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Covid-19 and the Virucidal Efficacy of Sanitizing Agents: A Review

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Abstract - The advent of COVID-19 pandemic, a viral disease caused by severe acute respiratory syndrome coronavirus-2 (SARSCoV-2) has almost brought the world to its knees. The virus is currently ravaging almost all the countries of the world, the Scientists are undergoing the rigorous processes of developing drugs and vaccines and evaluating their toxicity yet, there is no end in sight. In all these, it is important to contain the spread of this deadly disease. Sanitization agents in different formulations play a critical role in preventing the disease transmission. For these agent to be effective and efficient, the physiology of the causative virus, its nature, the chemistry of reaction of the chemical agents, the nature of the surfaces housing the virus, the manufacture, the regulation, the marking he applications and the environmental conditions are all important. Therefore, the efficacy of the disinfectants is a complex issue that is beyond the laboratory assurance tests. The review revealed the necessary factors that assist to guarantee the efficacy of sanitization agents and conclude that hand-hygiene and surfaces disinfection are very important means of preventing the transmission of diseases that are caused by pathogens.
Keywords: COVID-19, Disinfectants, Virus, Sanitization agents

1. Introduction

An acute respiratory disease caused by a novel coronavirus (Severe Acute respiratory Syndrome Corona Virus, SARS-CoV-2). The corona virus disease 2019 (COVID-19) was declared by the WHO as pandemic and the world has been battling with the problems occasioned by the outbreak. The emergence of SARS-CoV-2, since the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, marked the third introduction of a highly pathogenic and large-scale epidemic coronavirus into the human population in the twenty-first century. The disease has spread to over 200 countries of the world with the confirmed cases of over twenty million marks while the fatality stands at about a million (WHO). Genomic analysis revealed that SARS-CoV-2 is phylogenetically related to SARS-like bat viruses, therefore bats could be said to be the possible primary reservoir. The intermediate source of origin and transfer to humans is not known yet, however, the rapid human to human transfer has been responsible for millions of infections worldwide [1].

Viruses are responsible for a large number of clinical and subclinical infections in both humans and animals and it have been estimated that viruses account for approximately 60 % of human infections [2, 3]. Entero viruses and papilloma viruses are implicated as the causative agents of variety of chronic diseases [4].The continued virus evolution may change the spectrum of disease for which viruses are responsible. The clinical symptoms of COVID-19 patients include fever, cough, fatigue and a small population of patients appeared with gastrointestinal infection symptoms [1]. The elderly and people with underlying diseases are susceptible to infection and prone to serious outcomes, which may be associated with acute respiratory distress syndrome (ARDS) and cytokine storm.

Poor socioeconomic status, leading to crowded and unhygienic living conditions, increasing use of public facilities due to population explosion in urban centers, and practices of animal husbandry all favor transmission of virus infections. These conditions favour the likelihood of recombination events which may give rise to gene mutation and advent of viral strains with new manifestations as currently been witness in COVID-19 [5]. Some viral diseases can be prevented and controlled by vaccination programs, but, at the present time, there are few vaccines, safe and effective chemotherapeutic agents available against the majority of viral pathogens. Even when vaccines are available, they may fail to protect individuals for a variety of reasons and chemotherapeutic

drugs may not always be effective due to the presence or development of resistant virus strains [6]. Proper isolation of infected individuals is often difficult or impractical.
Chemical disinfection and antisepsis is a better approach to limit the spread of viral infections. Disinfectants are chemical germicides formulated for use on inanimate surfaces, in contrast to antiseptics, which are chemical germicides designed for use on the skin or mucous membranes. Disinfection of contaminated premises and equipment plays an important part in the control of diseases of livestock. Therefore, it is extremely important both to select suitable virucidal agents and to apply them effectively in reducing transmission of viral diseases. For virus to spread through environmental vehicles, it must be capable of surviving in or on those vehicles long enough for direct or indirect contact with a susceptible host. Infected hosts shed viruses into the environment through body secretions and excretions. The organic matrices and cellular debris present can protect the viral agents from degradation outside the host, and the chances of virus transmission increase in direct proportion to the extent of virus survival.

