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Original Research Article

A Study of the Effect of Sterilizers on the Bacterial Load Resulting from the Process of Administrative Exchange and Office Activities

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Abstract: The purpose is to compare the efficacy of various hand rub brands against specific skin-colonizing bacteria due to the widespread use of hand disinfectants, due to COVID-19's contagious nature, highlighting the importance of hand hygiene in reducing nosocomial infections and cross-transmission. Thirty workers from the Iraqi Scientific Research Commission's laboratories, split into two groups for administrative and scientific work to provide bacterial samples. A total of thirty samples of bacteria were acquired. Based on their popularity and frequent use in Baghdad, fifteen different hand sanitizers from various brands were purchased for this study. The well diffusion agar test was used in the experiment to assess the bacterial activity of the hand sanitizers used in the study. The majority of sanitizers were unable to eradicate every type of bacterial species identified during the study. The gel sanitizer with 93% ethyl alcohol was successful in eliminating every bacterial strain identified, except for one species. Meanwhile, a liquid sanitizer with 70% alcohol was unable to eradicate even a single strain of bacteria. Our study's findings demonstrated that the alcohol-free disinfectant outperformed the sanitizer with an alcohol content ranging from 60 to 93% in terms of bacterial control.

Keywords: Hand sanitizers, bacterial effectiveness, COVID-19, well diffusion agar test.

INTRODUCTION

Most infections are spread by hand (Kolhapure and Mondal, 2004). According to Oranusi *et al.,* (2013), these are the ensuing reasons for nosocomial infections and food-borne illnesses. Because COVID-19 (Coronavirus Disease-2019) is contagious, a worldwide problem, with serious social, health, and economic impact (Marin *et al.,* 2020). It has become a major global public health concern, prompting extensive use of hand disinfectants (Jairoun *et al.,* 2021). The fast dissemination of SARS-CoV-2 occurred swiftly across Iraq and various nations globally, leading to a widespread viral pandemic (Kareem and Aubaid, 2024).

SARS-CoV-2, the virus that causes COVID-19, can infect surfaces and spread for up to nine days (Chan *et al.,* 2019; Kampf *et al.,* 2020). Fomite and aerosol are two ways that SARS-CoV-2 can spread, according to recent studies. The virus can remain infectious in aerosols for hours and on surfaces for days, depending on the type of inoculum that is created (Van Doremalen *et al.,* 2020). Therefore, it is essential to break the virus's chain of transmission via tight infection control measures and contact isolation (Thomas *et al.,* 2014). In addition to face masks, proper hand hygiene is crucial because hands can become contaminated by respiratory droplets from coughs and sneezes of patients directly or indirectly through contact with surfaces, which can then help the disease spread and transmit (Seto *et al.,* 2003). The United States has supported and promoted hand hygiene through hand washing or the use of hand sanitizer due to the risks posed by this disease, according to the Centers for Disease Control and Prevention (CDC) (Am Med. 2020).

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Hand hygiene involves washing or sanitizing hands to kill microorganisms. It's a simple, effective, and economical procedure that reduces nosocomial infections and cross-transmission. Hand hygiene is sometimes said to be "an alternative to hand washing when there aren't any opportunities to use portable water or wash your hands in warm water and soap (Pickering. *et al.,* 2013) Hand hygiene is offered in a variety of formats (gel or liquid), as well as alcoholic and non-alcoholic compositions. The active components in antiseptics give them their antimicrobial properties. Ethyl alcohol and isopropyl alcohol are the two most commonly used active chemicals in alcoholic sanitizers, whereas propylene glycol, glycerin, and polyacrylic acid are some of the inactive ones (Ikegbunam *et al.,* 2013).

