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Applicability of Escherichia coli As an Indicator for Assessing Quality of Disinfectants and Antiseptic During the COVID-19 Pandemic

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ABSTRACT

Introduction: The application of disinfectant agents is a common way to fight against microorganisms. Although there are different types of disinfectant agents to fight COVID-19, many of them do not have the required quality and efficiency. The present work was aimed to evaluate the quality of the available disinfectant agents using gram-negative E. coli bacteria.

Materials and Methods: In the laboratory phase of the research, the gram-negative E. coli bacteria were used to evaluate the quality of disinfectants. According to the proposed laboratory method, microbial kit was prepared and used to evaluate the performance of disinfectants.

Results: According to the obtained findings, 1.0 mL of the prepared microbial suspension in the test tube, as microbial kit, was used for the quality assessment of the selected disinfectants. In case of growth of microorganisms in optimum conditions, the quality of disinfectants was undesirable, and if microorganisms were not grown, the quality of disinfectants would be appropriate in terms of its effect on gram-negative bacteria.

Conclusion: E. coli can be used as a reliable indicator for assessing the quality of the disinfectant and antiseptic agents used against COVID-19.

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Introduction

Chemical disinfectants and disinfectants are widely used to control pathogenic microorganisms [1]. The use of chemical disinfectants, in epidemics and pandemics situations, plays an important role in controlling pathogens in different environments, especially in hospitals [2]. Meanwhile, the quality of disinfectants plays an important role in disinfection efficiency. Evaluation of the strength of disinfectants is usually expressed in comparison with phenol [3]. There are various chemical and microbial analysis methods to control the quality of disinfectants. Chemical methods are usually carried out using

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molecular analysis and also determining the quality of the effective substance [4]. Microbial methods are evaluated based on the effect of disinfectant on an indicator microorganism. In epidemics associated with a particular microorganism, the disinfectants are evaluated based on their effect on the selected microorganism. The evaluation of the effect of disinfectants on bacteria is easier due to the ability of bacteria to grow in the environment and also the possibility of simple culture [5]. However, due to the lack of growth of the virus in the environment as well as the costly method of detecting the virus in the external environment, it is difficult to determine the disinfectant effect on the virus. If the type, species and morphology of the virus are well identified, other microbial indicators can be used to assess the quality of the disinfectant effect. In the past, bacteriophages have been used to evaluate the quality of disinfectants and their effect [6].

Depending on the target locations in the virus structure, factors affecting the chemical inactivation of viruses include lipid coating (Enlop), viral receptors, capsids, genome, and the functional proteins inside the capsid, which have less target location compared to other microbial agents due to the simplicity of building structure of the viruses, non-proliferation, and inactivity in the environment [7, 8].

COVID-19 is a new type of capsule-containing viruses that is very sensitive to disinfectant agents and has a very low resistance compared to other microorganisms. High transmission rate and unpredicted behaviors of this virus is considered as the most important concerns [9]. Rapid increase in the number of pandemic deaths caused by the virus in the world in a short period of time, making it the leading cause of death from microbial infections in 2020 [10, 11]. In the environment, virus can be efficiently controlled using disinfectant agents [12]. Using the biological indicators such as microorganisms is one of the methods to evaluate the effectiveness of disinfectants, microorganisms that are more stable than coronavirus can be used to evaluate disinfectants quality. Therefore, in the present work, the use of gram-negative E. coli bacteria to evaluate disinfectants quality was investigated. E.coli is an indicator of microbial contamination of water [13, 14]. Due to high resistance of E.coli bacteria to disinfectant agents, it has been selected as the water contamination index; if the conditions are unfavorable for the presence of this bacterium in water, the presence of other pathogens can be ensured with more confidence [13, 15] . This unique characteristic was used to the selection of this gram-negative bacterium for the present study to determine the quality of disinfectants.

The aim of this study was to evaluate gram-negative E. coli bacteria as an indicator to evaluate the quality of disinfectants. Herein, the minimum contact time, the minimum concentration of used disinfectant for testing with microbial suspension, and the shelf life of microbial suspension were evaluated.

Materials and Methods

According to the conducted literature review, the stability of various microorganisms against disinfectant agents was investigated. First, the resistance of pathogen agents based on the microorganism type and viruses were assessed against disinfectants agents. In the laboratory phase of the research, the gram-negative E.coli bacteria were used to evaluate the quality of disinfectants.

