Antimicrobial Efficacy of Various Hand Sanitizers Sold in Nigeria

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ABSTRACT

Background: The use of alcohol-based hand sanitizer that contains at least 60% alcohol prevents the spread of infections and thus prevents the spread of antimicrobial resistance. Aim: The aim of this study was to evaluate the antimicrobial efficacy of different brands of hand sanitizers sold in Nigeria. Materials and Methods: Antimicrobial efficacy of eleven popular brands of alcohol-based hand sanitizers sold in Nigeria was evaluated and comparatively analyzed using a combination of Agar well diffusion (Mueller-Hinton Agar for

bacteria and Sabouraud Dextrose Agar for fungi), Broth dilution (Nutrient broth) and In vivo Viable Microbial count reduction techniques. A total of 330 clinical isolates comprising 55 each of Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris and Candida albicans obtained from culture collection were used as test organisms. Results and Discussions: This study revealed a very high significant difference (P<0.001) on the susceptibility pattern of the test organisms against the various hand sanitizers used. The study showed a very high significant difference between the activity of the various hand sanitizers and the positive control (P<0.001). All hand sanitizers used in this study apart from Purit are less active against Proteus vulgaris but are more active against Staphylococcus epidermidis. The findings in this study showed that isopropyl alcohol based-gel form hand sanitizer had 82.2% efficacy while ethanol-based gel form hand sanitizers had 76.9% efficacy. This study revealed Significant difference (P<0.001) on Minimum Inhibitory Concentration (MIC) of all hand sanitizers used against test organisms except for Evree hand sanitizer which showed no significant difference (P>0.05) on Minimum Inhibitory concentration against test organisms. Of all the hand sanitizers used in this study only Purit has bactericidal activity against Proteus vulgaris not lower than 95% concentration. Conclusion: The results of this study suggest high significant difference in the antimicrobial efficacy of various hand sanitizers sold in Nigeria.

Keywords: Anitimicrobial, Concentration, COVID-19, Efficacy, Sanitizers.

INTRODUCTION

Globally, there are 119,094,168 corona virus cases, 2,640,822 deaths, 94,632,462 recovered and 21,820,884 active cases as at 12th March, 2021 [1][2]. The genome sequence of virus SARS-CoV-2 is similar to SARS Coronavirus (SARS-CoV) [3][4]. The CoVs are members of the same genus Beta coronavirus, sharing similar morphology in the form of enveloped, positive single-stranded RNA viruses [5]. They can be deactivated by certain lipid solvents such as ethanol, ether (75%), chlorine-containing disinfectants, and chloroform, except chlorhexidine [5]. Ethyl alcohol, at concentrations of 60%–80%, is effective viricidal agent inactivating all the lipophilic viruses (e.g., influenza, herpes and vaccinia virus) and many hydrophilic viruses (e.g., adenovirus, enterovirus, rhinovirus, and rotaviruses excluding hepatitis A virus (HAV) or poliovirus) [6].

Regular hand washing with soap and water prevent the spread of infections and decrease the risk of getting sick. When there is non-availability of soap and water, CDC recommends the use an alcohol-based hand sanitizer that contains at least 60% alcohol [6]. Hand hygiene prevents the spread of antimicrobial resistance. Alcohol-based hand sanitizers generally contain one or more alcohols (ethanol or 1propanol or 2-propanol) in quantitative ratios that are deferred by brand. The alcohols present in the Alcohol-based hand sanitizers formulations triggers bactericidal action by denaturation of microbial proteins [8]. Some Alcohol-based hand sanitizers may as well contain other chemical constituents that work in synergy with the bactericidal action of the alcohols. Other chemical compounds may confer independent bactericidal activities through unknown mechanisms.

Due to the continuing worldwide COVID-19 pandemic, the demand for hand sanitizers remains at a very high level. In Nigeria, many Pharmaceutical companies, cosmetics industries, even a soft drink bottling company and a University went to the production of hand sanitizers to meet market demand and for profit making. Majority of manufacturers claim 99.9% effectiveness of their hand sanitizers.

Different categories of hand sanitizers (alcohols, quaternary ammonium compounds and triclosan) possess different levels of sensitivities and therefore varying resistance by various infectious agents. For example, some species of Staphylococcus aureus carry a gene that allows resistance to some hand sanitizers [6]. Some hand sanitizers are fungistatic, others are bacteriostatic against gram-positive bacteria and bacteriostatic against some gram-negative bacteria [6]. Like ethanol hand sanitizers, those of the quaternary ammonium compounds are not active against non-enveloped viruses. More so, some are safer, more economical, less messy and more enjoyable to use for kids. This study intends to look into the usage and effectiveness of these different brands of hand sanitizers.