Benzoyl chloride is reported to be more susceptible to contamination from gram-negative bacteria and to be less effective against these pathogens than alcoholic hand sanitizers (Al-Salihy, 2011). In a way, sanitizers and microbial proteins are similar in that they both function by denaturing proteins. Additionally, alcohol denatures the fluids, leading to bacterial dehydration. Conversely, benzalkonium chloride exhibits anti-species activity and possesses the ability to alter protein and cell membrane structures (Dixit *et al.,* 2014). Alcohol-based hand sanitizer (ABHS) is advised by the World Health Organization (WHO) because of its demonstrated benefits of quick action and wide range of microbicidal activity that protects against viruses and germs (Sattar,2004). Its efficacy against non-enveloped viruses is still up for debate, but Manocha *et al.,* (2003). In this study, the efficacy of various hand rubs against bacteria that colonize the skin was compared. Since all products marketed as germicidal are inefficient in reducing the concentration of microorganisms on hands, statistics should be made available to the public to choose better and more protective hand antiseptics against disease-causing bacteria. The current study aimed to evaluate the efficacy of various hand rub brands against specific skin-colonizing bacteria. It is crucial to give the public information to enable them to select better and more protective hand antiseptics against disease-causing germs, as not all products marketed to the public as germicidal are effective in reducing the concentration of microorganisms on hands.

Materials and Methods

A total of thirty volunteers were chosen to gather bacterial samples for the research; prior consent was acquired from each subject before the experiment began. They were chosen based on the absence of any clinical signs of skin disorders, bruising, or infection, and they were split into two groups of volunteers from the Iraqi Scientific Research Commission administrative and scientific laboratories. After sanitizing their hands with a sterile swab, the thirty bacterial samples were obtained. They were then grown directly on culture media—Nutrient agar for the isolation and purification of positive bacteria and MacConkey agar for the isolation and purification of negative bacteria—and incubated for twenty-four hours at 37°C. In this study, fifteen distinct hand sanitizers were acquired from various brands and utilized from the numerous options available in the local marketplaces, taking into account their popularity and frequent usage in Baghdad.

All of the sanitizers that were chosen for the study had an alcohol content of 62% and above; all of them contained alcohol in their composition, except for sanitizer No. 6, which is alcohol-free and disinfectant, Table (1), which lists the chemical composition of the sanitizers used in this study. The sanitizers were arranged in a numerical sequence (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15).

Table 1: The chemical composition of sanitizers according to their sequence in the study

No	Type of sanitizer	Chemical structure and active material	Active ingredient
1	Liquid/commercial	70% alcohol	Ethanol
2	Gel/commercial	70% alcohol	Ethanol
3	Liquid/commercial	70% alcohol	Ethanol
4	Gel/local	93% alcohol	Ethanol
5	Gel/brand	Not exist	/
6	Liquid/brand/ disinfectant	Alcohol free	Benzylkonium chloride
7	Liquid/brand	70% alcohol	68% Ethanol 3.7% Methanol
8	Gel/children sanitizer/brand	70% alcohol	Ethanol
9	Liquid/brand	Not exist	/
10	Gel/brand	70% alcohol	Ethanol
11	Gel/brand	62% alcohol	Ethanol
12	Gel/brand	Not exist	Ethanol
13	Gel/brand	67%	Ethanol
14	Gel/brand	Not exist	Ethanol
15	laboratory preparation according to WHO	96%	Ethanol

Antibacterial efficacy evaluation of hand sanitizers experiment

To assess the bacterial activity of the hand sanitizers used in the study, the well diffusion agar test was utilized in the experiment (Magaldi *et al.,* 2004). Using a spectrophotometer set to 625 nm, the suspension turbidity of each type of bacteria was compared to that of the McFarland standard (0.5 McFarland standard, prepared in the lab). Bacterial strains were created by transferring the inoculum from recently stored cultures and growing them in a nutrient broth tube (Törün , 2023). Mueller-Hinton agar was prepared per the preparation fixed on the culture media box, sterilized in the incubator at 37°C, and cooled down on a flat surface before being streaked with inoculum; after solidification and streaked with all the test organisms, Mueller-Hinton agar was poured as a basic medium in the experiment to evaluate the sanitizers' effectiveness. Using a sterile 6 mm cork borer, four identically sized (4 mm) wells were removed from the agar plate, leaving a fifth well in the middle. The agar plugs were then disposed of with a sterile needle. Next, each of the four wells received fifty microliters of hand sanitizer, with the central well serving as the control and filled with an equal volume of sterile water; dishes were incubated 37 ° C for 24 hours. After each experiment, the width of any inhibition area was measured, and the inhibition zone, which showed the test organism's level of susceptibility or resistance to the antibacterial agent, was examined to record the results.

