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***Invitro* Assessment of Antibacterial and Antifungal Agents Efficacy of
Some Isolates from Hands Surface**

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ABSTRACT

Insufficient hand hygiene has been implicated in Hospital and community-acquired infections. Our hands are soiled when being used to touch contaminated surfaces and therefore serve as a vector for the transmission of infectious microorganisms. The efficacy of hand sanitizers sold out to the public is crucial to infection prevention and control since its one of the ways to break the chain of transmission of infections caused by pathogenic microorganisms colonizing our hands. Accordingly, five (5) brands of commonly used hands sanitizers was purchased in triplicate within Sokoto metropolis and were evaluated for their efficacy against selected transient bacteria and fungi isolates from hands surfaces. The test isolates were identified by cultural and microscopic examination, biochemically and serologically. The isolated test organisms include: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*0157:H7 and *Candida albicans*. The efficacy testing of the sanitizers was carried out by: 1) the time kill test and ii) the *in use* potency test. The result of the time kill test revealed that only DET-HS reduced the test isolates by a 3Log₁₀ and above within 30 seconds of application. The other hand sanitizers showed a lesser log₁₀ reduction of the test isolates at the said time. The efficacy of the Hand Sanitizers in reducing base line bacterial count from hands of subjects was computed. The mean percentage colony forming unit reduction shown by DET-HS was 99.7%, and therefore was the highest in efficacy observed. GMB-HS showed the least bacteria load reduction of 31.3%. Regulatory bodies are encouraged to carry out steady routine checks and constant efficacy testing of hand sanitizers sold within the public domain so as to inform the populace on hand sanitizers most suitable for hand hygiene practice, withdraw and destroy substandard products sold off the counter while enforcing stringent quality assurance measures during formulation, importation, storage, distribution and sales of the products.

Keywords: Hand Sanitizer, Efficacy, Bacteria and Fungi

1.0 INTRODUCTION

Hand hygiene is recognized as one of the most important measure to prevent nosocomial infections in the healthcare setting (Boyce and Pittet, 2002; Oke *et al.*, 2013). In non-clinical settings, hand hygiene is accepted as a key factor in helping prevent the spread of pathogenic microorganisms (Boyce and Pittet, 2002). About 88% of diarrhea and respiratory illnesses occurring worldwide are largely linked to insufficient hand hygiene which account for about 1.5 million deaths annually - mostly in young

children (Centre for Disease Prevention and Control [CDC], 2009). This is largely because, hands are a major vector in the transmission of pathogens (Rhonda and Jones, 2001). Therefore, good hand hygiene practice is critical to the control of infectious diseases (CDC, 2009).

Normal human skin harbors bacteria (between 10² and 10⁶ CFU/cm²) and during daily activities, this bio-burden is progressively increased (Sunanda and Kulpure, 2004). This is assumed to be as a result of

direct patient contact or contact with contaminated environmental surfaces and devices (Sunanda and Kulpure, 2004). Transient microorganisms become attached to hand, fingertip, and fingernail surfaces, mostly after using the toilet, changing diapers, cleaning up a pet, touching contaminated raw products (e.g. raw meat, poultry, fish, unwashed fruits and vegetables), pricking infected cuts and boils, shaking contaminated hands or an infected fingernail (Sally and Nath, 2006). Pathogens that may be present on the skin of hand as transient types and include: *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Clostridium perfringens*, *Giardia lamblia*, Norwalk virus, Hepatitis A virus, *Candida* spp., etc (Geoffrey *et al.*, 2012).

Hand hygiene carried out with soap and water alone is insufficient in maintaining hand hygiene, since solid soap may hold bacteria and other microbial agents acquired from previous wash due to its reusable nature (Oranusi *et al.*, 2013). Repeated hand washing equally compromises the natural skin barrier, leading to scaling or fissures of skin (CDC, 2009). To address these hand hygiene deficit, hand sanitizers are continuously being formulated to act as an infection control regimen. Hand sanitizers are liquid formulation designed for application to hands to remove or kill microorganisms. Alcohol (isopropyl alcohol, ethyl alcohol or propanol) is popularly used as the active ingredient in most hands sanitizers. At 60-80% concentration, alcohol shows great *in vitro* efficacy against Gram-positive and Gram-negative bacteria including multidrug-resistant bacterial strains and fungal pathogens (Jonny, 2015).

