The Efficiency of Hand Sanitizers Against Microbes From Coins

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Research

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Abstract

Objective:

This study aimed to examine the effectiveness of hand sanitizers against bacteria from circulating coins during COVID 19 era.

Method:

22-coin samples have been collected by the research group from taxi drivers in Nablus city, Then the samples were transferred in a sterile plastic bag to the Microorganism Laboratory (17B1140) at the Faculty of Medicine and Health Sciences at An-Najah National University. The collected coins were swabbed with normal saline and cultured on nutrient agar for enrichment. After their appearance, the grown colony has been sub-cultured on Blood, MacConkey, and Uri select, for identification with the assistance of microscopical examination and chemical tests for confirmation. To answer the question of the study, four different sanitizers have been applied to measure their antimicrobial activity against microbes from circulating metal coins.

Result:

The results showed that sanitizer A (Hand sanitizer gel) is the most effective one of the four sanitizers that were used, followed by sanitizer (D)(HiGeen )(JO), then sanitizer (C)(National ) (PS), while sanitizer (B)(Icona London) (UK) was the least effective one.

Conclusion:

The research group have conducted research on coins circulated from taxi drivers during COVID 19 era, hence the number of isolated bacteria was low, due to the current sterilization and prevention behaviors like the using of sanitizers containing 70% of alcohol, which was the best among the used types in the current study as well as it has been observed that excessive use of alcohol-based hand sanitizers can cause microorganisms resistant and or less affected to repeated use of hand sanitizer. Hence, Hand sanitizers may need to be used appropriately in combination with other procedures.

1- Introduction:

Multiple studies have been carried out to explore the existence of germs and microbes on the different types of currency, Gedik, Voss and Voss (2013) found that the Romanian currency showed higher levels of Staphylococcus aureus, Escherichia coli, and Vancomycin-Resistant Enterococci (VRE) survival that lasted 3 to 6 hours of drying. (1)

Furthermore, among the most present microbes on paper currency and coins, 98.4% of the bacteria were heterotrophic, 87.3% of the currency held coliform bacteria, and Staphylococci was present with a percentage of 79.4% (2).
The Sudanese currency included various germs living on its surface, 23% of it was Klebsiella pneumonia, 15% was Bacillus mycoides \(^{(3)}\). While the Indian currency was found to be contaminated with 6 different bacterial species, including Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Micrococci and Pseudomonas \(^{(4)}\).

As for the Palestinian currency and banknotes, Sawalha and Mowais (2012) found that Palestinian currency had 71% of Escherichia coli, 9% Staphylococcus aureus, 8% Proteus, 4% Bacillus, 3% Shigella, 3% Salmonella, and 2% Klebsiella, asserting that dirty currency and banknotes are highly contaminated compared to newly issued money \(^{(5)}\).

Researchers also found that coins are the most contaminated forms of currency, followed by polymer and paper currencies \(^{(2)}\). Where the currency used in public transport and by butchers and food sellers was the most transmitters of pathogens on currency \(^{(6)}\). Other risk health factors were found to be associated with the way people handle money, according to Chomba and Mwamainda (2016) keeping money undergarments directly contacted to the skin and using saliva to count money \(^{(7)}\).

It is good to know that kind of isolated bacteria can vary between studies, because of the difference in methods used, season, environmental conditions, sort of money (coin or banknote), or local community flora, in general, Gram-positive bacteria were the most predominant \(^{(1)}\). Germs and microbes grow on the surface of currency are related to its production and material, currency production input includes using organic material and its exposure to a sunbeam, humidity, and temperature \(^{(8)}\). The notable about these germs and microbes living on the surface of money is that they are penicillin, cephalosporins, and multidrug-resistant \(^{(3,9)}\).