To perform the work, Escherichia coli (PTCC No: 1399), purchased from the Industrial Microorganisms Collection Center of the Scientific and Industrial Research Organization of Iran, was used as an indicator. After preparing the bacteria, the agar was cultured on the nutrient agar and incubated for 24 hours. Then 10 colonies were removed from the grown colonies and added to 200 ml of water containing 0.5 mg of glucose and placed in the laboratory for 24 hours. In this work, 1.0 ml of the prepared solution was used as a microbial kit to evaluate the performance of disinfectants. In the first step, 1.0 mL of 0.05% sodium hypochlorite solutions and 70% ethanol alcohol as negative control and 1.0 mL of distilled water as positive control were added to the 3 prepared microbial kits and placed in the

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laboratory for half an hour. Then, samples were taken from each kit by sterile swab and cultured on EMB Agar culture medium. The culture medium was incubated for 24 hours. The obtained results were assessed based on the growth or non-growth of gram-negative bacteria on the culture medium; the growth of microorganisms on the EMB medium showed low quality and lack of growth indicated the high quality of disinfection. In order to determine the optimum contact time between disinfectant and microbial kit, the concentration level of 1.0 mL of 0.05% sodium hypochlorite disinfectants and 70% ethanol alcohol during 1, 5, 15, and 30 minutes were exposed to 1.0 mL of microbial suspension as kit. The growth quality was then evaluated on EMB agar medium after 24 h of incubation. After determining the optimum time, to investigate the minimum required amount of negatively controlled disinfectants, the microbial concentration levels of 1, 0.1, 0.01 and 0.001 of sodium hypochlorite disinfectants 0.05% and ethanol alcohol 70% were prepared and exposed to the synthesized microbial suspension within the optimized time. In all these stages, sterile distilled water and one ml of microbial suspension was used as a positive control to evaluate the viability of microorganisms. To evaluate the stability of the prepared microbial kit at laboratory temperature, within the time intervals of 7, 15, 30, 45, and 60 days since the preparation, the suspension was sampled and cultured on EMB medium and the results were analyzed. After determining the optimum contact time and the optimal concentration of disinfectant, 20 different types of disinfectants, which were currently used as effective disinfection in the control of Covid 19 epidemic, were gathered and their quality were assessed using the prepared kit.

Ethical Issue

The Ethics Committee of Yazd University of Medical Sciences approved the study protocol (Approval number: IR.SSU.SPH.REC.1399.073).

Results

Gram-negative bacteria to assess the quality of disinfectants agents

Using the results of previous studies (12,16,17), stability and resistance of microorganisms against disinfectant agents were investigated. Table 1 shows the vulnerability of micro-organisms to disinfectant agents, in which the most resistant organisms to disinfectant agents were listed at the top of the table, and the most vulnerable ones at the bottom of the table. In this way, viruses are considered the most vulnerable organisms.

Table 1: The resistance of virus to disinfecting agents compared to other micro-organisms [12, 16, 17].

Organism type	Example
Prions	PrPr, protein causing transferable encephalopathies
Bacterial endospores	Bacillus species spores or clostridium, geobacillus
Protozoan oocysts	Oocyst species of Cryptosporidium
Cysts or worm eggs	Ascaris or Enterobius species
Mycobacteria	Mycobacterium tuberculosis, Mycobacterium chelonei, Mycobacterium terrae
Small non-coated virus	Poliovirus, Parvovirus, Papilloma virus, Norovirus
Protozoan cysts	Giardia or Acanthamoeba species
Fungal spores	Aspergillus or Candida species spores, penicillium
gram-negative bacterium	Species of Pseudomonas or Escherichia coli
Spore-free fungi and algae	Trichophyton species, Candida, Aspergillus
Adult worms and Spore -free protozoa	Ascaris species, Cryptosporidium, Giardia
Non-enveloped virus	Adenoviruses, Rotavirus
gram-positive bacterium	Staphylococcus, Streptococcus, and Enterococcus species
enveloped virus	HIV, Vaccine, Herpes, Influenza, and HBV

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Rotavirus, human papillomavirus, bird flu virus, sever acute respiratory syndrome (SARS), coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), HTLV, HIV, HCV, etc. are considered new emerging viral infections [18, 19].

Transmission electronic microscope (TEM), using molecular or immunological instruments can be applied to identify these microscopic agents. When the micro-organism is a viral agent, using the results obtained from the size of the viral particle and its type of structure or previous available results, using tables 1 and 2, its sensitivity to environmental surface disinfectants can be estimated. After identifying the family and class of the viral agent, appropriate disinfectant agents are used[12, 20].

Table 2: Summarizes the sensitivity of human pathogenic viruses to disinfectant agents according to their size.