The chemical disinfectants can play a critical role in preventing vehicular transmission of viruses through the residual levels of disinfectant chemicals to which humans or animals are likely to be exposed. Both inanimate and animate surfaces are believed to play a critical role in the transmission of viral infections, and it is in this regard that chemical disinfectants may be best suited for limiting the spread of viral disease from infected persons to vulnerable individuals. The actual kinetics of disinfection are less important than whether the concentration of the particular chemical used can effectively decontaminate a surface during an appropriate contact time. Moreover, although the types of chemicals used may be restricted, depending on the nature of the surface material and considerations of toxicity on food-contact surfaces, the disinfectant concentration is usually considerably higher than that for water disinfection [7].
This review deals solely with the evaluation of disinfection of virus-contaminated surfaces, although some of the remarks are equally applicable to other areas of virus, and even bacterial disinfection. The term disinfectant is used in this review in its general sense, to cover all chemicals applied for the purpose of inactivating infectious agents. Chemosterilants, disinfectants, sanitizers and antiseptics (regarded as topical) are all disinfectants.

The surfaces likely to become contaminated with viruses include medical and veterinary instruments that cannot be steam sterilized between use and which come into direct contact with blood, body fluids, or internal mucosae of humans and animals. These surfaces are viable for virus transmission. These instruments are treated with concentrations of chemicals capable of providing chemo-sterilization or high-level disinfection [8]. Environmental surfaces are designated as critical only if they are in areas where highly vulnerable individuals are housed, such as neonatal intensive care units, burn units, operating suites in hospital settings. Environmental surfaces are generally considered to be less important as vehicles of virus transmission, and as such, only low-level disinfection is practiced for such surfaces.

Animate surfaces, especially hands, present a particularly difficult problem. They are frequently contaminated with viruses which may remain infectious for several hours [9]. Due the skin sensitivity only certain types of chemicals can be use to disinfectant hands and other skin parts of the body. Although there is a large body of scientific literature on the bactericidal properties of disinfectants, there is paucity of information on corresponding work with viruses. Information on the virucidal efficacy of disinfectant products is scattered through the scientific literature of the last 50 years, there are very few systematic studies, and most of the work appears to have been designed to determine whether specific products would be effective for one or more viruses under a particular set of conditions [10]. Most of these studies have failed to test the disinfectant under "in-use" conditions, and the conclusions drawn by the investigators may not be valid when applied to real situations requiring disinfectant application. Furthermore, tests have frequently been conducted using different methods and conditions and the results of such studies cannot truly be compared.
This review will attempt to draw together much of this information and will try to give a cohesive picture of virus disinfection, its potential, and its limitations. No attempt is made to correlate studies of specific virucidal agents with their bactericidal properties, although it is obvious that a wide spectrum of antimicrobial activity is a very desirable characteristic for chemicals used in disease control. Indeed, the designation of chemical antimicrobials as disinfectants or germicides implies, *de facto*, that they will be microbiocidal against all microbial pathogens except bacterial spores [11].

2 Factors Influencing the Efficacy of Chemical Disinfectant

The proper disinfection of any contaminating agent can only occur when there is *direct contact* for an *adequate time* between an *appropriate concentration* of disinfectant and the target agent(s). The disinfection of any contaminated surfaces will depend on the:

i. nature and properties of the surface to be disinfected;
ii. presence of other substances with which the disinfectant reacts which influences both the degree of disinfectant contact with the intended target(s) and the effective disinfectant concentration;
iii. diluent used for preparing the working concentration of disinfectant;
iv. disinfectant applicator;
v. natures of both the contaminating agent and the chemical disinfectant and;
vi. environmental parameters such as temperature and relative humidity.

2.1 Factors Affecting the Chemical Inactivation of Viruses

1. The Target Virus

Mature infectious virions of conventional viruses contain a nucleoprotein core and a structural protein coat. In many virus groups, this macromolecular structure is naked, but in others it is surrounded by a lipid-containing envelope which is usually essential for virus infection. There can be many potential points of attack for disinfectants in the mature virion, and most disinfectants are not sufficiently specific to react with only one virus component or functional group. The molecular mechanism of disinfectant action is not fundamentally different between bacteria and viruses. Nevertheless, many bacteria are usually disinfected more readily than viruses under the same conditions [12]. This is because initial reactions of bacteria with disinfectants lead to breaches of the bacterial cell wall, with consequent leakage of cellular constituents [13].