Now, in the era of COVID19, prevention is pricy more than ever, considering the events still taking place due to the worldwide spread of the various and its ferocity, the virus lives for several hours to days depending on the environment according to the WHO (2020) \(^{(10)}\). A study conducted by Akter et al. (2021) isolated the SARS covid-19 virus from Bangladeshi money and banknotes, found that the virus does exist on the currency, where the virus lives in 22° and 65% humid environment for 4 days on banknotes \(^{(11)}\).

Conclusively, circulating currency among people constitutes an opportunity for germs and bacteria to be transmitted from the infected person to the healthy person through it, which requires taking precautions and prevention to address the risks of infection and limit the spread of diseases, and washing hands is one of the most important of these measures, especially before eating, as banknotes contain many types of germs and bacteria. Hand sanitizer may have little effect in killing certain types of bacteria, such as Norovirus and Clostridium difficile, and it cannot remove physically harmful chemicals, unlike hand washing. In some cases, alcohol-based hand sanitizers may not be effective against microbes, for example, in cases which hands are greasy and soiled with visible materials from outdoor activities. So, in cases like dealing with food, playing sports on soil, we can't use hand sanitizers because contaminants produced from these activities and similar ones can't be removed by hand sanitizers. On the other hand,
some cases like hospital healthcare worker are more likely to be contaminated with pathogens which can be removed by hand sanitizers (12).

The efficiency of hand sanitizers against germs and microbes has been investigated thoroughly and widely across the world, where most of the literature reported a positive effect of hand sanitizers on fighting microbes (13,14). The sanitizers showed inhibitory activity against Klebsiella pneumonia and Staphylococcus aureus (5). And alcohol-based sanitizers are more effective than water-based sanitizers (15).

However, not all sanitizers work against all pathogens, in other words, one sanitizer is effective against one type of germs but not the other according to Ochwoto et al. (2017) (16). This effectiveness is determined by several factors including the type and concentration of alcohol, formulation and the nature of product, presence of excipients, applied volume, contact time and viral contamination load (17).

Hand cleanliness will be broadly perceived as a large portion vital in keeping the transmission of contamination especially in the case of disease (18). It keeps both endogenous and exogenous infections for patients (19). Those profit about hand sanitizer may be the capacity with battle germs the point when water and cleanser are not instantly available (20). This will decline the transmission of microorganisms as well as patients, morbidity, mortality, also costochondritis connected with healthcare-associated infections (HCAI) (21).

At fixation from claiming, 60% ethanol could worth of effort against germs generally, anyhow not coronaviruses particularly. Investigations indicated that 80% will be successful against concealed infections inside 30 seconds (22).

Hands are the essential method of transmission of organisms and contamination. Hand cleanliness is a straightforward and most affordable method for forestalling contamination (15). Hand-cleanliness medications affected lessening intense gastrointestinal ailment (23). Antimicrobial handwashing specialists were the strongest in bacterial evacuation, though waterless specialists showed variable viability (24). Type and concentration of alcohol, contact time, and viral contamination load are critical factors that determine the effectiveness of hand sanitizers (16).

The fact that germs and microbes can live for long periods on the surface of the different sorts of currency is frightening, it is denoting that pandemics such as COVID-19 can be fatal to all humanity, also, it is signifying the possibility of another pandemic that might be more lethal because as we know microbes, especially viruses that evolve with time and is becoming more drug-resistant, and considering that currency is a daily used item and handled by a limitless number of people, the risk is rising with time, therefore, studying the microbes living on the currency, in its different types, is vital as a preventative measure, such studies uncover the types of germs, living period and the environments of growth for these microbes, which by consequence, will aid the health authorities along with monetary authorities develop methods to decrease the risks associated with currency being a factor of pandemics source (25).
Many studies have been conducted investigating the microbes growing and living on the surface of currency around the world, however, as far as the researchers know, there is only study conducted in this field by Sawalha and Mowais (2012) in Palestine\(^5\), but no study has been conducted during COVID-19, which credits the study of novelty aspect. Moreover, with the rising rates of COVID-19 cases in Palestine, it is important to conduct this study and uncover safety habits among Palestinian people, as to provide authorities with the outcomes hoping they would take action to control it.

