Original Research Article

Hand hygiene practices and the effectiveness of hand sanitizers at controlling enteropathogens among the residents of a University community in Osun State Nigeria

ABSTRACT

Aim: To explore perceptions, attitudes and hand washing practices in relation to the effectiveness of hand sanitizers in controlling enteropathogens amongst residents of a Nigerian University with the purpose of creating awareness on the importance of hand hygiene to control the spread of communicable diseases.
Study Design: A simple random cluster sampling technique was used. A questionnaire designed to relate demographic and hand hygiene practices to the effectiveness of the practices to the control of enteropathogens was applied to the respondents.
Place and Duration of Study: The study was carried out between January and May, 2018 at the Redeemer's University, Ede, Osun State, Nigeria.
Methodology: Sterile swabs moistened with sterile normal saline were used in sampling the palms of 50 respondents and the normal transient flora was established, samples were again taken to determine effectiveness of hand washing at reducing the bacterial load and the diversity of organisms isolated from the samples after hand washing and application of hand sanitizers. Subsequently, the results were compared using one-way anova F-test at $p<0.05$.
Results: The results showed that at least 60% of the respondents were unaware of the WHO recommended way to wash hands and 72% of these do not wash their hands before eating food or after taking care of sick people. The predominant transient hand flora in the tested population were determined to be constituted by the following bacterial species, namely, *Enterobacter spp, Enterobacter aerogenes, Staphylococcus aureus, Yersinia pestis, Erwinia cactida, Klebsiella pneumonia, Enterobacter cloacae* and *Klebsiella oxytoca*. Hand washing with soap was found to be more effective at reducing these on the hands of the respondents at a degree similar to treatment with the hand sanitizer were PL® with a label claim of 70% alcohol contentand more effective than hand sanitizers CS® and GC® with 62% and 60% alcohol content respectively.
Conclusion: Hand washing with soap and water when done properly remains the most reliable means of breaking the cycle and spread of preventable enteropathogens in the community setting and it is perhaps more reliable than the use of alcohol-based hand sanitizers.

Key Words: hand hygiene, hand sanitizers, enteropathogens, skin flora

1.0 INTRODUCTION

The spread of disease-causing pathogens and reduction of disease burden is best achieved by improving hand hygiene in healthcare, communities and the general population [1]. Hand hygiene is defined as any method that removes or destroys microorganisms on hands. It is well-documented that the most important measure for preventing the spread of pathogens is effective hand washing [2].

A lot of research effort has been focused on the relationship between hospital acquired infections (HAI) and hand hygiene in the healthcare setting, however, the literature on hand hygiene in the community setting is scanty. In the community setting, the hand remains the most important vehicle for the transmission of diseases [2, 3]. In the home, school, places of worship and other public places, hands become readily contaminated through greetings (handshake), using the toilet, changing a baby's diaper, handling raw food, blowing the nose or sneezing into the hands, handling pets and domestic animals and after caring for infected persons [4]. There is abundant evidence to show that hand hygiene through hand washing with soap and running water or the use of hand sanitizers are proven means of affordable and impactful intervention to reduce morbidity and mortality due to infectious diseases [4,5].

There are three principal types of skin flora that have been described. The resident and transient flora [6]; in addition, the infectious flora, characterized by species such as *Staphylococcus aureus* or beta-haemolytic *streptococci*, which are frequently isolated from abscesses, whitlows, paronychia, or infected eczema [7].

Depending on the active ingredient used, hand sanitizers can be classified as one of two types: alcohol-based or alcohol-free. Alcohol-based products typically act as skin disinfectant by denaturing proteins of pathogens [8] and contain between 60 and 95 % alcohol, usually in the

form of ethanol, isopropanol or n-propanol [9]. At those concentrations, alcohol immediately denatures proteins, effectively neutralizing certain types of microorganisms. Alcohol-free products are generally based on disinfectants, such as benzalkoniumchloride (BAC), or on antimicrobial agents such as triclosan [9]. The activity of disinfectants and antimicrobial agents is both immediate and persistent. Many hand sanitizers also contain emollients (e.g. glycerin) that soothe the skin, thickening agents and fragrance [8].

The correct use of hand sanitizer does not require water, takes less time than hand washing and does not require drying hands with potentially contaminated surfaces [10]. A range of efficacy tests for hand sanitizer have been performed on hands artificially contaminated with bacteria and viruses. These studies have demonstrated hand sanitizers to be as or more efficacious than hand washing with plain (i.e. not antibacterial) soap and water [11]. Sanitizers must be used correctly to obtain the expected effect of pathogen control. According to Aiello et al, [12], the correct procedure for hand sanitizer is as follows: "apply the product to the palm of one hand (the correct amount to be applied should be obtained from the manufacturer's label); rub your hands together; rub the product over all hand surfaces and fingers until hands are dry".