Viruses, on the other hand, are non-metabolic units, capable of self-replication only when parasitizing their host cells. It is generally recognized that enveloped viruses are more readily inactivated by most chemical disinfectants than are non-enveloped viruses [14]. Although the mechanisms of virus inactivation by chemicals are poorly understood, lipophilic disinfectants and chaotropic agents should have little difficulty in breaking a normal lipid bi-layer [15]. It is probable, therefore, that the infectivity of enveloped viruses could be destroyed with little or no direct damage to the protein or nucleic acid of the virus. However, it is not known how much damage must be done to the virus envelope before virus infection is prevented. At high disinfectant concentrations, damage to the proteins and nucleic acids of the virion is also likely.

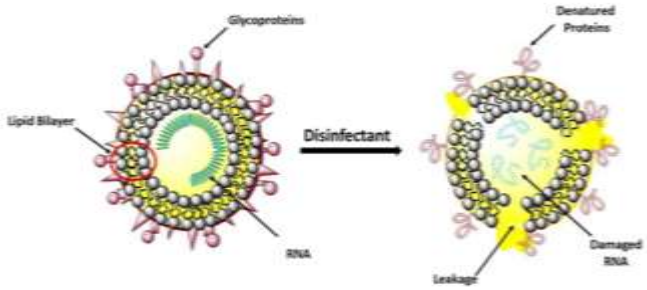

Fig. 1. Virus structure and it degradation

Non-enveloped viruses appear to differ markedly from one another in their sensitivity to many disinfectants [16]. Inactivation of these viruses by chemical disinfectants is assumed to involve damage to either the structural or functional proteins of the virion which are necessary for infection and replication, or to the nucleic acid, or both. The first contact of disinfectant and non-enveloped viruses is made with the protein coat, which usually forms 60 to 90% of the total virus mass [17]. There are obviously many specific sites in virion protein components which are critical for virus integrity, infectivity, or replication. For example, the site on the virus capsid which interacts with the cellular surface is obviously important in all viruses because its damage may interfere with virus infectivity. However, the symmetry of virus structure usually means that such sites are present in multiple copies and, because of this redundancy, damage to the majority or all of these sites may be necessary before virus infectivity is destroyed [18].

Virus types containing several functionally active proteins, such as replication enzymes, may be more vulnerable to the action of chemical disinfectants than those viruses which depend largely on cellular functions for their replication, merely because more intact critical proteins are required [ref]. The secondary and tertiary structure of proteins and nucleic acids leaves specific areas of the molecules more exposed to disinfectant attack.

Then vulnerability of these regions to disinfectants is governed not only by their location in the virion, but also by the hydrophobicity and other aspects of the immediate molecular environment which control disinfectant access [19]. In many viruses, the nucleic acid core of the virus may be well protected from the action of particular disinfectants by the overlying protein. However, damage to the nucleic acid core may account for virus inactivation in some cases where the coat protein is relatively refractory to damage and is permeable to particular disinfectants, or where prolonged access to the virus allows penetration of the disinfectant into the virion [20]. The ease with which disinfectants can penetrate the virus capsid is unknown, and probably varies dramatically between viruses and between disinfectants [21]. The number of potential sites for attack within the target virus increases with the use of disinfectants containing multiple active ingredients with different modes of action; either additive or synergistic effects may be observed.

Because of their extremely small size (approximately 20 to 300 nm in diameter), virus particles have a huge surface-to-mass ratio, approximately 10^7 times greater than man [22]. Therefore, a large proportion of the virion is in direct contact with its immediate surroundings and is greatly influenced by their physical and chemical nature. Larger non-enveloped viruses are often more readily disinfected than smaller ones although there are exceptions to this [23]. In some cases, this may be related to structural considerations in the virion itself which protect the nucleic acid from certain types of disinfectant, in others, it may be because the smaller the particle, the more easily it is shielded from disinfectant action by other contaminating materials [10].

Although the main focus of this review is the use of chemical disinfectants for direct prevention of human and animal viral disease by contaminated surfaces, it must be remembered that viruses of microorganisms important in the food industry can also contaminate surfaces and be transmitted thereby to their respective hosts. Within their target cells, viruses are usually produced as closely packed, crystalline-like arrays [15]. Enveloped viruses are often individually budded from the membrane of their host cells, but many may remain as clumps associated with cellular debris in sloughed cells. Non-enveloped viruses may be released when the host cell bursts, and although some can be found dispersed singly, many remain clumped and/or associated with cellular debris [1,4,7].