In the same filed, specialists and authorities have made it mandatory to use hand sanitizers in public places and all working sectors following the WHO guidelines, where many varieties of hand sanitizers are being used by the Palestinian population with no studies supporting or defining the most effective type, therefore this study is examining the effectiveness of hand sanitizers against microbes during COVID era, but the research group were not able to include the identification of COVID-19 presence due to the seriousness of the current situation.

2- Materials And Method

The research group have used personnel protective equipment and collected 22-coin samples from the category of shekel from taxi drivers in Nablus city, they put each coin separately in a sterile bag and then transported it to the Microorganism Lab (17B1140) in An-Najah National University, Nablus, Palestine.

2.1 Bacteria isolation from coins:

The research group have swabbed each coin totally with a cotton swab which was wet with normal saline, then cultured it on nutrient agar and incubated it for 24 hours at \(37^\circ\)C to observe the presence of bacteria. After incubation, the grown bacteria were sub cultured into Blood, MacConkey and, Uri select agar. Then the bacteria were identified microscopically and by biochemical tests.

2.2 Biochemical tests:

First, a catalase test on all isolates have done, the results showed that the samples have gram-negative which means that they belong to streptococcus genera and gram-positive which means that they belong to staphylococcus genera.

Second, to distinguish between staphylococcus aureus and staphylococcus epidermidis, a coagulase test has been conducted on the catalase-positive bacteria so, bacteria with coagulase positive is staphylococcus aurous and bacteria with coagulase negative is staphylococcus epidermidis.

After that, species have been inoculated on Uri-select agar to be sure of the types of bacteria that have been obtained by comparing the color of the colonies after 24hrs incubation period.

Finally, a wet mount for the isolates was done, and observed under microscope using the immersion lens (100x), and the detected bacteria have been presented in table 1.
2.3 Source of Hand sanitizers:

Four hand sanitizers (sanitizer A, sanitizer B, sanitizer C, and sanitizer D) certified for using by the Agency for Food and Drug Administration and Control (FDAC), (see table 2) were purchased from a pharmacy in Nablus and kept in the lab until tests have been conducted, (fig.1).

2.4 Isolated bacteria:

The isolated bacteria from coins were Staphylococcus saprophyticus, staphylococcus aureus, staphylococcus epidermidis, (see fig.2).

2.5 Assessment of sanitizers 'antibacterial effectiveness:

The agar diffusion method has traditionally been used to determine the antibacterial activity of any substance. Instead of using the agar dilution method, the Kirby-Bauer method (Agar disk diffusion method) was used in this investigation. The advantages of the agar disk diffusion approach include the sanitizer's chemical properties remaining intact, as well as the fact that it is a simple and less technique-sensitive process. It permits direct comparison of all sanitizer groups, demonstrating which group has the best chance of eradicating that specific test organism (26).

A concentration of bacteria that approximates the 0.5 McFarland turbidity standard have been prepared for each identified bacterium. A lawn culture was obtained by streaking the swab on Mueller-Hinton agar, and in order to have a uniform culture; the plate was streaked with the swab in one direction, then it was rotated 120° and was streaked again, was rotated another 120° and was streaked one last time. Then hand sanitizers were analyzed in pure forms for assessment of the antibacterial susceptibility by disc diffusion method under CLSI (Clinical and Laboratory Standards Institute). Sterile filter paper discs impregnated with sanitizer then placed on MH (Mueller Hinton) agar surface and incubated at 37°C for 24 hrs, after that the inhibition zones were measured in mm (see fig. 3).

and the results were compared to typical antibiotic discs for bacterial infections, such as Imipenem, (see fig.4).

The results were also compared by the 70% Ethanol discs diffusion method, (see Fig.5 here).