Virus structure type	Viral family	Virus size (nm)	Example
Small uncovered viruses	Astroviridae	27-30	Astrovirus
	Caliciviridae	35-39	Neurovirus
	Circoviridae	30	anelloviruses
	Parvoviridae	21-26	B19 virus, Boca Virus
	Picornaviridae	28-30	Enterovirus, Hepatitis A Virus, Rhinovirus
Large uncovered viruses	Adenoviridae	70-90	adenovirus
	papillomaviridae	55	Papilloma virus
	Polyomaviridae	40-45	SV 40
	Reoviridae	60-80	Rotavirus
Enveloped Viruses	Arenaviridae	110-130	Lymphocytic choriomeningitis virus
	Bornaviridae	80-125	Borna virus
	Bunyaviridae	80-120	Rift Valley Fever virus, hantavirus
	Coronaviridae	80-200	SARS
	Filoviridae	790-970	Marburg virus, Ebola virus
	Flaviviridae	45-60	Yellow fever virus, Hepatitis C virus
	hepadnaviridae	42-50	Hepatitis B virus
	herpesviridae	150-200	Cytomegalovirus, Varicella Virus
	orthomyxoviridae	80-120	Flu virus
	paramyxoviridae	150-300	Oreillon virus, measles virus
	Poxviridae	250 × 200 × 200	smallpox virus, vaccinia virus
	rhabdoviridae	180 × 75	Rabies virus
	Retroviridae	80-100	Immunodeficiency virus
	Togaviridae	70	rubella virus

Human pathogenic viruses which are in the top of the table have the lowest sensitivity and those that are at the bottom of table have the highest sensitivity to disinfectant agents [7, 20].

Coronaviruses are sensitive envelope virus among the weakest environmental microorganisms in terms of resistance to disinfectants (Tables 1 and 2). Due to vulnerability of covered viruses to disinfectant agents, compared to bacteria, especially gram-negative bacteria, it is expected that disinfectant agents that affect negative gram-negative bacteria can also destroy the coated viruses [6, 20]. Considering high resistance of the negative gram-negative bacterium *E.coli* to disinfectant agents, in this work it was used as an indicator to evaluate the quality of disinfectant agents to destroy COVID-19.

Laboratory phase of the study

The results of the used microbial suspension with negative control (0.05% sodium hypochlorite and 70% ethanol alcohol) and positive control (distilled water) are given in table 3. The culture results of microbial suspension in contact with negative and positive control on EMB agar medium showed the growth of microorganisms in positive control and no growth of microorganisms in negative control.

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Table 3: The results of E.coli bacterial growth in contact with positive and negative control

	Disinfectants		
	Distilled water	Ethanol alcohol (70%)	Sodium hypochlorite (0.05%)
Cultivation result of E.coli on EMB	Positive	Negative	Negative

The duration of disinfection contact with microorganism's kit was an important effective parameter. Therefore, different time durations were examined to determine the minimum time required to complete the reaction. The amount of required disinfectant to add the microorganism kit was another effective parameter which was investigated in the study.

Table 4 shows the culture status of microbial suspension in contact with positive and negative control at contact time of 1, 5, 15, and 30 minutes.

The results of the microbial suspension kit with different amounts of negative control are given in table 5.

Table 4: Results of microbial culture of microbial suspension with positive and negative control at different contact times on EMB medium

Time (min)	Disinfectants		
	1 ml of Ethanol alcohol (70%)	1 ml of Sodium hypochlorite (0.05%)	1 ml of Distilled water
1	Negative	Negative	Positive
5	Negative	Negative	Positive
15	Negative	Negative	Positive
30	Negative	Negative	Positive

Table 5: Microbial culture results of microbial suspension contact with different amounts of negative control at 1 minute contact time on EMB culture medium

Disinfectant type	Amount of disinfectant (ml)			
	1	0.1	0.01	0.001
Ethanol alcohol (70%)	Negative	Positive	Positive	Positive
Sodium hypochlorite (0.05%)	Negative	Negative	Positive	Positive

Investigation of the stability of the used microbial kit was another important variable of the study. Accordingly, the survival time of the bacteria in the kit at different times over a 60-day period was assessed. Table 6 shows the evaluation of the stability of the microbial suspension in the laboratory.

Table 6: Microbial culture results of microbial suspension at different time intervals on EMB culture medium

Result	Time (day)				
	7	15	30	45	60
Growth on EMB (+/-)	Positive	Positive	Positive	Positive	Negative
Number of colony	Many	Many	Many	Many	0

The results of the quality assessment of 20 disinfectants gathered from the market by the prepared kit showed that 4 disinfectants were not of good quality.