Furthermore, individual virus particles often have a tendency to adhere to other particulate matter and surfaces. Therefore, viruses in body fluids and on naturally contaminated surfaces are often found as clumps or aggregates [16]. It is obvious that viruses located at the center of such clumps are likely to be much more inaccessible to disinfectants by virtue of their physical protection by the virus particles or particulates in the outer layer(s). Further protection of the target virus on contaminated surfaces may be afforded by the secretions or excretions in which viruses are invariably embedded when they are shed from the infected host. It is very rare for viruses be the only microorganisms present, although they may be the only pathogen in the contaminated surfaces [25].

Natural virus suspensions may be somewhat viscous and not readily penetrable to disinfectants, especially when dried onto the surface. In addition to the physical protection provided to viruses contaminating surfaces, the matrix of organic molecules and inorganic salts which comprise the body fluids, together with the cellular debris from virus infected or uninfected cells, are likely to react chemically with most disinfectants. This will tend to neutralize the disinfectant and reduce its effective working concentration [20]. The mass of protective material may considerably exceed the mass of the contaminating virus and this may present a severe challenge to the disinfectant [12]. Disinfectant efficacy against a target virus must be measured in terms of the degree of lost infectivity. Such measures are usually feasible only in *in vitro* cell cultures may not necessarily representative of *in vivo* infectivity of the same virus preparation [ref]. It is not always possible to predict, even with knowledge of the chemical structure of a disinfectant, the disinfectant that will most efficiently inactivate which virus. However, under most in-use conditions, it is not practical to use different products for different viruses and therefore, caution should mandate that the choice of infection control product be one that will potentially inactivate all viruses of concern in a particular setting [22].

The difficulty of predicting virucidal efficacy of disinfectants against a variety of viruses is partly due to the unrecognized differences in susceptibility and to variation in the overall properties of the virus preparations. Virus preparations obtained from natural infections differ in the target organs in which the viruses were produced, the composition of the medium in which the viruses were shed, the degree of cell association of the virus, and in the number of infectious virus particles. Even in virus pools produced in the laboratory for the express purpose of disinfectant testing, where the same cell strain has been used for growing the different viruses and the number of infectious virus particles has been standardized, differences can be expected in the degree of virus-cell association [25]. Repeated exposures to inadequate dosage of disinfectants could result to generation of resistant virus strains. It would be interesting to see whether regular use of chemical disinfectant during a prolonged outbreak of a viral infection in an institution could results to differences in the sensitivities of isolates obtained at different periods during the disease outbreak.

It must be emphasized that disinfectant failure cannot be equated with microorganism resistance. Disinfectant failure may be more probably due to the improper use of disinfectants or antiseptics. It can be theorized that viruses may show increased resistance to disinfectants either by changes in the vulnerability of critical points within the virus or by changes in pathogenicity to the host, which may provide them with more passive protection from inactivation [26]. If sub-lethal damage to viruses results in strains with increased resistance to disinfectants, then these changes must maintain or increase the infectivity of the virus in order to be selected for.

3 Types of Chemical Disinfectant

1. Phenols

Phenolic compounds have been in widespread use as germicides over many years, although their popularity has declined due to controversy surrounding some toxicity tests performed in the U.S. and the fact that they cannot be used on food-contact surfaces [27]. Simple and substituted phenols are a complex group of chemicals which are often formulated in combinations in commercial disinfectants. Such phenolic disinfectants often contain alcohols, chelating agents, and anionic detergents or soaps. The antimicrobial activity of phenols is very dependent on the exact formulation and concentration of the active components, temperature, pH, level of organic matter, and other physical and chemical factors.
In general, the more highly substituted phenol derivatives, some of which have antifungal activity, are more selective in their action than the cruder mixed-tar acids. The more selective phenolics, such as triclosan, are often used for skin antisepsis, whereas the less refined products are used for general disinfection [28]. Phenolic compounds can only be used in conjunction with anionic detergents because both cationic and non-ionic surfactants destroy their germicidal activity[ref]. This could lead to practical difficulties if phenolic disinfectants are used on surfaces where residuals from other disinfectant types are still present. The reactive moiety on the phenol molecule is the hydroxyl group, and alkyl substituents affect such properties as partitioning coefficient, reduction of surface tension and species selectivity. Halogen substitution probably has similar effects, but, in addition, it affects the electrolytic dissociation of the phenol derivative, increasing the acid character of the compound with the increasing number of halogen substituents [29].