Enteropathogenic bacteria are those that cause infection or diseases in the intestinal tract and employ a variety of sophisticated strategies to colonize the intestinal epithelium. In essence, ingested pathogens have evolved the abilities to: resist non-specific host defenses, such as acidity, peristalsis, mucosal cell exfoliation, intestinal mucins and bacteriocins; adhere to intestinal epithelia and ultimately colonize the epithelia. Colonization may or may not involve cellular invasion. When cellular invasion occurs, it can be followed either by intracellular multiplication and spread of the bacteria to other tissues or by bacterial persistence [13]. The

presence of enterobacteria on the hands could lead to serious infection, illness and possible mortality.

The aim of the present work is to explore perceptions, attitudes and hand washing practices in relation to the effectiveness of hand sanitizers in controlling enteropathogens originating from the transient flora amongst residents of Redeemer's University, Ede, Osun State, Nigeria. The University community is considered to be ideal for this type of study since socio-economic factors have been linked to non-compliance with hand hygiene and its effectiveness (or lack thereof) in infectious disease control [14]. The University community is populated by persons of varied socio-economic background, ranging from the highly educated, semi-illiterate artisans, traders and students.

2.0 MATERIALS AND METHODS

Sample collection, experimental design and microbiological analyses

50 residents of the Redeemer's University community were randomly selected from the different age groups and sexes as shown on Table 1 and these persons from now on are referred to as respondents. A simple random cluster sampling technique was used in sampling the 50 respondents from the University population. A questionnaire containing information on bio-demographic characteristics and hand hygiene practices was applied to the individuals in the study population. Hand swabs from the respondents were collected in order to determine the resident flora and subsequently, the respondents were taught the W.H.O standard of hand washing and proper use of hand sanitizers. Three brands of alcohol-based hand sanitizers were purchased from the University's CRM supermarket, the products were PL® with a label claim of

70% alcohol content including CS® and GC® with 62% and 60% alcohol content respectively. The hand sanitizers were offered to the respondents, two weeks later, another hand swab was taken from the respondents within 20 mins of hand sanitizer application.

Microbiological samples were obtained from the respondents using sterile swab sticks. Sterile saline was prepared and swab sticks were dipped in the test tubes and samples were collected from the palms of the individuals that completed the questionnaire. Distinct isolates were identified using cultural, cell morphological, and Biochemical Tests [15]. Data was collected, entered and analyzed using SPSS-16 statistical software. One way anova test was employed to examine the differences in log10 reduction in bacteria due to the application of the various brands of hand sanitizers.

1.0 RESULTS

Fifty members of the Redeemer's University community were studied. Among these did males and females constitute 38% and 62% respectively. These were further classified into children (0-18 years old) and adults (19 years old and above) constitute 30% and 70% respectively. Moreover, the levels of educational attainment of the respondents ranged from primary school at 14%, high school at 22%, undergraduate at 50% and postgraduate levels at 14% (Table 1).

3.1 Hand hygiene and hand washing practices

Table 1: Socio-demographic characteristics of the respondents

Variable	Frequency (Percentage)
Age	
0-18	**15 (30)**
19-22	**21 (42)**
25 and above	**14 (28)**
Total	**50 (100)**
Gender	
Male	**19 (38)**
Female	**31 (62)**
Total	**50 (100)**
Level of Education	
Preschool/ Primary	**7 (14)**
High School	**11 (22)**
Undergraduate	**25 (50)**
Postgraduate	**7 (14)**
Total	**50 (100)**

A majority of the sampled population (60%) indicated that they were not aware of the W.H.O standard for hand washing. When compared on the basis of gender, a larger percentage of the persons oblivious of the W.H.O standard were males (Table 2a). Moreover, when probed for the reasons for non-compliance to frequent hand hygiene, 10% of the respondents claimed not to care (i.e. nonchalant), 4% were unaware of the health importance of hand washing, none of the respondents claimed that they did not know how to wash their hands, the majority of the

respondents (44%) claimed they were too lazy to be committed to frequent hand washing while 42% claimed the non-availability of cleaning agents such as soap and water as reason for non-compliance to hand washing (Table 2b). In addition, a larger proportion of males claimed that they never wash their hands throughout the day after taking their bath in the morning while none of the respondents ever bother to wash their hands after handling money (Table 2c).