3- Results:

The results showed that sanitizer (A) (Hand sanitizer gel) was the most effective one against 7 isolates (mostly Staphylococcus epidermidis and Staphylococcus Saprophyticus), followed by sanitizer (D) (HiGeen ) (JO) against 5 isolates (mostly Staph epidermidis), followed by sanitizer (C)(National ) (PS) which has maximum inhibition against 3 isolates (mostly Staphylococcus aureus and Staphylococcus epidermidis), while sanitizer (B) (Icona London) (UK) has no antimicrobial activity against any of the bacterial isolates, (see table 3).
The antibiotic (Imipenem) was found to be effective against the identified bacteria, (see table 4).

**Discussion:**

The Palestinian Ministry of Health has devised protocols to eliminate the Covid-19 pandemic, and following these guidelines has resulted in a decrease in other diseases, which is why just a few species of bacteria were found during our research. The coins samples used in our study came from taxis cars drivers, and the number of bacteria isolated from the coins samples was quite low compared with similar study conducted in Pakistan in the year of 2015\(^{(14)}\), indicating the Palestinians keenness and commitment to prevention procedures.

The skin serves as a contact with the outside world, and it is inhabited by a wide range of microorganisms the most of which are harmless or even beneficial to their host \(^{(27)}\). Hand sanitizers reduce the incidence of health-care-associated infections by reducing the proliferation of microorganisms, thereby lowering infection risk and considered the simplest and cheapest way \(^{(28)}\).

Furthermore, as we demonstrated in the results, some sanitizers were effective against particular types of bacteria while were ineffective against others. We concluded that sanitizers should include 70% or more alcohol, thus the effective concentrations and their activity should be assessed, as a result, we need regulatory bodies and stringent oversight of the quality and effectiveness of sanitizers to verify sterilizer manufacturers’ reliability and give the protection that Palestinian consumers demand.

Microorganisms have evolved strict ways to evade the fatal effects of antimicrobial compounds as a result of some mutations, which lead to occurrence of many diseases and spread of infection \((2018)\) \(^{(29)}\). The composition of WHO-recommended hand sanitizer formulations contains, either ethanol (96%; final concentration 80% v/v) or isopropyl alcohol (99.8%; final concentration 75%) along with hydrogen peroxide (0.125% v/v as a preservative to inactivate bacterial spores) \(^{(30)}\). Despite claims on the label to reduce "dangerous germs and bacteria" by 99.9%, some antimicrobial hand sanitizers do not contain alcohol \(^{(31)}\).

We also found that the sanitizers that have been conducted in the study, contain benzalkonium chloride among their components, as well as alcohol, in addition to several other substances such as Carbomer, Acrylates, Triethanolamine.

Sanitizers containing benzalkonium chloride are less irritating than those containing alcohol, yet new research reveals that they may cause contact dermatitis more frequently than previously assumed. Despite the fact that alcohol-based hand sanitizers (ABHS) is less skin friendly than non-alcohol-based hand sanitizers (NABHS), (ABHS is more commonly used in healthcare settings due to its inexpensive cost and effectiveness in preventing disease transmission. NABHS, on the other hand, is less worrisome \((2020)\) \(^{(32)}\).
Many articles discussing the effect of sanitizers on bacteria isolated from money found a large number of different types of bacteria, some of which were harmful, compared to the results of the current study conducted in Nablus City that we came up with, as the types of detected bacterial community were few and limited, which may be attribute to the pandemic situation and people tendency toward continuous sterilization. In addition to the differences in religion and hygiene culture from one country to another, population density has a role also, and another factor is an individual consciousness and development as time passes. These factors, as we believe, have combined to explain why the bacteria we found were limited and few.

Cleansing the hands with alcohol-based sanitizers has constantly proven equivalent or elevated efficacy than antimicrobial soaps for eradication of most microbes, with exception of bacterial spores and protozoan oocysts. And it is necessary to recognize which kinds of hand sanitizers work best (2020) \(^\text{33}\).