Some studies have demonstrated poor efficacy of some hand sanitizers against common infectious microorganisms (Reynoid *et al.*, 2006; Oke *et al.*, 2015). Leading to considerable impacts on individuals such as prolonged hospitalization, long-term disability, increased risk of antimicrobial resistance (as seen in methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus*), huge financial burden for patients and their families and deaths (CDC, 2013). To determine whether hand sanitizers are safe and effective for use in hand sanitizing and therefore reinforce consumers' confidence when buying a product, a variety of laboratory methods are used to assess its *in vitro* antibacterial and antifungal

activity, among which are; the time-kill test and the *in use* potency test.

Both of which provide information on the nature of the inhibitory effect, time-dependent or concentration-dependent inhibition and the cell damage inflicted to the test microorganism (Balouiri *et al.*, 2016). This research therefore is aimed at *in vitro* assessment of Antibacterial and Antifungal Efficacy of five (5) Brands of Hand Sanitizers on Bacterial and Fungal Isolates from hand Surfaces. The objectives of the study are to: **i)** Isolate, Characterize and identify transient bacteria and fungi isolates associated with hand surfaces. **ii)** Perform the time kill *in vitro* antimicrobial assay on the test isolates. **iii)** Perform the *in use* potency test on the selected isolates. **iv)** Guide the public as to which hand sanitizer is most suitable for hands sanitizing based on the information gathered from this work.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

A total of five (5) most popular hand sanitizers used at homes were randomly purchased in triplicate from Sokoto central market and pharmaceutical outlets within Sokoto metropolis. Moist and sterile swab sticks were used for swab collection from hundred undergraduate students of Usmanu Danfodiyo University, Sokoto who participated in this studies. Samples collected were capped, labeled appropriately and quickly taken to the Microbiology laboratory of Usmanu Danfodiyo University Sokoto for further analysis.

2.2 Method of Sample Collection

Samples of hand sanitizers were purchased and inspected for manufacturer and expiry dates. Those within their labeled expiry dates were chosen by lottery sampling technique. Certification or otherwise of the samples by the National Agency for Food, Drug Administration and Control (NAFDAC) was examined and recorded. Further inspection of samples was made into those manufactured in Nigeria and elsewhere including names of manufacturers. The labeled chemical composition of the samples was examined and recorded including their specific antimicrobial constituents. Samples were tagged appropriately and taken to the Microbiology laboratory of Usmanu Danfodiyo University Sokoto for further analysis

2.3 Sterilization of Material

All glass ware used were properly washed, dried and sterilized in the oven at 160°C for one hour. Surfaces of laboratory work bench were disinfected with 70% ethanol to reduce contaminants (Dimphna, 2016).

2.4 Media preparation

Nutrient agar and other selective media used were prepared according to the manufacturers' instructions accompanying the label on the containers.

2.5 Isolation and Identification of Test Bacteria from Hands of Study Subjects

This was carried out according to the manual of the UK standards for Microbiology investigation (2015). The swabs were collected from a total of one hundred undergraduate students of Usmanu Danfodiyo University Sokoto. Swabs were cultured on plates of Mannitol Salts agar medium, MacConkey Sorbitol agar medium, Lysine Xylose Deoxycholate agar and Cystine Lactose Electrolyte Deficient agar medium. They cultured media were then incubated at 37°C for 24 hr. The colonial characteristics of the isolates was examined by Microscopy, Latex kit agglutination test and biochemically.

2.6 Isolation and Identification of Fungi Isolate from hands of study Subjects

This was carried out according to the UK standards for Microbiology investigation (2015). Swabs collected from undergraduate students of Usmanu Danfodiyo University Sokoto, were streaked on the surface of Sabouraud dextrose agar medium for the presence of *Candida albicans*. Cultured plates were there after incubated in an incubator at a temperature of 25°C for 2 days. Colonial characteristics of the cultured medium was studied including Microscopy, Biochemical characteristics and Germ tube test. Fungal isolate was thereafter sub-cultured on Potato Dextrose Agar slant medium maintained at temperature of 5°C until when needed.