Table 2a: Awareness of W.H.O standard for hand washing/ frequency of hand washing prior to sampling the population

Awareness of W.H.O standard for hand washing		
I am aware of W.H.O's recommended way to wash hands?	Yes	No
Female	13 (42%)	18 (58%)
Male	7 (37%)	12 (63%)
Number of individuals	20 (40%)	30 (60%)
Total 50 (100%)		

Table 2b: Reasons for non-compliance with W.H.O standard for hand washing

	Reason for non-compliance				
	Frequency/ (percentage)				
	0	1	2	3	4
Female	2 (4)	1(2)	0	12 (24)	16 (32)
Male	3(6)	1(2)	0	10 (20)	5 (10)
Number of individuals	5 (10)	2 (4)	0	22 (44)	21 (42)
Total	50 (100)				

Where 0= Nonchalant; 1= lack of awareness of the health significance of hand washing; 2= little or no idea of the proper way to wash hands; 3= laziness; 4= lack of availability of water and soap

Table 2c: Frequency of hand washing

Frequency of hand washing: Questionnaire item- When do you wash your hands?

Frequency/ (percentage)

	0	1	2	3	4	5
Female	2 (6)	4 (13)	12 (6)	2 (6)	11 (36)	0
Male	3 (16)	3 (16)	6 (39)	0 (0)	7 (37)	0
Number of individuals	5 (10)	7 (14)	18 (36)	2 (4)	18 (36)	0
Total 50 (100)						

Where 0= I never wash my hands after bathing in the morning; 1= before, during and after preparing food; 2 = after using the toilet; 3= after taking care of sick people; 4= before eating food; 5= after handling money

A total of 113 distinct bacterial isolates were obtained from the sterile swab sample of the palms of the respondents and these were grouped according to cultural characteristics into eight (8) groups with group identification A-H. Representative samples from these groups were identified using cell morphological and biochemical characteristics (Tables 3 and 4).

Table 3: Grouping of bacterial isolates from sterile swab samples of the palm of 50 randomly selected respondents within the Redeemer's University community according to cultural characteristics

Group ID	Cultural characteristics	Presumptive identities of isolates using biochemical tests
A	Moderate, yellow, opaque, circular, entire, flat	*Enterobacter spp*
B	Moderate, cream, opaque, circular, filiform, flat	*Enterobacter aerogenes*
C	Moderate, cream, opaque, irregular, undulate, flat	*Staphylococcus aureus*
D	Moderate, cream, opaque, circular, entire, flat	*Yersinia pestis*
E	Moderate, cream, opaque, circular, undulate, flat	*Erwinia cactida*
F	Moderate, cream, opaque, irregular, lobate, flat	*Klebsiella pneumonia*
G	Moderate, cream, opaque, rhizoid, lobate, flat	*Enterobacter cloacae*
H	Moderate, cream, opaque, circular, entire, raised	*Klebsiella oxytoca*

Table 4: Biochemical identification table of bacterial groups A- H isolated from transient flora of the palms of respondents

Group ID	Gram staining	Lactose Fermentation	Gram staining	Citrate	Motility	Indole	Methyl red	Vogues Proskauer	Lysine Decarboxylase	H_2S Production	Orthinine Decarboxylase	Presumptive organism
A		-	+	+	-	-	-	+	-	+	-	*Enterobacter spp*
B		-	+	+	+	-	-	+	+	-	+	*Enterobacter aerogenes*
C												*Staphylococcus aureus*
D		-	-	+	-	+	-	+	-	-	-	*Yersinia pestis*
E		-	+	+	+	-	-	+	-	+	+	*Erwinia cactida*
F		-	+	+	-	-	-	+	+	-	-	*Klebsiella pneumoniae*
G		-	+	+	+	-	-	+	+	+	+	*Enterobacter cloacae*
H		-	+	+	+	-	-	+	+	-	+	*Klebsiella oxytoca*

As shown in Table 5, the transient organism with the highest percentage occurrence was *Staphylococcus aureus*, found in all the age groups and sexes but with the highest amount among the adult male category. This was followed by *Yersinia pestis* which showed the second highest percentage occurrence and found to be most abundant on the adult female respondents. The least occurring transient organism among the respondents was *Klebsiella oxytoca*, found only among the adult female in the study population (Table 5).