Statistical assay

The SAS (2018) software was utilized to ascertain the impact of variance variables on the study parameters. In this study, the chi-square test was utilized to compare percentages (0.05 and 0.01 likelihood), statistically significant.

Results and Discussions

The COVID-19 pandemic caused significant rates of infection and mortality, posing a major threat to the whole society. As a result, hand sanitizers are frequently utilized in residences, public spaces, and medical facilities (Marumure *et al.,* 2020). Following the global health conditions caused by the corona virus outbreak as an epidemic, the significance of utilizing hand sanitizers has increased (Matatiele *et al.,* 2022); The World Health Organization has authorized the use of alcohol-containing hand sanitizers as one of the most important methods for protection against the virus, as well as for maintaining clean hands at all times, particularly when soap and water are not available. This will lessen the risk of the virus spreading from the hand to one area of the face, which serves as a host for it because the virus depends on living membranes for reproduction and population growth (Golin *et al.,* 2020). In this study, nine different bacterial species were isolated and purified from the hands of the Iraqi Scientific Research Commission employees, table 2.

Table 2: The different bacterial species isolated from the Iraqi Scientific Research Commission employees' hands

Gram stain	No	Percentage (%)
Positive	4	44.44
Negative	5	55.56
Total	9	100%
Chi-Square (χ^2)	---	0.112 NS
P-value	---	0.782

NS: Non-Significant.Gram Positive: Staphylococcus sciuri, Staphylococcus lentus, Staphylococcus vitulinus, Dermacoccus nishinomiyaensis* and *Kocuria rosea*). Gram Negative: *Pseudomonas luteola, Pseudomonas fuorescens, Sphingobacterium thalpophilum* and *Sphingomonas paucimobilis*. Fifteen hand sanitizers were purchased from Iraqi local markets. Each one had given a serial number from 1 to 15 during evaluating their microbial activity, table (3), fig (1&2).

Table 3: The results of examining the biological efficacy of the sanitizers used in the study with their effect on the isolated bacterial species

Bacterial Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pseudomons luteola	R	R	S	S	R	S	R	R	R	S	R	S	S	R	S
Staphylococcus sciuri	R	R	S	S	R	S	R	S	S	S	R	S	R	R	R
Dermacoccus nishinomiyaensis	R	R	R	S	R	S	R	R	R	R	R	R	R	R	S
Sphingomonas paucimobilis	R	R	R	R	R	S	R	R	R	R	R	R	S	R	S
Pseudomonas fuorescens	R	R	R	S	S	S	R	R	R	R	S	R	R	R	R
Kocuria rosea	R	R	R	S	S	S	R	R	R	R	R	R	R	R	S
Sphingobacterium thalpophilium	R	R	R	S	S	S	R	R	S	R	R	R	R	R	R
Staphylococcus lentus	R	R	S	S	R	S	R	R	S	S	S	R	R	R	R
Staphylococcus vitulinus	R	R	R	S	R	S	R	R	S	R	R	R	R	R	R

S: Bacteria sensitive to sanitizer. R: Bactria resistant to sanitizer

Fig. 1: The antimicrobial efficiency of some hand sanitizers on different bacterial genus

Fig. 2: The Antimicrobial efficiency of some hand sanitizers on different bacterial genus

Most of the sanitizers failed to kill all the bacterial species diagnosed in the study, the sanitizers (1, 2, 7, 8) failed to kill half the number of diagnosed bacteria, as the hand sanitizers No. 1, 2, and 7 failed in giving any inhibition zone against bacteria, where all the species were resistant to it, while hand sanitizer No. 8 had given (1 mm) inhibition zone against *Staphylococcus sciuri*, While the sanitizers (3, 5,10) had a slightly greater effect in killing the bacterial species, as it gave an inhibition zone with different measurements of less than 2 mm against different species, while the sterilizer No. 9 gave four inhibition zones, three of them were against different bacterial species of the genus Staphylococcus, while the hand sanitizer No. 4 recorded its ability to kill all bacterial species except the genus *Sphingomonas paucimobilis*. While hand sanitizer No. 6 recorded absolute microbial activity in killing all bacterial species without exception by recording inhibition zones of different measurements, Table (3).