2.7 Preparation of McFarland Turbidity Standard

McFarland Turbidity Standards are set of tubes with increasing concentration of barium sulfate suspension. A 0.5ml McFarland Standard was prepared by mixing 0.05 ml of 1% anhydrous barium chloride (BaCl_2) with 9.95 ml of 1% sulfuric acid (H_2SO_4) which forms barium sulfate precipitate

(turbid solution). The tube was tightly sealed and kept until required. The standard is used to determine approximate bacterial and fungal density in a culture suspension.

2.8 Standardization of the Inoculum

The standardization of the inoculums was carried out in accordance with the methods of Oyeleke and Manga, (2008). The isolates were revived in freshly prepared Mueller Hinton agar medium and incubated at 37°C for 24 hours. After the incubation, 5 ml of sterile distilled water was placed into different universal bottles and a sterile loop was used to pick a loopful of inoculum from a pure culture of each of the test organisms. This was then transferred and suspended in tubes of sterile distilled water used to prepare the size of the inoculums. The tubes were compared with the turbidity standard and the density of the organisms was adjusted to that of the standard by adding more bacterial/fungi or more sterile distilled water. The McFarland scale of 0.5 was used which is equivalent to 1.5×10^8 cfu/ml.

3.0 RESULT

Table 3.1a: Colonial Characteristics and Cell Micro Morphology of Bacteria and Fungi Isolates

S/NO	Bacterial & Fungal Isolates	Colonial Characteristics	Cell Micromorphology (Microscopy)
1	<i>Salmonella typhi</i>	On Lysine Xylose Deoxycholate agar, Opaque and translucent colonies	Gram Negative straight Rods
2	<i>Staphylococcus aureus</i>	On Mannitol Salt Agar, Golden yellow colonies were produced with yellow halo	Gram positive Round shaped colonies in clusters
3	<i>Escherichia coli</i> , o157:H7	On Sorbitol MacConkey agar medium, colourless colony, slightly raised and has an entire, fixed margin and a steady growth pattern, creating concentric growth rings in the colony	Gram Negative Straight Rod
4	<i>Candida albicans</i>	On SDA, creamy-grey colonies filamenting along their outer border	Gram +ve Round to Oval Cells

Table 3.1b: Biochemical and Serological Test of Bacteria and Fungi Isolates

Bacteria Isolates	Biochemical and Serological Test for the Characterization and Identification of Bacteria and Fungi Isolates									
	Agglutination with O, Vi & H antisera		Oxidase		Urease	Indole	Gram Reaction	Citrate	Motility	
<i>Salmonella typhi</i>	+ for O Antisera		-		-	-	-	-	-	
Bacterial Isolate	Serology with Staphilsideantisera	Gram Reaction	Catalase test	No vo bio cin	Coagulas e	Citrate			Urease	
<i>Staphylococcus aureus</i>	+	+	+	+	+	+			-	
Bacterial Isolate	Latex agglutination test with specific antisera	Gram Reaction	Oxidase		La ctose	Sorbitol MacCon key medium	Indole	MR	VP	Citrate
<i>Escherichia coli</i>	+	-	+		+	-	+	+	-	-
Fungal Isolate	Gram Reaction	Lactose	Sucrose	Maltos e	Dulsitol	Raffinose	Melibio se	Inositol	Germ tube	
<i>Candida albicans</i>	+	-	-	+	-	-	+	-	+	

**Bacterial and Fungal Isolates-Salmonella typhi, Staphylococcus aureus, Escherichia coli and Candida albicans
Test Bacteria and fungi Isolates Were Characterized and Identified using the UK standards for Microbiology Investigation (2015)**

3.2 Assessment of Antibacterial and Antifungal Efficacy of Hand Sanitizers

3.2.1 Determination of the Time-Kill *in vitro* Antimicrobial Assay (E 2315)

The efficacy of hand sanitizers was tested according to the American Standard for Testing and Material (ASMT E 2315) - A standard guide for the assessment of antimicrobial activity using a time-kill procedure (2015) with slight modification in the time of exposure of the isolates.