2. Alcohols

Aliphatic alcohols are among the commonly used disinfectant compounds and the mechanism of their antibacterial action is presumed on: denaturing, lytic, and anti-metabolic. The last of these can be ruled out for all viral agents since virues are not metabolic system, and the second for non-enveloped viruses. Therefore, most of the action of alcohols on non-enveloped viruses at least is primarily protein denaturation. Alcohols efficacy depend on it concentration. If the concentration of an alcoholic disinfectant is dropped below a certain critical level, then they rapidly become ineffective [30]. It has long been known that the bactericidal action of the aliphatic alcohols increases in the series methyl < ethyl < propyl < butyl < amyl. However, for hydrophilic picornaviruses, the reverse was generally true. Ethanol with a minimum concentration of 70% was found to be the only alcohol capable of inactivating all of the viruses tested [31]. Several observations have been made on the susceptibility of human and animal rotaviruses to alcohol disinfection. This may be of particular importance because of the frequent inclusion of ethanol or isopropranol as constituents of topical antiseptics, and human hands may be one of the primary vehicles in rotavirus transmission. Alcohol and alcohol containing disinfectants are effective against animal rotavirus while simple alcohols were ineffective against rotavirus dried in fecal matter [32].

3. Aldehydes

The two principal aldehydes that are commonly used as disinfectants are formaldehyde (methanal) and glutaraldehyde. The former is available as an aqueous solution of approximately 37% (w/w). However, this solution is relatively unstable and undergoes polymerization to form the solid paraformaldehyde. It is, therefore, usually supplied in a stabilized form containing 10 to 15% methyl alcohol. Formaldehyde gas is also used for fumigation purposes because of its antifungal properties. Glutaraldehyde (pentanedial) is a dialdehyde in which both free aldehyde groups react readily under suitable conditions, especially with proteins. In acidic aqueous solution, it is relatively stable, but much less active than at alkaline pH, where it rapidly loses biocidal activity; this is probably due to irreversible polymerization. This discrepancy disappears as the temperature is increased, and at 70°C it is active over a wide range of pH [33]. Commercial preparations of glutaraldehyde, therefore, are usually supplied in acid solution, but many of them also contain corrosion inhibitors and alkaline-buffered

activators which provide disinfectant solutions with a limited stability (14 to 28 d). Specific modifications have been described which either improve biocidal capacity in acid solution or increase the stability at alkaline pH.126.

Formaldehyde and glutaraldehyde are often considered as chemical sterilants due to their extremely broad spectrum of activity. In this regard, formaldehyde, in particular, has been widely used in the production of inactivated viral vaccines. It has been established that most viruses are susceptible to formaldehyde inactivation though, the reaction may be relatively slow. The antimicrobial actions of glutaraldehyde have been reviewed, with emphasis on the broad spectrum and it has been reported that faster inactivation occurred against viruses as well as vegetative and spore forms of bacteria and fungi [34]. Glutaraldehyde was found to be effective disinfectant against rotavirus and other enveloped and non-enveloped viruses even when the virus is dried on a surface in the presence of fecal material or mucin. It has also been reported to be an effective disinfectant for hepatitis B virus [35]. In spite of this, some viruses are more refractory than others to the action of glutaraldehyde [28].

4. Acids

Many organic and inorganic acids are well known as antimicrobial substances, but they are not generally recognized as a class of disinfectants in their own right. In the case of the strong mineral acids, this may be due to problems of both toxicity and corrosion associated with their use. Acids are only in disinfectant formulations pH modulator to enhance the biocidal capacity of other active ingredients. The weaker organic acids often function as preservatives in the food and pharmaceutical industry, but are not now widely used in formulations for surface disinfection [36]. The weak organic acids often have a greater effect than can be accounted for by pH alone, and can be effective constituents of certain disinfectants [20].