Through our experiment and from Table (3), it was discovered that the sanitizer number 4 in the form of gel with an ethyl alcohol concentration of 93% succeeded in killing all the bacterial strains diagnosed in the study except for one bacterial species, while the same sanitizer brand in the liquid form with an alcohol concentration of 70% (sanitizer no. 13) had no ability to kill As a single strain, this supports that a gel formulation of the sanitizer may be successful at a higher alcohol concentration in killing bacteria and this is consistent with the results of Greenaway *et al.* (2018). They found that gel and foam formulas were more desirable than liquid. In this study; the administrative employs had a common bacterial species of (*Kocuria rosea*) due to the widespread use of public user interfaces, such as computer keyboards and mice, which can operate as fomites reservoirs for the spread of microbes due to regular cutaneous contact with hands.

It has already been shown that computers can function as fomites in the healthcare industry (Huber and Pelon, 2005); in the workplace, it is also known that germs can contaminate the office space, which includes the computer and mouse (Enemuor *et al.,* 2020). It is also known that germs can contaminate workplace spaces, such as computers and mice (Enemuor *et al.,* 2020); keyboards can operate as pathogen reservoirs in non-hospital settings (Eguia and Chambers, 2003). Hand cleaning before and after contact with keyboards and mice, together with the possibility of bacteria being transferred from mouse devices and computer keyboards, should greatly lower the risk of contamination and cross-transmission (Hasan *et al.,* 2015). It has been demonstrated that surface bio-contamination contributes to outbreaks of nosocomial and community-acquired illnesses by either serving as a reservoir or as a food source for disease transmission (Nwankiti *et al.,* 2012). The importance of studying the biological effectiveness of hand sanitizers available in the local Iraqi markets, because the World Health Organization (WHO) has developed a protocol that regulates the manufacture of an effective hand sanitizer with a high killing rate of microorganisms generally, whether they are viruses or bacteria (WHO, 2010), Most of the commercial hand sanitizers that are marketed contrary to that protocol to achieve material profits away from the consumer interest. And since the hands are the most important means of disease transmission, dirt and pathogens from different microorganisms, it is the only way to follow up and complete all the vital activities of the human being, so it is the real source of danger. The study proved that the results of the bacterial diagnosis from the hands of administrative staff of the Iraqi Scientific Research Commission are similar in bacterial genus, according to the type of administrative work and the use of hands in transferring paper and the use of office materials such as notebooks, pens, desks and others, while the hands of scientific staff from the same ministry had different bacterial genus from one volunteer to another, and differed between pathogenic genus and normal flora, despite that some ministry volunteers do not use hand sanitizer.

The sterilizer No. 4 was effective in killing microbs, despite being a disinfectant and not a sanitizer; it does not contain any alcohol percentage in its components. our study showed the bacterial genus Sphingomonas paucimobilis was the most resistant bacterial genus to sanitizers, *Sphingomonas paucimobilis* shown by Canvas Prints as in fig. (3).

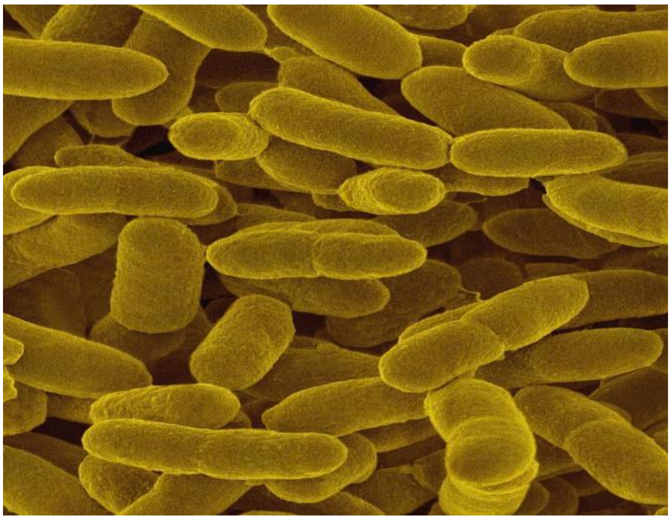

Fig. 3: Sphingomonas paucimobilis shown by Canvas Prints

It is a Gram-negative bacterium that is known to be resistant to many chemical disinfectants. This bacterium shows remarkable resistance against a wide range of disinfectants, posing a challenge in infection control, especially in hospitals and health facilities, this is due to several mechanisms:

- The structure of the outer membrane: S. paucimobilis has a unique outer membrane that contains sphingolipids instead of lipopolysaccharide (LPS), which reduces the permeability of disinfectants (Naka *et al.,* 2003).
- Secretion of lysosomal enzymes: Some strains of S. paucimobilis produce enzymes such as dehydrogenase that break down toxic compounds (Stolz, 2009).
- Biofilm formation: These bacteria form biofilms that enhance their resistance to disinfectants (Teitzel & Parsek, 2003).