A 0.1mL aliquot of a challenge suspension containing 1.5×10^8 cfu/mL of each of the isolates was transferred serially into five sterile test tubes containing 9.9 mL each of sterile nutrient broth to give a fivefold dilution. A 0.1mL aliquot was removed from the last diluent and mixed with 5mls of the hand sanitizers contained in a test tube for 20, 30, 40, and 60 seconds contact time. A 0.1 ml of the bacterial and fungal suspension each was aliquoted into a solution containing 100 ml phosphate buffered saline and plated in duplicate on Mueller Hinton agar medium. With sterile distilled water used as control. The plates were incubated at 37°C for 24 hours for the test bacteria and 24-48 hours' incubation at 25°C for fungal isolate. Colonies were thereafter counted and the average \log_{10} reduction calculated using the following equation: $\text{Log}_{10} \text{Reduction} = \text{Log}_{10} \text{IP} - \text{Log}_{10} \text{PEX}$, Where: $\text{Log}_{10} \text{IP} = \text{Log}_{10}$ to base 10 of Initial population of challenge species (cfu/mL); $\text{Log}_{10} \text{PEX} = \text{Average population after exposure to each of the test formulations (cfu/mL)}$.

The result of the time kill *in vitro* antimicrobial assay is as shown in **table 3.2.1a-e**. The result shows the killing effects of the hand sanitizers against the test isolates at different time interval. IMJ-HS showed a less than $3\log_{10}$ reduction of the isolates at 30 seconds exposure time (Table 3.2.1a). ALV-HS caused a less than $3\log_{10}$ reduction of the test isolates at 30 seconds exposure time (Table 3.2.1b). DET-HS largely caused a $3-4\log_{10}$ reduction of the test isolates at 30 seconds exposure time except against *Salmonella typhi* which was reduced by $2.8\log_{10}$ at the said time (Table 3.2.1c). SPT-HS caused a $3.2\log_{10}$ reduction of *Salmonella typhi* at 30 seconds exposure time, while causing a less than $3\log_{10}$ reduction of the remaining isolates at 30 seconds exposure time (Table 3.2.1d). GMB-HS caused a $2.1-2.9\log_{10}$ reduction of the test isolate

at 30 seconds exposure time. A hand sanitizer which showed a $3\log_{10}$ reduction and above of the test isolates at 30 seconds exposure time are said to cause a killing effect of 99.9% ($3\log_{10}$ reduction) of the isolates, which is the conventional standard for bactericidal activity (Aiyegoro *et al.*, 2008; CDC, 2012

Table 3.2.1a: Time Kill *In Vitro* Antibacterial and Antifungal Assay of IMJ Hand Sanitizers

S/N	HS	B&F Isolates	CT (s)	Initial Population of Challenged Spp (cfu/ml)	Average of Final population of Challenged Spp (cfu/ml) ($\times 10^4$ cfu/ml)	Log ₁₀ Initial population on Challenged Spp	Log ₁₀ Final Population on Challenged spp	Log ₁₀ Reduction
1	IMJ	<i>S. typhi</i>	20	1.5×10^8	122	8.18	6.08	2.1
			30		84		5.92	2.3
			40		29		5.46	2.7
			60		8		3.90	4.5
		<i>S. aureus</i>	20	1.5×10^8	148	8.18	6.17	2.0
			30		122		6.08	2.1
			40		44		5.64	2.5
			60		34		5.53	2.6
		<i>E. coli</i>	20	1.5×10^8	124	8.18	6.09	2.1
			30		64		5.80	2.4
			40		59		5.77	2.4
			60		36		5.55	2.6
		<i>C. albicans</i>	20	1.5×10^8	200	8.18	6.30	1.9
			30		69		5.83	2.4
			40		82		5.91	2.3
			60		66		5.83	2.4

Key: IMJ-Imaj; *Salmonella typhi*; *Staphylococcus aureus*; *Escherichia coli*, *Candida albicans*; HS- Hand Sanitizer; B & F Isolates- Bacteria and Fungi Isolates; CT- Contact Time

Table 3.2.1b: Time kill *In Vitro* Antibacterial and Antifungal Assay of ALV Hand Sanitizers