In our study, the potent of used alcohol-based hand sanitizers were amazing by referring to table 3 and 4 as the hand sanitizers potent were low and this may be interpreted by the coronavirus pandemic which noticed to enhance environment of antimicrobial resistance of to hand sanitizers. And there were many health care facilities are dealing with sanitizers to reduce bacterial infections, while the excessive use of sanitizers can cause resistant bacteria to become more common.

Similar results have been observed in other parts of the world due to excessive use of alcohol-based hand sanitizers \(^\text{34}\).

139 strains of Enterococcus faecalis isolated from hospitals were tested for alcohol tolerance between 1996 and 2015. The results showed that Enterococcus faecalis was 10 times more resistant to alcohol after 2010 than older strains. In the early 2000 s, Australian hospitals began to install more hand sanitizers \(^\text{34}\). Hayat and Munnawar, (2016) have documented that Almost all gram-negative bacteria are resistant to Cool & Cool, Safeguard, Purell, Fresh up, Insta foam disinfectants \(^\text{35}\).

So, using sanitizers indeed us to think about how best to protect ourselves and the community from COVID-19 and other diseases as well as to avoid creating antimicrobial-resistant micro-organisms.

**Conclusion:**

We’ve completed our scientific study on the antibacterial effect of hand sanitizers, since we preferred to pick and choose it among the subjects, as the country is going through the Covid-19 pandemic when people were more likely to use disinfectants and follow preventive protocols to prevent the virus from spreading.

To be fair, many people are very committed to following health protocols and prevention methods. We seek perfection in this and make recommendations and call on anyone in the community who cares about his life, the lives of his family, friends, and the world around him to follow all of the health
protocols established by the Palestinian Ministry of Health. Whether in a closed state or in a state of cohabitation with the Covid-19 virus.

There are various areas of sterilizer impact that academics can explore in their scientific studies, including how to make sterilizers more efficient and tougher against bacteria.

**Recommendations:**

Based on the results of this study, a strict protocol should be established to make the appropriate usage of alcohol-based sanitizers mandatory in all sectors (health, education, commerce...).

More studies are needed over the years to evaluate the efficacy of hand sanitizers after we adapt to the coronavirus safety protocol and to compare the results with the results in this study.

Everyone in the community must adhere to the instructions of the Ministry of Health because everyone is susceptible to infection and transmission.

**Limitations:**

The study was conducted during the Corona pandemic, which hindered work in several ways, such as reaching the laboratory, virtual meeting with group members, collecting samples, and repeating the work several times to reach the desired results.

**Declarations**

**Declaration of Conflicting Interests:**

The author(s) declared that they have no potential conflicts of interest with respect to the research, authorship and publication of this article.

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**Authors' contributions**

All authors listed have contributed and approved the work for publication. The authors worked in an orderly manner. Dr. Wafaa designed the study and supervised the work, reviewed and approved the manuscript.

Sandy Yasser Hashash, Soha Mohammad Azaam, Iman "Mohammad Ali" Namrouty beside Noor Nader Abdullah, and Hanaa Kher Bashoti worked at the same pace, taking turns collecting samples, working in the lab, writing investigations, and observing the results.
Supplemental Material

The data are documented directly in tables and figures, and the samples have been disposed of according to the most appropriate safety measure.

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Tables

Table-1: Identification of Microbiological Community on Circulated Coins.

* No Applicable (do not apply)