This study examined the antimicrobial efficacy of eleven brands of hand sanitizers sold in Nigeria against Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris and Candida albicans, and also to assess and compare their antimicrobial effectiveness.

MATERIALS AND METHODS

Type of Study

This is a cross sectional descriptive study and was conducted to comparatively analyze some hand sanitizers sold in Nigeria.

Study Location

The research was conducted in the Biology Laboratory of the Department of Biological Sciences, Faculty of Science, University of Medical Science, Ondo, Nigeria.

Ethical Considerations

Written/verbal informed consent was received from subjects who participated in the study at the study center. The Ethical committee of the Edo state Ministry of health, Benin City, reviewed and approved the study protocol, HA. 737/83.

Population and Subjects

The population of this study was centered on users of hand sanitizers among health workers, students and civil servants in Benin City and Ondo town of Nigeria.

Test Organisms

A total of Three hundred and thirty clinical isolates comprising fifty-five each of Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, and Candida albicans obtained from Don Bosco Health Centre, Akure, Ondo State, Central Hospital, Benin City and Stella Obasanjo Hospital, Benin City, Edo State were used as test organisms in this study. These micro organisms were chosen because they are commonly implicated in community-acquired infections [8][9].

Test Subjects

A total of Two hundred Subjects comprising of Health Workers, Civil Servants and Students participated in this research after they consented to participate.

Hand Sanitizers

Eleven brands of alcohol-based hand sanitizers (10 waterless gel form – Lifebouy, Purit, Dettol, Carex, Tetmosol, Sanitol, Wind, Seed, Tribute, Evree, and one spray form – 2sure) commonly sold and used in Nigeria were chosen for this study based on their popularity and availability at retail outlets across Nigeria. Each hand sanitizer was stored as recommended by its manufacturer and was used well before the expiration date.

Test Organisms Standardization

Turbidity variability of Bacterial suspensions may affect result, thus the need for standardization of microbial testing; McFarland standards was used as a reference to adjust the turbidity of bacterial suspensions. The McFarland 0.5 turbidity standard was prepared by adding 0.5 ml of 1.175% w/v barium chloride dihydrate (BaCl2·2H2 O) solution to 99.5 ml of 15 w/v sulfuric acid (H2 SO4) [10]. This was mixed uniformly and aliquoted into test tubes identical to the ones used in preparing inoculum suspensions of the test organisms. The accuracy of the density of the standard was verified using a spectrophotometer. The absorbance of the 0.5 McFarland standards at wavelength of 625 nm was 0.08–0.10. The tubes were stored in a well-sealed container in the dark at room temperature until when required [11].

A sterile loop was used to pick a loopful of inoculum from a pure culture of the test organism. This was then transferred and suspended into a tube containing sterile normal saline (NaCl 8.5 g, distilled water 1 L). The tube was compared with the turbidity standard, and the density of the organism was adjusted by adding more bacteria or sterile saline until standardization was attained [12].

Susceptibility of Test Organism to Hand Sanitizers

Disk agar diffusion technique (well-variant) as described by Valgas et al. was used for determining antimicrobial efficacy of hand sanitizers [13]. Sterile Mueller-Hinton agar plates were inoculated with standardized test organisms. A sterile cotton swab was dipped into a test tube containing the inoculum and was rotated properly to allow maximum contact. Excess inoculum was removed by pressing and rotating the swab firmly against the inside wall of the tube above the liquid level. The swab was then streaked over the surface of the medium three times while rotating the plate at 60° angle after each application. The swab was also passed around the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed.