S/No	HS	B & F Isolates	CT (s)	Initial Population of Challenged Spp (cfu/ml)	Average Final Population of Challenged spp (cfu/ml) ($\times 10^4$ cfu/ml)	Log ₁₀ Initial population of Challenged Spp	Log ₁₀ Average Final Population of Challenged Spp	Log ₁₀ Reduction
2	ALV	<i>S. typhi</i>	20	1.5×10^8	154	8.18	6.19	2.0
			30		154		6.19	2.0
			40		126		6.10	2.1
			60		124		6.09	2.1
		<i>S. aureus</i>	20	1.5×10^8	98	8.18	5.99	2.2
			30		74		5.87	2.3
			40		36		5.56	2.6
			60		14		5.15	3.0
		<i>E. coli</i>	20	1.5×10^8	216	8.18	6.33	1.9
			30		160		6.20	2.0
			40		120		6.08	2.1
			60		100		6.00	2.2
		<i>C. albicans</i>	20	1.5×10^8	56	8.18	5.75	2.4
			30		38		5.58	2.6
			40		24		5.38	2.8
			60		20		5.30	2.9

Key: ALV- Aloe Vera Lotion; *Salmonella typhi*; *Staphylococcus aureus*; *Escherichia coli*, *Candida albicans*; HS- Hand Sanitizer; B & F Isolates- Bacteria and Fungi Isolates; CT- Contact Time

Table 3.2.1c: Time kill *In Vitro* Antibacterial and Antifungal Assay of DET Hand Sanitizers

S/No	HS	B & F Isolates	CT (s)	Initial Population of Challenged Spp(cfu/ml)	Average Final Population of Challenged spp (cfu/ml) ($\times 10^4$ cfu/ml)	Log ₁₀ Initial population of Challenged Spp	Log ₁₀ Average Final Population of Challenged Spp	Log ₁₀ Reduction
3	DET	<i>S. typhi</i>	20	1.5×10^8	36	8.18	5.6	2.6
			30		28		5.4	2.8
			40		12		5.0	3.2
			60		4		4.6	3.6
		<i>S. aureus</i>	20	1.5×10^8	20	8.18	5.3	2.9
			30		18		5.2	3.0
			40		10		5.0	3.2
			60		2		4.3	3.9
		<i>E. coli</i>	20	1.5×10^8	38	8.18	5.6	2.3
			30		14		5.1	3.1
			40		14		5.1	3.1
			60		8		4.9	3.3
		<i>C. albicans</i>	20	1.5×10^8	21	8.18	5.3	2.9
			30		16		4.2	4.0
			40		9		5.0	3.2
			60		6		4.8	3.4

Key: DET-Dettol; *Salmonella typhi*; *Staphylococcus aureus*; *Escherichia coli*, *Candida albicans*; **HS**- Hand Sanitizer; **B & F Isolates**- Bacteria and Fungi Isolates; **CT**- Contact Time

Table 3.2.1d: Time *In Vitro* Antibacterial and Antifungal Assay of SPT Hand Sanitizers

S/No	HS	B & F Isolates	CT (s)	Initial Population of Challenged Spp(cfu/ml)	Average Final Population of Challenged spp (cfu/ml) ($\times 10^4$ cfu/ml)	Log ₁₀ Initial population of Challenged Spp	Log ₁₀ Average Final Population of Challenged Spp	Log ₁₀ Reduction
4	SPT	<i>S. typhi</i>	20	1.5×10^8	122	8.18	6.09	2.1
			30		110		6.04	2.1
			40		56		5.75	2.4
			60		20		5.30	2.9
		<i>S. aureus</i>	20	1.5×10^8	44	8.18	5.64	2.5
			30		20		5.30	2.9
			40		10		5.00	3.2
			60		-		4.00	4.2
		<i>E. coli</i>	20	1.5×10^8	122	8.18	6.09	2.1
			30		102		6.00	2.1
			40		50		5.69	2.5
			60		10		5.00	3.2
		<i>C. albicans</i>	20	1.5×10^8	24	8.18	5.38	2.8
			30		10		5.00	3.2
			40		-		4.00	4.2
			60		-		4.00	4.2