Table-2: The Used Of The Tested Sanitizes.
| #No. Of Swabbed Coins | Nutrient Agar | Blood Agar | MacC- oncky Agar | Catal- asE test | Coag- ulaEe test | Type Of Bacteria |
|-----------------------|--------------|------------|-----------------|----------------|----------------|-----------------|
| 1                     | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 2                     | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 3a                    | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 3b                    | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 4                     | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 5                     | +            | +          | -               | +              | +              | Staphylococcus auruse |
| 6                     | +            | +          | -               | +              | +              | Staphylococcus auruse |
| 7                     | +            | +          | -               | +              | +              | Staphylococcus auruse |
| 8                     | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 9                     | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 10                    | +            | +          | -               | +              | +              | Staphylococcus auruse |
| 11a                   | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 11b                   | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 12                    | No Bacterial Growth |            |                 |                |                |                 |
| 13                    | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 14                    | No Bacterial Growth |            |                 |                |                |                 |
| 15                    | +            | +          | -               | +              | -              | Staphylococcus epidermidis |
| 16                    | No Bacterial Growth |            |                 |                |                |                 |
| 17                    | +            | +          | -               | +              | +              | Staphylococcus auruse |
|   |   |   |   |   |   |                             |
|---|---|---|---|---|---|------------------------------|
| 18| + | + | - | + | - | Staphylococcus epidermidis   |
| 19| + | + | - | + | * | Staphylococcus saprophyticus |
| 20| + | + | - | + | * | Staphylococcus saprophyticus |
| 21| + | + | - | + | * | Staphylococcus saprophyticus |
| 22| + | + | - | + | - | Staphylococcus epidermidis   |

| Brand Name                        | Chemical Composition                                                                 |
|-----------------------------------|----------------------------------------------------------------------------------------|
| Sanitizer-A (IL)                  | SD Alcohol 40, water, Fragrance, Linalool, Carbomer, Glycerin, Triethanolamine,       |
|                                   | Panthenol, Ethanol 70%                                                                 |
| Sanitizer-B (Icona London) (UK)   | Carbomer, Benzalkonium Chloride Solution, Propylen glycol, Glycerol, Ethyl Alcohol    |
|                                   | 70%, Triethanolamine, Fragrance, Water.                                               |
| Sanitizer-C (National) (PS)       | 70% Ethyl Alcohol.                                                                     |
| Sanitizer-D (HiGeen) (JO)        | Ethanol 70%, Water, Glycerin 2%, Carbomer, Acrylates, Triethanolamine, Hydroxypropyl   |
|                                   | methylcellulose, Retinyl palmitate, Tocopherl acetate, Cellulose, Lactose, Benzophenone |

**Table-3: The Susceptibility Of Bacterial Isolates Against Sanitizers (Zones Of Inhibition (mm))**
| Bacterial species | Sanitizer A | Sanitizer B | Sanitizer C | Sanitizer D |
|-------------------|-------------|-------------|-------------|-------------|
| 1                 | 13          | --          | 12          | 11          |
| 2                 | 13          | 8           | 10          | --          |
| 3a                | --          | 11          | 9           | 12          |
| 3b                | 8           | 10          | --          | 11          |
| 4                 | 14          | 10          | 11          | 11          |
| 5                 | 16          | 9           | 10          | 11          |
| 7                 | 15          | 9           | 12          | 10          |
| 8                 | 8           | 9           | 13          | 10          |
| 9                 | 12          | 8           | 10          | 7           |
| 10                | 8           | 8           | --          | --          |
| 11a               | 11          | --          | 13          | 11          |
| 11b               | --          | 10          | --          | 10          |
| 13                | 12          | 10          | 11          | --          |
| 15                | --          | 8           | --          | 10          |
| 17                | --          | --          | 10          | 8           |
| 18                | 10          | --          | 9           | 7           |
| 19                | 13          | 8           | 10          | 9           |
| 20                | --          | 12          | 13          | 7           |
| 21                | 11          | --          | 7           | --          |
| 22                | --          | --          | 10          | 12          |

—: Resistance.

Table-4: Some Of The Susceptibility Of Bacterial Isolates Against antibiotic discs (Imipenem) (Zones Of Inhibition (mm))
Figures

Figure 1
Type of Hand Sanitizers
Figure 2

The Isolated Bacteria
Figure 3

Activity of Sanitizers Against Bacterial Isolates
Figure 4

The Susceptibility Of Bacterial Isolates Against Antibiotic Discs
Figure 5

The Susceptibility Of Bacterial Isolates Against 70% Ethanol Discs.