Table 5: Determination of the predominant transient flora: percent occurrence of bacteria obtained from 50 respondents classified by age group in the University community

Transient flora (Bacteria)	Adult Female Age 19 and above (%)	Adult Male Age 19 and above (%)	Male children Age 0 to 18 (%)	Female children Age 0 to 18 (%)
Enterobacter spp	0	0	9	6
Enterobacter aerogenes	10	10	3	0
Staphylococcus aureus	14	48	34	34
Yersinia pestis	43	24	34	37
Erwinia cactida	18	9	0	0
Klebsiella pneumoniae	0	9	6	14
Enterobacter cloacae	5	0	14	9
Klebsiella oxytoca	10	0	0	0
Total 100%				

As shown in Table 6, when the percentage occurrence of transient microorganisms obtained from the palms of respondents was compared within 2 weeks of consistent washing with or without soap, the bacterial load diminished significantly when compared with the data when the respondents were not committed to hand hygiene (Table 5). In most cases the bacterial load diminished to zero count for many organisms earlier predetermined as part of the transient flora on the palms of the respondents. However, the degree of the ability to reduce the bacterial load differed between the treatments when a comparison was made between when the respondents washed their hands with or without soap. When the respondents washed without soap, the data indicated that five of the transient organisms remained on the hands of the respondents, these

organisms included *Enterobacter aerogenes, Staphylococcus aureus, Yersinia pestis, Klebsiella pneumoniae, Enterobacter cloacae*, whereas for the hand washing with soap treatment, only two species of organisms remained, namely, *Staphylococcus aureus* and *Yersinia pestis*.

Table 6: Percentage cfu reduction of microorganisms obtained from the palms of respondents after regular washing of hands without or with soap when samples were taken at 2 weeks intervals. Respondents' palms were sampled 2 weeks after WHO standard of hand washing was taught to the respondents.

Transient flora (Bacteria)	**Adult Female Age 19 and above (%)**	**Adult Male Age 19 and above (%)**	**Male children Age 0 to 18 (%)**	**Female children Age 0 to 18 (%)**
Enterobacter spp	100 (100)[1]	100 (100)	100 (100)	100 (100)
Enterobacter aerogenes	75 (80)	100 (86)	100 (100)	100 (100)
Staphylococcus aureus	100 (60)	60 (57)	60 (100)	100 (100)
Yersinia pestis	75 (60)	80 (57)	60 (100)	70 (100)
Erwinia cactida	100 (100)	100 (100)	100 (100)	100 (100)
Klebsiella pneumoniae	75 (100)	100 (100)	100 (100)	100 (100)
Enterobacter cloacae	75 (100)	60 (100)	80 (100)	50 (100)
Klebsiella oxytoca	100 (100)	100 (100)	100 (100)	100 (100)
Total 100%				

[1]Data for percentage occurrence of transient microorganisms for hand washing with soap are shown in parentheses.

A comparative assessment of the three popular brands of hand sanitizers available within the Redeemer's University community showed that the hand sanitizers were able to exert a cleansing effect similar to hand washing with soap, with hundred percent colony forming units (cfu) reduction observed for most of the bacterial organisms earlier predetermined as members of the transient flora. The effectiveness of the hand sanitizers at reducing the bacterial loads on the respondents' palms however varied along the lines of alcohol content of the respective brands of hand sanitizers. PL® with a label claim of 70% alcohol content was most effective at sanitizing the hands of the respondents, followed by CS® (62% alcohol content) and GC® (60% alcohol content) in descending order of effectiveness from the most effective to the least effective (Table 7). However, for two organisms earlier predetermined as members of the transient flora on the hands of the respondents, namely, *Staphylococcus aureus* and *Yersinia pestis* the percent cfu load reductions varied between 20- 100% even in the case of the most effective hand sanitizer, PL® with a label claim of 70% alcohol content (Table 7). In some cases, the amount of cfu load reduction was as low as 13% for the GC® brand with the alcohol content of 60% (Table 7). These differences in log10 reduction were found to be statistically significant different when the three treatments of hand sanitizers were compared using the F test at $P < 0.05$.

Table 7: Comparative assessment for effectiveness of three popular brands of hand sanitizers available in the University community based on percentage cfu reduction of microorganisms obtained from the palms of respondents after consistent application of sanitizer for at least 2 weeks. Respondents' palms were randomly sampled 2 weeks after the recommended standard of sanitizer was taught to the respondents.