Key: SPT-Septol; *Salmonella typhi*; *Staphylococcus aureus*; *Escherichia coli*, *Candida albicans*; **HS**- Hand Sanitizer; **B & F Isolates**- Bacteria and Fungi Isolates; **CT**- Contact Time

Table 3.2.1e: Time kill *In Vitro* Antibacterial and Antifungal assay of the GMB hand sanitizers

S/ No	HS	B & F Isolates	CT (s)	Initial Population of Challenged Spp (cfu/ml)	Final Population of Challenged spp (cfu/ml) ($\times 10^4$ cfu/ml)	Log ₁₀ Initial population of Challenged Spp	Log ₁₀ Average Final Population of Challenged Spp	Log ₁₀ Reduction
5	GMB	<i>S. typhi</i>	20	1.5×10^8	48	8.18	5.68	2.5
			30		20		5.30	2.9
			40		12		5.08	3.1
			60		5		4.69	3.5
		<i>S. aureus</i>	20	1.5×10^8	66	8.18	5.81	2.4
			30		30		5.48	2.7
			40		24		5.38	2.8
			60		12		5.08	3.1
		<i>E. coli</i>	20	1.5×10^8	120	8.18	6.08	2.1
			30		110		6.04	2.1
			40		100		6.00	2.2
			60		92		5.96	2.2
		<i>C. albicans</i>	20	1.5×10^8	110	8.18	6.04	2.1
			30		68		5.83	2.4
			40		32		5.50	2.7
			60		26		5.41	2.8

Key: GMB- Germ Blast; *Salmonella typhi*; *Staphylococcus aureus*; *Escherichia coli*, *Candida albicans*; HS-Hand Sanitizer; B & F Isolates- Bacteria and Fungi Isolates; CT- Contact Time

3.3 Determination of the *In use* Potency of Hand Sanitizers

The methods of Oke *et al.*, (2013) and Rita *et al.*, (2014) were used to determine the efficacy of hand sanitizers in reducing viable counts of bacteria on the hands of subjects.

A total of thirty-five (35) individuals were randomly selected for the study. This number was divided into seven groups of five individuals and labeled (A-G). Guided by the procedure outlined in the European Norm (EN 1500), a standard guide for *in vivo* testing of hygienic hand rubs that are designed to reduce the level of transient flora on the hands, 3 ml of the hand rub was applied to subjects' hands and rub thoroughly for 30 seconds until it dries. Verbal informed consent was obtained from all participating subjects prior to the conduct of the test. The hands of subjects were examined for baseline bacterial count reduction with the hand sanitizers. Sterile nutrient agar plates were serially numbered and each divided into 2 halves A and B. The test was carried out with unwashed hands of the subjects. Subjects' right hands were gently used to make a finger impression on the agar by pressing and rolling the finger on the agar in the part labeled A on the plates. Three milliliters (3 ml) of the sanitizer was applied to the hand and then rubbed thoroughly on the palm, hands and fingernails until hands became dry. The finger impression was then repeated on the B part of the plates. This was done for all subjects. The plates were incubated at 37°C for 24 to 48 hours and the number of colonies counted.

The Percentage reduction in the bacterial load, %R was calculated as:

$$\% R = \frac{BBS - BAS}{BBS} \times 100$$

Where: BBS is bacterial load before sanitizing and BAS is bacterial load after sanitizing. Alcohol rub sanitizers containing 70% alcohol kills 99.97% of the bacteria on hands, 30 seconds after application (CDC, 2012).