Mineral acid-containing disinfectants fall into two main groups. The first type is used primarily for the disinfection of toilet bowls, urinals etc., and may contain quaternary ammonium compounds. The second group consists of general-purpose sanitizers which are classified as either anionic surfactants or iodophores. In both cases, the acid serves to potentiate the action of the other active ingredient. The widespread use of these compounds in the food and dairy industry dictates the choice of phosphoric acid as the mineral acid to avoid problems of toxicity and corrosion. Many viruses are known to be sensitive to low pH, but differential sensitivity of certain viruses to mineral acids is known to occur. Although phosphoric acid was reported to be completely ineffective for swine vesicular disease virus [25], it could readily inactivate foot-and-mouth disease virus and Human rotavirus [32].

5. Bases

Like acids, basic compounds are not generally considered to be a disinfectant class on their own, but, rather, are included in disinfectant formulations as a means of modulating pH. In spite of this, many viruses are susceptible to high pH and many disinfectants are strongly basic in reaction. One has to wonder, therefore, whether all or part of the antimicrobial effects of certain products might be due to the alkaline character of the commercial formulations rather than to other active ingredients present. An example of this may be a domestic bathroom cleaner-sanitizers which have tested against a variety of virus-contaminated surfaces [30].

6. Anionic surfactants

Anionic surfactants are common constituents of phenolic disinfectants, and acidic anionic surfactant sanitizers are widely used in the dairy and food industries and for institutional cleaning. The mechanism of action of anionic surfactants in the inactivation of bacteria is believed to be either through disruption of membrane permeability or by denaturation of enzymes and other cellular proteins [37]. The efficacy of these compounds against enveloped viruses suggest that the primary effects of these compounds may be on the lipid components of membranes, with consequent effects on membrane structure and permeability. Sattar *et al*., investigated the disinfection of surfaces contaminated with two enveloped and two nonenveloped viruses. The addition of sodium lauryl sulphate to the ineffective phenolic formulation improved virucidal efficacy against the enveloped viruses. Similarly, some anionic surfactants were able to inactivate some viruses (rabies and vaccinia) but not polio-1, polio-2, or echo-9 viruses [38]. Acidic anionic surfactants tested against human rotavirus showed poor activity [37] but it should be noted that these commercial products contained phosphoric acid which, as discussed above, was unable to inactivate human rotavirus under the conditions tested. Poliovirus, resistant to synthetic detergents, as noted above, became sensitive when it was exposed to them at a pH of 3 to 5 rather than 7 or greater [38].

7. Cationic surfactants

Quaternary ammonium compounds (QAC) are a large group of cationic surfactants where the hydrogen atoms in the ammonium group are replaced by alkyl and/or aryl substituents. Typically, at least one of the alkyl groups is a long hydrophobic carbon chain, which increases its surface-active properties. Because of their widespread bactericidal activity against both Gram-positive and Gram-negative bacteria, these compounds are widely used as germicides and sanitizers. Although dilute solutions are used in medicine as topical antiseptics for the skin, conjunctivae, and mucous membranes, the most widespread use of QAC is as hard-surface disinfectants. For this purpose, QAC have gained wide acceptance in restaurants, dairies, food plants, laundries, and hospitals, and represent numerically the largest group of disinfectant products available on the market. The efficiency of disinfection by QAC is reduced by soap and most proteins on the alkaline side of their isoelectric points. In spite of this, QAC are commonly applied directly after use of soap and water [26].

8. Amphoteric compounds

Amphoteric agents are more widely used in Europe than in North America. Chemically, they are amphoteric surfactants containing amino acids substituted with long-chain alkyl amine groups, and appear to be less affected by protein than some other disinfectant types. The published literature on the virucidal properties of these compounds indicates that they are effective virucides against a variety of enveloped viruses (herpes simplex, vaccinia, influenza, and vesicular stomatitis); adenoviras 2 was also inactivated, but poliovirus 1 was unaffected. [34.]

9. Peroxides and peracids

Hydrogen peroxide has long been known as a disinfectant, but early preparations were impure and unstable. The production of pure hydrogen peroxide and the addition of stabilizers to the finished product have resulted in a highly stable disinfectant with potential application in a number of different areas. It may be particularly useful for the disinfection of plastics such as surgical implants and soft contact lenses, and has been suggested as an in-line sterilant for the aseptic packaging of liquid food 28]. The mode of action of hydrogen peroxide is through oxidation, not by the hydrogen peroxide molecule itself, but by the hydroxyl free radical formed during metal (optimally, copper)-catalyzed decomposition of the hydrogen peroxide. The hydroxyl radical is highly reactive and can oxidize lipids, nucleic acids, and many other compounds [32].