With the aid of a sterile 4mm cork borer, 3 equally spaced holes were bored in the agar plate. The agar plugs were discarded using a sterile needle. A 100 microliters volume of undiluted hand sanitizer was then introduced into the first well, the second well was filled with an equal volume of sterile water to serve as negative control, while the third well was filled with 90% ethanol as positive control. This was done for all the test organisms and hand sanitizers. The plates were incubated for 24hours at 37°C in an upright position. They were then examined for zones of inhibition which indicated the degree of susceptibility or resistance of the test organism to the antibacterial agent. The point of abrupt diminution growth which corresponds to the complete growth inhibition was taken as the zone edge. The test was carried out five times, and the average of all readings was taken as the zone of inhibition in each case.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that completely inhibits the growth of a test organism as seen by the unaided eye [14]. To determine the MIC, increasing concentrations (5% – 100% with 5% intervals) of each Hand sanitizer were prepared in 9ml tubes of sterile nutrient broth. Exactly 100microlitre of each standardized test organism was then introduced into each tube of Hand Sanitizer. A tube containing only nutrient broth and bacteria without hand sanitizer served as negative control while another tube containing just the sanitizer and broth without bacteria served as positive control. Each tube was incubated for 18hours for bacteria and 48hours for fungi, then

examined for visible growth or turbidity. The concentration of the hand sanitizer in the tube in which no visible growth was observed when compared with the controls was taken as the MIC.

Determination of Minimum Cidal Concentration

Minimum bactericidal concentration is the lowest concentration of an antimicrobial that can kill the test organism [11]. To determine the cidal for each Hand sanitizer, samples from the test tubes used in MIC test that showed no visible growth after the period of incubation were inoculated on sterile nutrient agar plates (which had no antimicrobial incorporated) for bacterial and Sabouraud Dextrose Agar for fungi in them using sterile swab sticks. The plates were incubated at 370C for 18 to 24hours and were then observed for growth. The concentration at which absence of growth was observed (bactericidal activity) was then taken as MBC.

In vivo Reduction of Viable Bacteria and Fungi counts on Hands of Subjects

The Hand sanitizers evaluated for their efficacy in reducing baseline bacteria and fungi counts on the hands of subjects. Ten individuals were selected for each products and verbal informed consent was obtained from each subject prior to the conduction of the experiment. Subjects did not apply antimicrobial substance to their hands prior to the experiment. Sterile nutrient agar and Sabouraud Dextrose agar plates were divided into two halves with one half labeled BF (before) and the other labeled AF (After). Subjects were asked to gently make an impression on the surface of the BF side of the agar with the three unwashed fingers. After this, 3ml of the hand sanitizer was then applied to the hands and rubbed thoroughly on the palms, fingers and the back of the hands until the hands became completely dried. Subjects were then asked to repeat the finger impression on the AF part of the plate. This was done by all subjects. The plates were incubated at 370C for 24hours and the numbers of colonies were counted. The percentage of CFU reduction was calculated as follows:

%CFU reduction = CFU/mL on Bf Section – CFU/mL on AF Section x 100 CFU/mL on BF Section [15].

Statistical Analysis

Analysis of Variance (ANOVA), Duncan Multiple Range Test, Unpaired T-Test and Chi-Square were used to Test for Significant Difference on Susceptibility Pattern of Test Organisms to the various Hand Sanitizers.

Paired T-test was used to test the significant difference of Percentage CFU Reduction of Viable Bacteria and Fungi from hands of Subjects by the hand Sanitizers.

Minimum Inhibitory Concentration (MIC), Minimum Cidal Concentration (Bacteriocidal) (MBC) and Fungicidal (MFC) of the Hand Sanitizers against Test Organisms are presented in Charts.

RESULTS

Data Analysis and Presentation

In this study, a total of Three hundred and thirty clinical isolates comprising fifty-five each of Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, and Candida albicans were tested against eleven alcoholbased hand sanitizers commonly sold and used in Nigeria (Table 4.1) for their efficacy.

It was observed that all the hand sanitizers used in this study have varying inhibitory activities against all test organisms and that each of the hand sanitizers used had different level of inhibition against each of the test organisms. A sanitizer very active against a particular test organism is less active against another test organism (Table 4.2).

The study revealed that all the hand sanitizers used in this study have varying Minimum Inhibitory concentration (MIC) against the susceptible test organisms (Table 4.3).

