGLI, 2020; ISS, 2020). In 2015, the Italian Institute of Health updated the national guidelines for the control and prevention of legionellosis (Linee Guida per la Prevenzione e il Controllo della Legionellosi, 2015) and highlighted that a well-designed clinical-environmental surveillance program is the most important tool for early identification of diseases and sources of infection in the hospitals (Napoli et al., 2019). In Italy, the conservation of hospitals is highly critical. It is estimated that more than two-thirds of the hospitals have exhausted their life cycle, and more than half is not adequate for new organizational models (Dell'Ovo and Capolongo, 2016). The hospital in this study is also characterized by a general state of obsolescence; therefore, old water pipes with limestone and biofilm problems further complicated the management of closed wards during the COVID-19 pandemic. In this regard, to guarantee the safe reopening of the wards closed for more than a month during the COVID-19 pandemic, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *Legionella* Infections has published recommendations for the management of *Legionella* in hospital water systems (ESGLI, 2020).

The need to periodically disinfect the hospital water network and cisterns to make hospitalization safe for patients highlights another problem: the need to identify *Legionella* at the level of species and serogroups. In our case, *L. pneumophila* sg 1 and sg 6 were isolated from the water network of all three enrolled wards. The detection of different serogroups of *Legionella* allows a more accurate assessment of *Legionella* spread in the environment and an adequate remediation plan. Some authors (Marchesi et al., 2011) demonstrated that the use of biocides for water treatment might select for resistant *L. pneumophila* serogroups (De Giglio et al., 2015). Thus, culture-based methods, supported by monovalent serotyping of strains, would avoid problems associated with improper disinfection methods.

The presence of other bacteria (coliforms, *E. coli*, Enterococci, and *P. aeruginosa*) in the hospital water system indicate that the water supplied by the water system is not sterile. According to Legislative Decree 31/01, only *E. coli* and Enterococci are considered mandatory parameters to evaluate potable water quality (Decreto Legislativo 2 Febbraio, 2001, n. 31). However, the presence of thermophilic coliforms and *P. aeruginosa* also raises concerns about the use of this water by healthcare personnel and hospitalized patients. Coliform bacteria include the genera *Escherichia, Citrobacter, Klebsiella, Enterobacter*, *Serratia,* and *Hafnia*. Some of these bacteria colonize the human and animal intestines and thus are excreted in the feces, but many can also multiply in the environment, including water and soil (De Giglio et al., 2016). They are often used to evaluate the cleanliness and integrity of water networks, the presence of biofilms, and the effectiveness of disinfection processes (Bumadian et al., 2013). For this reason, they must be absent in the system after remediation; their presence indicates an inadequate treatment (Cunliffe et al., 2011).

*P. aeruginosa* remains one of the most common opportunistic pathogens acquired in hospitals and is endemic in many intensive care units (ICUs) (Rosenthal et al., 2012). Infections caused by *P. aeruginosa*, in particular pneumonia or sepsis, are associated with high mortality, especially if these microorganisms are drug-resistant. In ICUs, these infections are generally considered endogenous (i.e., coming from the pre-existing colonization of patients). Some studies, however, showed that the clinical strains of *P. aeruginosa* were genetically related to the strains found in the patient's room, e.g., in tap water, sinks, and hand wash stations (Coppry et al., 2020).

These data highlight that it is essential to survey the water network of a building or portion of a building that has been previously closed for longer than one week (ESGLI, 2020) before starting normal hospital services so that the risk of increasing waterborne diseases, including Legionnaires' disease, can be reduced.

## 5. Conclusion

As our study confirms, the risk of waterborne diseases has been exacerbated due to stagnation of water in unused buildings during the lockdown.

According to the incoming rules of the revised drinking water directive, a water safety plan should be developed and implemented in all hospitals, as recommended by the World Health Organization in 2004. It should include a system maintenance plan, staff training, and implementation of a more strict environmental microbiological surveillance.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Credit author statement

Osvalda De Giglio: Conceptualization, Methodology, Validation, Writing - original draft. Giusy Diella: Conceptualization, Methodology, Validation, Writing - original draft. Maria Teresa Montagna: Conceptualization, Methodology, Validation, Writing - original draft. Fabrizio Fasano: Software, Data Formal analysis. Marco Lopuzzo: Investigation. Francesco Triggiano: Investigation. Carla Calia: Investigation. Chrysovalentinos Pousis: Investigation. Giuseppe Calabrese: Visualization, Investigation. Vincenza Rafaschieri: Visualization, Investigation. Federica Carpagnano: Visualization, Investigation. Giuseppina Caggiano: Review & editing. Matilde Carlucci: Review & editing. Loreto Gesualdo: Review & editing. Maria Luisa Ricci: Review & editing. Maria Scaturro: Review & editing. Maria Cristina Rota: Review & editing. Lucia Bonadonna: Review & editing. Maria Teresa Montagna: Supervision. Luca Lucentini: Supervision.

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