10. Chlorhexidine and polymeric biguanides

Chlorhexidine is a cationic biguanide, available as dihydrochloride, diacetate, or gluconate salts. The gluconate salt is freely soluble in water and is the most commonly used in disinfectant formulations. However, contact with inorganic anions such as sulphate, phosphate, carbonate, nitrate, and chloride may result in a reduction of the disinfectant activity due to the precipitation of less soluble salts. These anions are commonly found in hard water and many biological fluids. In practice, the commercial formulations may contain cationic surfactants to minimize such precipitation. Chlorhexidine shows a wide spectrum of activity against vegetative cells of Gram-negative and Gram-positive bacteria. It is mainly used as a topical antiseptic in either aqueous (hygienic hand wash) or alcoholic (waterless hand wash or preoperative skin preparation) solution because it combines a rapid action with persistence on the skin surface, and also has a low oral and percutaneous toxicity [27]. Aqueous solutions of chlorhexidine salts are sometimes recommended for general-purpose disinfection, and alcoholic solutions (60 to 90% ethanol or isopropanol) are also widely used in more critical areas.

The main biocidal effect of chlorhexidine is at the level of the microbial cell membrane, causing leakage of cell constituents. It has been shown by a number of investigators that alcoholic chlorhexidine solutions are superior bactericides to aqueous ones. When chlorhexidine compounds were tested in aqueous solution, alone or with a low-level quaternary ammonium compound, they were almost completely inactive against human rotavirus, even in the absence of an organic load [39].

3.1 The Need for Virucidal Testing of Disinfectants

It is important to consider the necessity for virucidal tests on disinfectants and highlight the essential components of suitable testing protocols. The viral diseases which are potentially most amenable to control by disinfectants and antiseptics are those which spread mainly through contact with virus-contaminated animate and inanimate surfaces. Application of effective chemicals to such virus-contaminated vehicles should, theoretically, be able to limit disease transmission. The modes and vehicles of spread for many viral infections are unknown, but it should be assumed, especially in conditions where many humans or animals are housed in close proximity, that *all* viral agents can potentially be spread by contamination of common vehicles as well as by direct contact.

The necessity for chemical disinfection of invasive medical devices which cannot be heat sterilized is undeniable. However, it has often been argued that disinfection may have limited value in controlling the transmission of human viral diseases by fomites, and that lack of compliance with hand-washing guidelines rather than choice of hand-washing agent is to blame for virus transmission by hands. This attitude may have arisen because of the belief that direct contact is much more important than fomite-borne transmission of infections and that many nosocomial virus infections are not preventable. The two notions are valid; however, there also appears to be some misconceptions amongst those who deny the value of disinfecting fomites; the widespread views that many viruses do not survive for long periods outside their natural hosts, and that those that do are often resistant to disinfection, are both incorrect [8].

3.2 Testing Methods for Disinfectants and Antiseptics

Disinfectants can act both by killing microorganisms *in situ* and by removing them during contact. No laboratory test can determine whether disinfectants are truly effective in the field; it can only examine the potential of the products for their proposed use(s). The primary objective of a disinfectant testing protocol, therefore, is to determine whether commercial disinfectants, at the manufacturer's recommended in-use concentration(s), have the potential to accomplish the claims which are made regarding their efficacy under *realistic test conditions.*

The criterion for establishing a claim of virucidal efficacy under laboratory test conditions should depend on the starting titer of the virus, and that all infectious virus should be eliminated. In the field, different viruses will be in varying numbers, which could vary as much as 10^2 to 10^{13}. Even for the same virus, the numbers may differ among different hosts, and the degree of contamination will also affect the number of viruses to be inactivated. This would make for enormous practical difficulties in regulation and standard tests, and the criterion usually set for virucidal efficacy is a 99.9, 99.99% [28, 32.36] reduction in virus titer. However, experience has set the efficacy criterion (3 log10) reduction in organism population. It is very difficult to determine when all viruses have been inactivated. The criteria set for efficacy of hard-surface disinfectants may not be technically achievable by *in vivo* tests for virucidal product efficacy.