Presumptive organisms	[1]GC® (60% alcohol content)				CS® (62% alcohol content)				PL® (70% alcohol content)			
	[2]Adult Female	Adult Male	Male Children	Female Children	Adult Female	Adult Male	Male Children	Female Children	Adult Female	Adult Male	Male Children	Female Children
Enterobacter spp	100[3]	92	100	100	100	100	100	100	100	100	100	100
Enterobacter aerogenes	100	100	100	100	100	100	100	100	100	100	100	100
Staphylococcus aureus	27	31	25	25	38	100	33	100	70	80	70	100
Yersinia pestis	33	38	25	75	50	100	67	100	60	80	70	100
Erwinia cactida	100	100	100	100	100	100	100	100	100	100	100	100
Klebsiella pneumoniae	13	23	50	100	22	100	100	100	100	100	100	100
Enterobacter cloacae	27	100	100	100	100	100	100	100	100	100	100	100
Klebsiella oxytoca	100	100	100	100	100	100	100	100	100	100	100	100
Total												

1. Three brands of hand sanitizers, namely, GC®, CS® and PL® were assessed
2. Age 0-18 were classified as children while age 19 and above were classified as adults
3. There was a statistically significant difference in log10 reduction in comparing the three treatments (F test, $P < 0.05$).

4.0 DISCUSSION

Results from the present study showed that hand hygiene, both by hand washing with water or with soap and water is an effective means controlling the spread of disease-causing pathogens and reduction of disease burden, particularly enteropathogens known to cause gastrointestinal illnesses such as diarrhea and flu-like diseases such as upper respiratory tract infections in particularly in children [16, 17]. The present report is one of the very few studies linking hand hygiene to the spread of enteropathogens in the community setting; most of the previous reports have been in the healthcare setting.

Apart from providing information that may create awareness on the importance of proper hand washing and the correct use of waterless alcohol based hand sanitizers, the present study provides much needed information on the effectiveness of these sanitizers in stemming the spread of preventable diseases in the community. There appeared to be a correlation between the concentration of the alcohol contained in the hand sanitizers and their effectiveness at reducing the total count and the diversity of transient flora organisms isolated after the application of the hand sanitizers. As shown in Table 7, GC® (60% alcohol content) was the least effective of the hand sanitizers, followed by CS® with an alcohol content of 62% alcohol content, followed by PL® with a label claim of 70% alcohol content being the most effective at reducing the total bacterial count and at limiting the diversity of organisms isolated from the respondents' hands after the hand sanitizer treatment.

Alcohols are known to exert disinfectant activity in bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids [18]. The present report demonstrates the effectiveness of alcohol based hand sanitizers and corroborates previous report by Oke et al, [19] where various branded alcohol based sanitizers with alcohol content of 62% demonstrated bacteriostatic activity when tested against laboratory test organisms such as *Staphylococcus aureus, Streptococcus pneumoniae* etc in vitro. Moreover, that limited reductions in bacterial count may be observed in the instance of some specific organisms such as *Staphylococcus aureus* perhaps due to the impact of added excipients used in formulating the hand sanitizers that may diminish the effect of alcohol in providing the desired bacteriostatic activity depending on the strain of microorganism [18].

According to Kaya and Pittet et al, [20], the resident flora colonizes deeper skin layers and is more resistant to mechanical removal than the transient flora. This flora is characterized

by coagulase-negative *staphylococci* and *corynebacteria* that multiply in hair follicles and remain relatively stable over time. The resident flora is known to possess lower pathogenic potential to the transient flora and present colonization resistance to potentially more pathogenic organisms. On the other hand, the transient flora is known to colonize the superficial skin layers for short periods, usually acquired through contact with contaminated persons, objects or environment. The microorganisms are easily removed by mechanical means such as hand washing. The transient flora is known to be responsible for most contact-associated infections and the spread of antimicrobial resistance [20].

In the community setting, hand washing as a means of hand hygiene is often limited when community members are unaware of the correct procedures for the removal of common pathogens from the hands of residents. This includes instructions on proper hand hygiene, including the use of soap and water and or hand sanitizers, followed by effective hand drying [21].

The correct procedure for hand washing as prescribed by the WHO is as follows: "Wet hands with water; apply enough soap to cover all hand surfaces; rub hands palm to palm; right palm over left dorsum with interlaced fingers and vice versa; palm to palm with fingers interlaced; back of fingers to opposing palms with fingers interlocked; rotational rubbing of left thumb clasped in right palm and vice versa; rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa; rinse hands with water; dry thoroughly with a single use towel; use towel to turn off faucet" [22].

5.0 CONCLUSION

The present results confirm that hand washing with soap and water is perhaps the most cost effective and reliable way to prevent the spread of pathogenic diseases in the community setting. Moreover, the results show that soap and water may provide better cleansing effect than certain brands of hand sanitizers. In addition, this work as expected has created more awareness of the importance of hand hygiene in breaking disease cycles within the Redeemer's University community and perhaps may serve as model for other communities elsewhere.

Conflict of Interest

None declared.

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