Mensah et al., *Cogent Chemistry* (2017), 3: 1317317
https://doi.org/10.1080/23312009.2017.1317317

cogent chemistry

MEDICINAL CHEMISTRY | RESEARCH ARTICLE

Relative bactericidal efficacies of a selection of alcohol-based hand sanitizers (ABHS) available in Ghana

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Received: 05 December 2016
Accepted: 05 April 2017
First Published: 18 April 2017

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Reviewing editor:
Youcef Mehellou, Cardiff University, UK

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Abstract: The liberal use of alcohol-based hand sanitizers (ABHS) for hand disinfection has raised questions regarding the bactericidal efficacies of brands that are widely available in Ghana. Ten different brands of hand sanitizers bearing different chemical constituents and originating from six different countries were purchased in Kumasi, Ghana. The 10 samples represent the only available brands in the retail market. Purchased brands were examined for their relative bactericidal efficacies with the combined use of agar well diffusion, broth dilution, and viable bacterial count reduction assays. Serially diluted solutions of the ABHS displayed variable brand-specific bactericidal efficacies against a panel of bacteria specimen that comprised three gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*, *Streptococcus pneumonia*) and three gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*) in the agar well diffusion and in the broth dilution assays. Neither gram-positive nor gram-negative bacteria strains showed a clear pattern of preferential susceptibility to the growth inhibitory activities of any of the examined brands of ABHS. Only HS9 displayed a full spectrum bactericidal activity against all bacteria species in agar diffusion assay. Full strength ABHS formulations demonstrated brand-based rapid bactericidal action on hands of volunteers in a pattern best described as a post-ABHS-treatment reduction in levels

ABOUT THE AUTHORS

John Kenneth Mensah's multidisciplinary research focuses primarily on the assessment of antimicrobial and antioxidants activities of secondary metabolites isolated from terrestrial plants and from pure-cultured fungi.

The thrust of this study and the author's multidisciplinary interests have a common focus on the activities of antibacterial compounds. The study results provide new insights into the development and characterization of compounds and formulations with antibacterial activities.

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PUBLIC INTEREST STATEMENT

This study utilized three distinct bactericidal assays to offer a scientific support base for the relative bactericidal efficacies of ABHS. Although ABHS fulfill a useful role in antiseptic practice today, the findings of this study raise doubts about the effectiveness of some branded formulations. The study also dampens the hygienic dependability of hand disinfection that is based on the sole and intermittent use of ABHS.

of viable bacteria hand counts. HS9 displayed the highest efficacy and this relative estimation held true in all the three assays utilized for the assessment of bactericidal efficacies.

Subjects: Chemistry; Health & Society; Public Health Policy and Practice

Keywords: alcohol-based hand sanitizer; bactericidal efficacies agar diffusion; broth dilution; viable bacteria counts reduction

1. Introduction

Judicious use of alcohol-based hand sanitizers (ABHS) has been reported to provide multiple beneficial effects including a reduction in infection-related illnesses that leads to absenteeism among elementary school children (Hammond, Ali, Fendler, Dolan, & Donovan, 2000; Meadows & Le-Saux, 2004). ABHS use has also been implicated in a substantial decline in nosocomial transmission of infectious agents among hospital workers (Doebbeling et al., 1992). Accordingly, use of ABHS has been endorsed by some health professionals as alternatives to handwashing (Bloomfield, Aiello, Cookson, O'Boyle, & Larson, 2007). ABHS use has proven, with empirical evidence, to provide a first line of defense against the risk of transmission of multiple infections, including influenza, pharyngitis, and diarrheal illnesses (Bloomfield et al., 2007).

ABHS uniformly contain one or more alcohols (ethanol or 1-propanol or 2-propanol) in quantitative ratios that are distinct by brand. The alcohols present in the ABHS formulations triggers bactericidal action through denaturation of microbial proteins (Paulson, Fendler, Dolan, & Williams, 1999). Some ABHS may also 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. In Ghana, ABHS use is most prevalent among the urban-educated demography. ABHS users rely on its rapid bactericidal effects for transient and quick hand disinfection, particularly, in cases where soap and water use are either inconvenient or inaccessible.

The results shows that available brands have varying degrees of efficacies that manifests in a concentration-dependent growth inhibition of bacterial strains in agar diffusion and broth dilution assays. Observed moderate levels of relative inhibition by ABHS in agar diffusion and broth dilution assays suggest that hand disinfection with available brands might not be as thorough and as efficient hand sanitization practice that consumers envisaged. Viable bacterial counts reduction assays led to brand-dependent bactericidal activities that mirrors the trend in growth inhibition of agar diffusion and broth dilution *in vitro*. Since the viable bacteria count reduction assay shows that all brands failed to eliminate all bacterial pathogens on treated hands, the sole use of available ABHS can be considered as an inefficient hand disinfection method. Observed inefficacies of the studied brands leads to the proposition that the practice of effective hand hygiene should involve the transient use of ABHS that is complemented by conventional soap and water washing whenever possible.

2. Materials and methods

All chemicals were of analytical grade.