The two types of tests are generally used to assess the virucidal potential of chemical disinfectants:

a. *Suspension test:* A known quantity of virus suspension is mixed with a disinfectant at the manufacturer's recommended concentration for a finite contact period. The virus disinfectant mixture is then detoxified by dilution, neutralization, or removal of the disinfectant before virus assay [39].
b. *Carrier test:* A known quantity of virus suspension is dried onto a surface before exposure to a disinfectant for a finite contact period. The virus is then eluted from the carrier and the eluent titrated to assess the degree of virus inactivation. If dilution in the eluent is adequate to stop disinfectant activity, a further detoxification step may not be required.Since the loss of virus infectivity due to the initial drying step and the possible irreversible binding of the virus to the carrier must be taken into account, the procedures for conducting quantitative carrier tests are considerably more complex and time consuming than those for suspension tests.

Most studies on virus disinfection use the suspension test. The suspensions tested range from purified virus in distilled water to naturally shed virus in blood or diluted fecal material. In general, it can be said that the more organic matter present, the less effective the disinfectant in a given set of conditions. Even though the true kinetics of virus inactivation by a given chemical can only be assessed in the absence of any organic and inorganic impurities in the reaction mixture, the results of such tests have little *practical* significance; under

field conditions, disinfectants are always expected to inactivate viruses in the presence of a variety of organic and inorganic materials.

Disinfectant manufacturers acknowledge the problems of disinfecting soiled surfaces, and many of the products marketed carry warnings that they should be applied to pre-cleaned surfaces. However, from the point of view of safety for the cleaning staff and prevention of virus disease transmission as well as practicality, there is an obvious need for products which can be applied directly to a contaminated surface without pre-cleaning.

Chemical disinfection is not the answer to limiting the spread of viral, or other, infections; good hygienic practice should always be the first line of defense. Nevertheless, judicious use of reliable disinfectants and antiseptics can play an important role in this regard. There is a general lack of understanding of disinfectants, their strengths and weaknesses, and the factors which affect their efficacy. It is necessary to consider how a purchaser of disinfectants can have a reasonable assurance that the product which has been purchased is suitable and effective for its intended use.

Most countries regulate disinfectant use in some manner, usually by the registration of products with an appropriate government agency; disinfectant and antiseptic products are often considered to be either pesticides or drugs. In the U.S., hard-surface disinfectants must be registered with the EPA as pesticides, and topical antiseptics are governed by the Food and Drug Administration (FDA). In Canada, some hard-surface disinfectants are considered to be pesticides and others to be drugs, depending on their intended use. In Nigeria, drugs and pesticides are regarded as regulated products and are registered by the National food and drugs administration and control (NAFDAC).

Current disinfectant regulation and testing practices have come under severe criticism from a number of concerned infection control experts, many of whom feel that regulatory authorities have abandoned their mandate to ensure product efficacy [28]. Disinfectant manufacture and sales is a very competitive field. New formulations are introduced frequently and old ones withdrawn, so that the disinfectant purchaser is faced with a bewildering array of choices.

4. Conclusion

Good hygienic practice should always be the first line of defense in disease control. The notion of disbelief in disinfectants could be traced to the observed rate of nosocomial infections and most of the currently marketed disinfectants may be unreliable under field conditions. The inadequacy of the products could result from poor product design and unreliable efficacy test which may lead to the acceptance of ineffective disinfectants. Lack of compliance with hand-washing guidelines is probably the major factor in the spread of infections by hands, but even a high degree of compliance will not help if the hand-washing agent is incapable of removing or inactivating the microorganism(s) causing the infections. Furthermore, even if hands are freed of viral pathogens, inadequate disinfection of fomites can rapidly lead to their recontamination.

There is increasing awareness of the fact that both contaminated hands and fomites as potential vehicles of human pathogenic virus transmission. Contaminated hands of attendant personnel are commonly accepted as the most important factor in the acquisition of nosocomial disease, and effective hand-washing may be the single most important measure in limiting the spread of many infections.

The importance of hand hygiene among food handlers cannot be overemphasized in the prevention of food-borne viral diseases. Very little is known about the effectiveness of hand-washing, either with soap and water or with antimicrobial hand-washes, in preventing disease spread. There is a general lack of understanding of disinfectants, their strengths and weaknesses, and the factors which affect their efficacy. Nevertheless, judicious use of reliable disinfectants and antiseptics can play an important role in this regard.

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