Discussion

The results of the previous studies revealed that among microorganisms, prions have the highest resistance to disinfectant agents; parasites, fungi, some types of bacteria and viruses are less resistance to disinfectants, respectively [12, 16]. Compared to gram-positive and gram-negative bacteria, enveloped viruses such as corona viruses are less resistance against disinfectant agents [16, 17, 20].

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Due to the problems regarding the growth and detection of viruses in the environment outside the body, other microorganisms, especially bacteria that can easily grow and detect in the environment, have the potential to be used as an indicator to evaluate the effect of disinfectants. Considering the resistance to disinfectant agents, *E.coli* is much stronger than coronavirus[16]; therefore there is a potential that disinfectant agents that affect *E.coli* can effectively eliminate the COVID-19 virus.

Considering that the growth and reproduction of *E.coli* bacteria is simply possible, exposing this bacterium to the disinfectant agents is an easy way to evaluate the quality of the disinfectant agents.

Therefore, due to the high stability of this microorganism against disinfectants in comparison with coronaviruses, *E.coli* was used as a suitable biological indicator to evaluate the quality of disinfectants affecting coronaviruses in this study. The results of the laboratory phase of the present study showed that the disinfectants introduced by the World Health Organization (WHO) to control COVID19 could well affect the E. coli bacterium at the concentration determined by this organization.

Based on the obtained results of the growth of *E. coli* bacteria on the EMB culture medium, this bacterium has the potential as an indicator to identify and evaluate the quality of disinfectant agents. According to the obtained results, the duration of 1 minute of contact of the microbial suspension with disinfectant is sufficient. Therefore, in order to evaluate the quality of disinfectants, after adding a sufficient amount of disinfectant with a residence time of 1.0 minute, the solution was cultured on EMB agar medium.

The results of disinfectant amount in contact with the microorganism suspension in the kit showed that for assessing the quality of the disinfectants in both negative controls, 1.0 mL of disinfectants is sufficient.

The results of examining the duration of usability of the prepared microbial kit showed that the microbial suspension inside the kit can be used for 45 days. After 45 days, the number of microorganisms in the kit is reduced so that on the day of 60th, the result of culture of microbial suspension on EMB agar was negative. According to the results of the laboratory phase and considering the following conditions: adding 1.0 mL of disinfectant to the microbial kit with residence time of 1.0 minute then culturing the solution inside the kit on EMB agar medium, if the microorganism did not grow on the culture medium, it can be concluded that the disinfectant has a good quality in terms of bactericidal. The prepared suspension in the kit can be used at laboratory temperature for 45 days for relative evaluation of the antimicrobial ability of disinfectants. After 45 days, the microbial suspension must be prepared again.

The results of the present study showed that disinfectants introduced by the guidelines of the WHO can completely eliminate the *E.coli* bacteria in the kit in the specified concentration, and if the quality of the disinfectant is reduced, the ability to completely remove this bacterium is eliminated and bacteria is growth on the culture medium. Based on the results of this study, the prepared kit can be evaluated to determine the quality of disinfectants (in terms of their effect on gram-negative bacteria and other weaker microorganisms). However, it seems that more studies are needed to study a wider range of disinfectants, as well as more detailed laboratory studies in terms of microbiology and disinfection processes. It should also be noted that the prepared kit can only determine the effect of disinfectant. It is necessary to consider other methods for evaluating the toxic effects on eukaryotic cells like human cells.

Conclusion

Assessing the quality of disinfectants and their effect on viruses is difficult due to the high cost and technology required and is not possible in a short time. Because of the high variety of disinfectants and the possibility of providing fake disinfectant agents, it is require determining the quality of disinfectants especially in epidemics. This issue became especially important during the coronavirus epidemic, which first confronted the

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market with a shortage of disinfectants and then introduced a large volume of disinfectants into the market. Due to the high resistance of gram-negative bacteria compared to enveloped viruses such as Covid-19, and the possibility of rapid viability testing, this bacterium can be used as an indicator to assess the quality of disinfectants. In this work a rapid, simple, inexpensive, and reliable method for quality assessment of the disinfectants was introduced. This method can be simply used in routine laboratories for rapid test of the disinfectants quality. Based on the obtained results, 1.0 mL of microbial suspension in the test tube, as microbial kit, is used for quality assessment of the disinfectants. Also, based on the results and considering the optimum conditions, in case of growth of microorganisms, the quality of disinfectant is evaluated as unfavorable, and in the absence of growth of microorganisms, the quality of disinfectant is considered appropriate in terms of its effect on gram-negative bacteria and weaker microorganisms. The results of this study showed that E. coli is a good choice for evaluating the effects of disinfectants on coronavirus.

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Conflict of interest

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

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