It was observed that not all the hand sanitizers used in this study had cidal acitivity against test organisms and where it did have, the Minimum cidal concentrations were mostly 100% (Table 4.4)

The study revealed varying In-vivo reduction of Viable Bacteria and Fungi from hands of subjects by three hand sanitizers that have cidal activities on test organisms. It was observed that Purit hashigher (82.2%) viable bacterial count reduction than fungi (31.8%). The study also revealed that Evree has equal in-vivo reduction of viable bacteria and fungi (76.9%) while Lifebuoy has highe in-vivo reduction of viable fungi (64.5%) than bacteria (41.6%) (Tables 4.5 and 4.6)

Purit and Lifebouy have inhibitory activity against test organisms not below 60% concentration while Dettol has inhibitory activity not below 70% concentration. Sanitol, Seed and Tribute have inhibitory activity not below 75% concentration. Carex, wind and Evree have inhibitory concentration not below 80% concentration while Tetmosol and 2Sure hand sanitizers have inhibitory activity against test organisms not below 90% concentration

DISCUSSION

The use of alcohol-based hand sanitizers has become more popular with the emergence of Coronavirus pandemic. Many brands of alcohol-based hand sanitizers flooded the Nigerian markets with the manufacturers of various hand sanitizers claiming 99.9% effectiveness against infectious organisms. This study became necessary due to increasing reliance on alcohol-based hand sanitizers to prevent community spread of infectious diseases.

This study revealed a very high significant difference (P<0.001) on the susceptibility pattern of the test Organisms (Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, and Candida albicans) against the various hand sanitizers used in this study, this is in line with the finding of [16], who also reported significant difference (P<0.001).

The study also show a very high significant difference between the activity of the various hand sanitizers and the positive control (P<0.001). This finding is different from the finding of [17] who reported no significant difference between the activity of the various hand sanitizers and the positive control. Though he did not revealed the brand name of the hand sanitizers used in his study, they were also alcohol-based hand sanitizers, the difference may be due to other ingredients in the hand sanitizers, mores so [17] used a commercial hand sanitizer as his positive control while 90% ethanol was used as positive control in this study.

All hand Sanitizers used in this study apart from Purit are less active against Proteus spp (a Gram-negative bacilli) but are more active against Staphylococcus epidermidis (a Gram-positive cocci), this may be due to differences in their cell wall components.

The findings in this study that isopropyl alcohol based-gel form Hand sanitizer (Purit) has higher efficacy than ethanol-based gel form hand sanitizers is the same with what was observed by [15] that isopropyl alcohol solution has higher efficacy than ethanol solution, but contrary to findings of [18] which was in reverse.

This study also showed Significant Difference (P<0.001) on Minimum Inhibitory Concentration (MIC) of all Hand Sanitizers used against Test Organisms except for Evree Hand Sanitizer which show no significance difference (P>0.05) on Minimum Inhibitory concentration against test organisms.

This study revealed that none of the hand sanitizers sold in Nigeria, used in this study have inhibitory activity (MIC) below 60% concentration which is lower than below 75% concentration that was reported by [17].

2Sure the only Liquid form hand sanitizer used in this study has lower antimicrobial efficacy than other gel form hand sanitizers used, this is the same with the report of [18].

Of all the hand sanitizers used in this study only Purit has bactericidal activity against Proteus spp not lower than 95% concentration. Lifebuoy, Dettol, Sanitol, Seed, Tribute and Evree have bactericidal effects not lower than 100% concentration on other test organisms. Carex, Temosol, 2Sure and Wind hand Sanitizers have no bactericidal effect on test organisms.

This study showed that Purit, Lifebuoy, Tetmosol and Evree have fungicidal effects on Candida albicans at concentration not lower than 100%. Dettol, Carex, Sanitol, 2Sure, Wind, Seed and Tribute hand Sanitizers sold in Nigeria, used in this study did not have fungicidal effect on test organism.

The mean percentage in vivo reduction of viable bacteria and fungi counts observed in this study is higher than what was observed by [17] but lower than 99.9% claim by manufacturers of hand sanitizers.

Conclusion

Based on the findings of this study, the Manufacturers' claim of 99.9% of antimicrobial efficacy of various hand sanitizers sold in Nigeria, used in this study is in doubt. There is significant difference on the antimicrobial efficacy of various hand sanitizers sold in Nigeria, used in this study and the various hand sanitizers sold in Nigeria are more active against Gram positive bacteria than Gram negative bacteria with more potency against bacteria than

fungi. Isopropyl alcohol based-gel form Hand sanitizer (Purit) has higher efficacy than ethanol-based gel form hand sanitizers

Recommendation

The revelation of this study suggests that no hand sanitizer sold in Nigeria, used in this study have 99.9% antimicrobial efficacy, therefore hand washing with water and soap should be practiced more often to physically remove the infectious microbes from the hands then combine with rubbing hand with hand sanitizers.

Conflict of Interest

Authors declare no conflict of interest

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