As it showed its resistance to all sanitizers except sanitizer No. 6, and the bacterial genus Pseudomonas luteola was the more sensitive bacterial genus to sanitizers, which was killed by seven sanitizers from the 15 sanitizers used in the study, as shown in figs (2 and 3). Which is contrasted with search results Kumar *et. al.* (2015) Where they found that some brands of sanitizers are effective on a limited range of bacteria used in their study, and that the large number of sterilizers randomly manufactured in the local markets makes them useless in use because they are ineffective.

Evaluating the effects of sterilizers on some viruses and bacteria

The use of alcohol-based hand sanitizers (60-95%) has become one of the mainstays of COVID-19 prevention. However, the effectiveness of these sanitizers against microorganisms varies based on their genetic makeup and cell wall properties. In this study, the effect of disinfectants on a sample of these bacteria was analyzed, focusing on the mechanism of their resistance or susceptibility to disinfectants.

Gram-positive bacteria (e.g. Staphylococcus sciuri and Staphylococcus lentis)

Gram-positive bacteria, such as Staphylococcus sciuri and Staphylococcus lentus, are more sensitive to alcohol-based sanitizers than Gram-negative bacteria, because their cell wall contains a thick peptidoglycan layer that reacts with alcohol, resulting in the stripping of proteins and lipids and destruction of the cell. A study showed that alcohol at a concentration of 70% reduces the effectiveness of these bacteria by up to 99.9% within 30 seconds (Kampf *et al.,* 2018).

Gram-negative bacteria (e.g. Pseudomonas luteola and Pseudomonas voricensis)

Gram-negative bacteria such as Pseudomonas luteola and Pseudomonas fluorescens possess a lipopolysaccharide (LPS)-containing outer membrane that reduces alcohol permeability, which may increase their resistance. However, studies indicate that alcohol at a concentration of 80% or higher is able to penetrate this membrane and inactivate essential proteins (McDonnell & Russell, 1999).

Dermacoccus nishinomiyaensis and Sphingomonas paucimobilis

Dermacoccus nishinomiyaensis belongs to Gram-positive bacteria, but may show relative resistance due to its biofilm formation. *Sphingomonas paucimobilis* (Gram-negative) has a unique LPS-free membrane, which may make it less resistant to alcohol than other Gram-negative bacteria. A study found that 70% alcohol is 95% effective against them (Rimstad *et al.,* 2020).

Sphingobacterium and Thalassolituus

Some genera such as Sphingobacterium show resistance to disinfectants due to their production of alcohol-degrading enzymes (e.g. catalase). Thalobacterium, which lives in saline environments, may react differently to disinfectants due to its adaptation to osmotic stress. There are not enough studies on the effect of alcohol on them, but higher concentrations (90%) are assumed to be more effective (Boyce, 2016).

Staphylococcus aureus and Kocuria rosea

For Staphylococcus aureus, a common human pathogen, alcohol is effective against it, but some methicillin-resistant strains (MRSA) may develop biofilm mechanisms that reduce the effectiveness of disinfectants. Cocoria rosea (Gram-positive) is sensitive to alcohol at a concentration of 60% (Fraise *et al.,* 2013).

Resistance of viruses and bacteria to alcohol-based sanitizers: The role of genetic mutations and the effect of alcohol concentration

Microbial resistance to alcohol-based disinfectants is a growing health challenge, especially as the effectiveness of disinfectants varies based on alcohol concentration (ethanol or isopropanol) and microbial genetics. Studies show that some viruses and bacteria can develop resistance mechanisms via genetic mutations that allow them to survive even when exposed to conventional alcohol concentrations (60-90%), which are typically considered to be inhibitory to microbes.