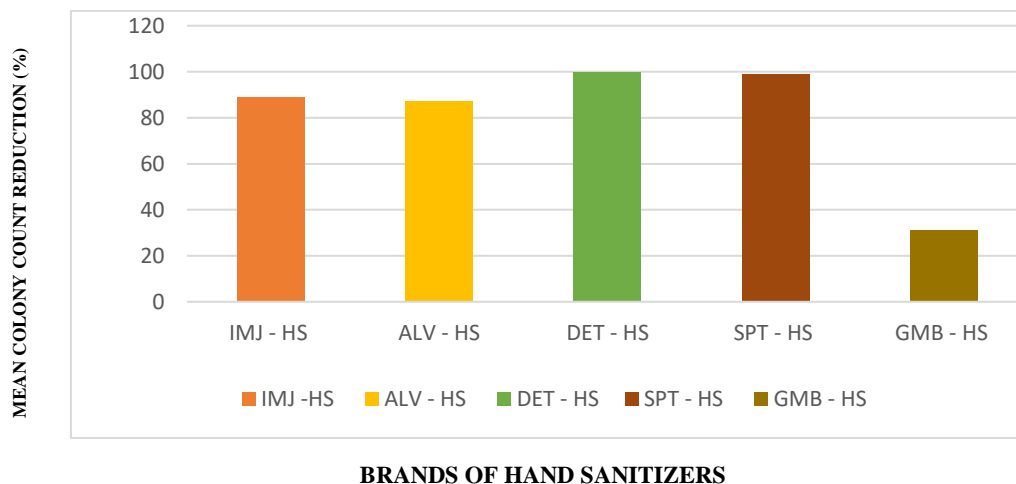
The result of the *in use* potency test is presented in **table 3.3.1**. It shows the percentage (%) reduction of viable microbial count on hands of subjects before and after treatment with hand sanitizers. When hands of the study groups were treated with IMJ-HS, ALV-HS, DET-HS, SPT-HS and GMB-HS, the baseline microbial load reduction in percentage was 89.0, 87.4, 99.7, 98.9, and 31.3 respectively. From

the result obtained, DET-HS showed the highest potency of 99.7% reduction of viable microbial load. Yet does not meet the standard specified for an effective antimicrobial hand sanitizer whose killing effect on test bacterial agents is 99.9% within 30 seconds after application (CDC,2012).

Study Group \ HS	IMJ-HS			ALV-HS			DET-HS			SPT-HS			GMB		
	BBW	BAW	%R	BBW	BAW	%R	BBW	BAW	%R	BBW	BAW	%R	BBW	BAW	%R
A	260	60	76.9	98	20	79.5	234	1	99.5	120	4	96.7	84	52	38.0
B	240	20	91.7	96	14	85.4	248	0	100	112	2	98.2	68	44	35.3
C	256	34	86.7	100	8	92.0	182	0	100	204	0	100	104	88	15.4
D	232	18	92.2	162	15	90.7	168	1	99.4	124	2	98.4	60	54	10.0
E	140	16	88.6	96	16	83.3	160	0	100	94	1	98.9	204	88	56.7
F	144	13	90.9	110	13	88.2	198	0	100	88	0	100	34	18	47.0
G	204	10	96.0	120	05	95.8	196	2	98.9	96	0	100	120	100	16.7
% Mean Reduction	89.0			87.4			99.7			98.9			31.3		

Table 3.3.1: Percentage (%) Reduction of Viable bacteria Count from Hands of Subjects before and after Treatment with Hand Sanitizers
Key: BBW- Bacterial Load Before wipe use, BAW- Bacterial Load After wipe use, % R – Percentage (%) Reduction of Bacterial Load

3.3.2 A Bar Chart showing the Percentage (%) Reduction of Viable Bacterial Count on Hands of Subjects before and after Treatment with Hand Sanitizers



4.0 DISCUSSION

It is pertinent to isolate and identify transient bacteria and fungi agents associated with the hands of study subjects. So as to test the efficacy of commonly sold hand sanitizers on the isolates. This is to ascertain their susceptibility to commonly used hand sanitizers within the study area and thus help break the chain of transmission of community based acquired infections. In this study, the isolates involved include: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 and *Candida albicans*, which are all associated with contaminated hands of subjects and are implicated in causing nosocomial and community-acquired infections. This result correlates with the findings of Montravers *et al.*, (2009), who showed that the test isolates were associated with the palm of apparently healthy subjects. Mondal and Kolhapure (2004) in India; and Aranusi *et al.*, (2013) at Ilorin- Nigeria, in their separate studies further corroborated these facts.

The result on table 3.2.1a-e shows the speed of killing of the test isolates within a given contact time with the hand sanitizers. DET-HS showed a 3 to 4log₁₀ reduction of the test isolates.

Representing the most effective among the hand sanitizers used. As an ideal antiseptic, DET-HS

hand sanitizer showed a 3log₁₀ reduction of the isolates within 30 seconds exposure time.