2.1. Hand sanitizer sampling

Ten retail samples of alcohol-based hand sanitizers (ABHS) comprising ten different brands (Carex™, Pharmaderm™, Deva™, Purell™, Sivoderm™, Progel™, Bactigel™, Deb™, Hygenic gel™, and Delglo™) and representing six countries of origin (Canada, China, Ghana, Indonesia, India, and Ivory Coast) were randomly purchased from different local outlets in Kumasi, Ghana, in 2015. The retail outlets include street markets, grocery shops, and supermarkets sited at Adum, Kejetia, and the central market of Kumasi, Ghana. The 10 samples represent the only available brands in the retail market. All samples were 60 mL in volume and were packaged in plastic bottles. Purchased samples were transported to the laboratory at the KNUST and kept at room temperature until analysis. Prior to analysis, samples were coded by brand as HS1, HS2, HS3, HS4, HS5, HS6, HS7, HS8, HS9, and HS10

(not in any particular order with stated brand names). Since the emphasis of sampling is in obtaining samples that reflect the broad range of hand sanitizers used by Ghanaians, production lots and the storage times of samples were not controlled in this study. All samples were within the expiry date displayed on the respective containers.

2.2. Culture, maintenance, and standardization of test microorganisms

Six bacteria strains acquired from ATCC (USA) and available in the culture collection of the Department of Microbiology, School of Pharmacy of KNUST were used as test microorganisms for the assessment of antimicrobial activities of the hand sanitizers with the agar diffusion and broth dilution assays. The panel of test bacteria included *Staphylococcus aureus* (ATCC 25923) *Streptococcus pneumonia* (ATCC 49619), *Enterococcus Feacalis* (ATCC 51299) (gram-positive bacteria) and *Pseudomonas aueruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 14028), *Escherichia coli* (ATCC 25922) (gram-negative bacteria). Bacteria specimen was stored in liquid nitrogen in 20% glycerol and were regularly maintained by sub-culturing on nutrient agar followed by storage at 4°C prior to experimental use.

2.3. Agar diffusion

The agar diffusion assays were performed with methods reported elsewhere (Mensah et al., 2016). Hand sanitizer concentrations for the agar diffusion assay were prepared in 10% increments and ranged from 10 to 50% for all brands (sterile distilled water used as diluent). In all, five dilutions (10, 20, 30, 40, and 50%) were made from each of the 10 different ABHS and utilized as the antibacterial agents against the panel of six pathogenic bacterial strains that comprises three gram-negative (*S. typhi, E. coli, Pseudomonas aeruginosa*) and three gram-positive (*S. pneumonia, E. feacalis, S. aureus*). Positive control test was made with Ciprofloxacin, a standard antimicrobial drug. Inhibition zone diameters were measured to the nearest whole millimeter from the center to the point where there was no visible growth (clear zones) after 24 h incubation. Plates were analyzed individually to determine zone of inhibition of bacterial growth and the average values from three repeats were taken in determination of the final diameters of zone inhibition.

2.4. Broth dilution

Broth dilution assay was performed as previously described (Mensah et al., 2016). Hand sanitizer concentration for broth dilution assays were 1.25, 2.5, 5, 10, 20, 30, 40, and 50%. Dilutions were made with sterile distilled water. Broth dilution assay utilized the same six pathogenic bacterial strains used for the agar diffusion assay. Ciprofloxacin was used as a positive control. Broths were analyzed individually for the estimation of MIC and the average values from three repeats were taken as the final determination of MIC. Through visual examination, sample MICs was taken as the lowest ABHS concentrations that showed 100% growth inhibition compared to the growth control after a 24 h incubation.

2.5. Study volunteers and hand cultures

Study volunteers were 10 students (all were males and all were right handed). Students were chosen because of their familiarity with ABHS use, their reliance on ABHS use for hand disinfection and because their hand flora is likely representative of the hand flora of the ABHS-user demography. Each volunteer was given one hand sanitizer as the test sample. Volunteer hands were tested for viable bacterial counts immediately before and immediately after treatment with ABHS. The thumb was used as representative of the hand.

A “before ABHS use” thumb culture was obtained by manually placing the left thumb of volunteer on one-half of a sterile agar plate and gently tilting the thumb from side to side. Great care was taken to avoid smearing the thumb across the agar surface as this error would produce “tailed” or duplicate colonies. Next, volunteers squirted approximately 3 mL of hand sanitizer into the palm of the left hand and were instructed to rub their hands in a pattern that interlaced the fingers with palms opposite each other; placed the backs of one hand in the opposing palms with interlocked fingers and then rotationally rubbed the right thumb clasped in the left palm and vice versa. This

hand rubbing method ensured that an even distribution of the ABHS on all areas of both hands was achieved before the squirted ABHS became completely dry.

Subsequently, an "after ABHS use" hand culture was made, after 2 min, on the other half of the agar plate using the right thumb imprint. This methodological choice is based on the rational that the "before ABHS use" sampling method applied to the left thumb would invariably lead to less microbial contamination on the left thumb. All plates were incubated at 37°C for 24 h. After incubation, colonies of bacteria were identified and counted by standard microbiological techniques.