Bacterial Resistance to Alcohol Sterilizers

Bacteria such as Staphylococcus aureus (Staphylococcus aureus) have shown increased resistance to low-concentration alcohol sanitizers (less than 60%), through genetic mutations that promote the production of proteins called Efflux Pumps, which expel alcohol molecules out of the cell, or changes in cell membrane structure to become less permeable. A study published in the Journal of Antimicrobial Chemotherapy (2020) indicated that methicillin-resistant S. aureus (MRSA) strains developed mutations in the mprF gene, which modifies the electrical charge of the cell membrane, reducing alcohol binding, (Adame-Gómez *et al.,* 2020).

Virus resistance to sterilizers

Unenveloped viruses (e.g. norovirus) are more resistant to alcohol than enveloped viruses (e.g. influenza virus), due to the absence of the lipid layer that alcohol targets. However, recent research has shown that some enveloped viruses may develop mutations in envelope proteins (such as the Spike protein in coronavirus) that make them less susceptible to high alcohol concentrations. A study in PLOS Pathogens (2021) showed that mutations in the S-protein gene led to increased stability of the viral envelope, reducing the effectiveness of alcohol by 70% in inactivating the virus, Laporte, *et al.,* (2021). In some Iraqi patients which had COVID-19; levels of miR-23a-3p were markedly elevated, but the levels of the serum miR-195-F1 gene were shown to be substantially downregulated (AL-Waely and AL-Ahmer, 2024).

Role of alcohol concentration in inducing resistance

The optimal alcohol concentration for killing microbes is 70-90%, as higher concentrations (e.g. 95%) cause rapid dehydration of the cell membrane without penetrating it. With prolonged use of sub-optimal concentrations (below 60%), microbes are under evolutionary pressure to develop genetic mutations that promote survival. According to a World Health Organization (WHO, 2022) report, improper use of sterilizers has contributed to the emergence of increasingly resistant bacterial and viral strains, World Health Organization (2022).

Genetic Mechanisms of Resistance

The main genetic mechanisms include: Mutations in genes responsible for DNA repair (such as the RecA genes in bacteria). Modification of enzymes involved in alcohol metabolism (e.g., Alcohol dehydrogenase). The formation of biofilms that protect microbes from alcohol penetration, as demonstrated by a study in Applied and Environmental Microbiology (2019). Jones, L. & Patel, K. (2019).

CONCLUSIONS

Based on the findings of the study and previous discussions, the following conclusions can be drawn:

- Superiority of alcohol-free disinfectants: The results showed that alcohol-free disinfectants were superior in controlling bacteria compared to disinfectants with an alcohol concentration between 60% and 93%. This suggests that relying on alcohol as the main sterilizing agent may not always be the best option, especially in the context of resistant or biofilm-forming microbes.
- The role of physical composition in sanitizer efficacy and user acceptance: The physical formulation of the disinfectant (gel, liquid, foam) plays a crucial role in consumer acceptance. Gel formulations have been associated with discomfort or dirtiness for users, despite being the most effective in eliminating bacteria.

In contrast, liquid or foam formulas may be more suitable for daily use because they are easy to apply and leave no visible residue, enhancing users' adherence to hygiene protocols.

- Effect of combined concentration and formulation: Although gel formulations may be more effective at killing bacteria when used with high alcohol concentrations (e.g., 93%), this formulation may reduce user adherence due to undesirable side effects (e.g. stickiness or residue). This discrepancy between microbiological efficacy and user experience suggests the need to balance scientific and behavioral factors when designing sanitizing products.

Practical Recommendations:

Develop Hybrid Formulations: Combine effective non-alcoholic ingredients with optimized physical formulations (such as foams or fast-drying liquids) to enhance efficacy and user acceptance.

User Education: Educating the public on the importance of optimal concentrations and proper use of disinfectants, especially in medical settings where infection control is critical.

Future Studies: Testing the interaction of physical formulations with different types of active ingredients (such as oxidizing agents or enzymes) to strike a balance between efficacy and convenience.

These findings highlight the need for an integrated approach to hand sanitizer design, combining chemical innovation with an understanding of human behavior to sustainably achieve public health goals.

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