Which represent the conventional standard for germicidal

activity (Aiyegoroet *al.*, 2008; CDC, 2012). This could be attributed to the presence of the required concentration of alcohol denat and glycerol in the hand sanitizer whose combined antimicrobial effect eliminated the test organisms used unlike the other hand sanitizer.

The result of the colony count reduction is as shown in **table 3.3.1**. From the result, the bacterial count reduction by DET-HS was 99.7%, representing the highest among the hand sanitizers studied. This however does not conform to the conventional standards, i.e., (99.9% reduction of bacterial colony count). This could be attributed to the innate potential or Intrinsic mechanisms possessed by the test bacteria agents. Such as formation of efflux pumps, biofilm secretion, spore formation or double layer lipid membrane which primarily act to mediate antimicrobial resistance. For example, many strains of *Salmonella* Spp., harbor a genomic island known as *Salmonella* genomic island that contains a cluster of genes that may confer resistance to a variety of antimicrobials including hand sanitizers (Alakxandria, 2015). Some of these gene code for pumps which are transport proteins involved in the extrusion of foreign substrates (i.e. antibiotics, disinfectants and antiseptics) (Alakxandria, 2015).

Escherichia coli, secretes biofilm, a dense protective substance (extracellular polymeric substances [EPS]) which does not only enable communication via the chemical signaling pathway (quorum sensing [QS]) but also enhance their survival efficiency in the presence of a variety of antimicrobials.

The poor activity of the hand sanitizers could also be attributed to their active ingredients being inadequately formulated in the required concentration (65-80% for higher germicidal activity) or are chemically bounded with other ingredients (emollient, perfumes, colorants and humectants) making them insufficiently available to kill the test isolates in the suspension. This is in agreement with the work of Reynolds *et al.* (2006) who reported that a number of products in American stores contained alcohol in concentrations as low as 33% and 40% and are reported as being effective for use in hand antisepsis by their manufactures. In the study conducted by Oke *et al.*, (2013), using hygel and dettol hand sanitizers at Ilorin in Nigeria. None of the two Hand Sanitizers used met the conventional standard of usage specified by the center for disease prevention and control (CDC, 2012). The two hand sanitizers used by them showed a bacterial count reduction of 89.90% and 73.80% respectively. Odebisi-Omokanye *et al.*, (2013) in their study of hand sanitizers sold out commercially in Ilorin-Nigeria, further assert that their hand sanitizer studied could only cause a mean colony forming unit reduction of 72%. Which is Far less than the conventional standard specified by the Centre for disease prevention and control which specifies a killing effect of 99.9% of the bacteria on hands, 30 seconds after application (CDC, 2012). The percentage reduction of bacterial load observed in this study could also be attributed to the alcohol content of the hand sanitizers, getting readily volatilizes at high environmental temperature (daily environmental temperature could rise to 40°C during the day within the study area) resulting in decreased concentration and activity of the active components. With an increase in environmental temperature, the alcohol content of the sanitizers is further degraded and its germicidal activity weakened.

5.0. CONCLUSION

The intro assessment of antibacterial and antifungal efficacy of hand sanitizers is an important step in ascertaining the effectiveness of hand sanitizers use in infection prevention and control.

Findings from this research shows that all the isolates used to assess the efficacy of the hand sanitizers were transient flora all of which are of medical importance. The laboratory techniques employed to evaluate or screen for their *in vitro* antimicrobial activity provided information on the mean colony bacterial count reduction at base line and the Log₁₀ reduction of the test bacterial and fungal isolates within a given contact time. Of the five brands of hand sanitizers studied, DET-HS proved to be the most effective. This is largely due to the alcohol and propylene glycol contained in it whose combined antimicrobial effect caused a higher killing effect than the other hand sanitizers used. Being most promising of the products studied. Regulatory bodies are encouraged to carry out steady routine checks and constant efficacy testing of hand sanitizers sold within the public domain so as to inform the populace on hand sanitizers most suitable for hand hygiene practice, withdraw and destroy substandard products sold off the counter while enforcing stringent quality assurance measures during formulation, importation, storage, distribution and sales of the products

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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