3. Results

3.1. Chemical content of ABHS

Largely because of its proven bactericidal activities primarily attributed to its denaturation of microbial proteins, alcohol was found to be consistently present in the list of active constituents of all of the examined ABHS provided by the manufacturer. Besides alcohols, some labels indicate the presence of other chemical compounds with unknown bactericidal mechanistic mode of action as well as other additives that likely synergizes with the bactericidal activities of alcohol. Neither the alcohol content nor the content of the other constituents was stated in the label. Table 1 shows the complete list of the chemical constituents provided on the labels of studied ABHS.

3.2. Agar well diffusion assay

Agar well diffusion assay was used to assess the growth inhibitory effects of ABHS on the panel of bacterial strains. Zone diameters of growth inhibition at different ABHS dilution ratios, estimated in millimeters, became correlates of its bactericidal activity. Table 2 depicts the utilized range of ABHS concentrations along with their corresponding zone diameters of growth inhibition triggered against the panel of tested bacteria strains in the agar well diffusion assay.

HS1 displayed a narrow spectrum of bactericidal activity that featured a meager growth inhibition of only *Enterococcus faecalis*, a gram-positive bacteria species. While all applied concentrations of HS1 demonstrated bactericidal action against *E. faecalis*, the quantitative pattern in the zone diameters of inhibition was erratic and was devoid of a dose-dependent effect. For example, a 20%

Table 1. Chemical constituents and country of origin of ABHS

Code name	Chemical constituent	Country of origin
HS1	Alcohol denat, aqua, glycerin, gossypium herbaceum seed extract, tocopheryl acetate, carbomer, aminomethyl propanol, benzophenone-1, parfum, benzyl, salicylate, coumarin, limonene, geraniol, hexyl cinnamal, butylphenyl methylpropional, linalool, CI 77891	Indonesia
HS2	Alcohol denat,acqua, glycerin, carbomer, aminomethyl propanol	Cote d'Ivoire
HS3	Alcohol denat, aqua, propyl alcohol, BIS-PEG 12, dimethicone, coco-glycoside, glyceryl oleate, PEG-200 hydrogenated glycerul palmate, PEG-7 glyceryl cocoate, behentrimonium chloride, dihydroxypropyl, PEG-5 linleammomium chloride, isopropyl alcohol	Ghana
HS4	Alcohol denat, aqua, glycerin, gossypium herbaceum seed extract, tocopheryl acetate, carbomer, aminomethyl propanol, benzophenone-1	Canada
HS5	Alcohol denat, aqua, propyl alcohol, BIS-PEG 12, dimethicone, coco-glycoside, glyceryl oleate, PEG-200 hydrogenated glycerul palmate, PEG-7 glyceryl cocoate, behentrimonium chloride	Cote d'Ivoire
HS6	No active ingredient stated on container	India
HS7	Alcohol denat, aqua, glycerin, carbomer, aminomethyl propanol, behentrimonium chloride, isopropyl alcohol	Not stated
HS8	No active ingredient stated on container	Ghana
HS9	Alcohol denat, aqua, glycerin, carbomer, aminomethyl propanol	China
HS10	No active ingredient stated on container	China

Table 2. Zone diameters of bacterial growth inhibition (mm) in agar well diffusion assay

Hand sanitizer	Hand sanitizer concentration (%)	Test organisms					
		E. coli	*P. aeruginosa*	*S. typhi*	*E. feacalis*	*S. pneumoniae*	*S. aureus*
HS1	10	–	–	–	10	–	–
	20	–	–	–	14	–	–
	30	–	–	–	12	–	–
	40	–	–	–	14	–	-
	50	–	–	–	11	–	-
	Cipro	59	47	51	49	51	45
HS2	10	11	29	25	–	–	28
	20	14	30	27	–	–	29
	30	17	30	27	–	–	31
	40	21	32	28	–	–	30
	50	23	35	29	12	–	31
	Cipro	39	54	47	40	45	32
HS3	10	31	–	27	30	–	34
	20	32	–	29	31	–	34
	30	32	–	31	32	–	38
	40	35	–	36	34	–	39
	50	36	–	38	35	–	39
	Cipro	51	51	55	55	54	30
HS4	10	–	–	–	–	–	-
	20	–	–	-	–	–	–
	30	–	–	–	–	–	–
	40	–	–	–	–	–	–
	50	–	–	–	–	–	–
	Cipro	44	42	54	36	31	37
HS5	10	17	16	13	–	12	–
	20	21	22	18	–	16	11
	30	23	23	19	–	19	15
	40	24	25	19	–	21	17
	50	26	28	23	–	25	20
	Cipro	41	44	45	32	49	38
HS6	10	–	–	–	–	–	–
	20	–	–	–	–	–	–
	30	–	–	–	–	–	–
	40	–	–	–	–	–	–
	50	16	22	20	–	18	22
	Cipro	45	44	41	43	49	41
HS7	10	–	–	–	–	–	–
	20	–	–	–	–	–	–
	30	–	–	–	–	–	–
	40	–	–	–	–	–	–
	50	–	–	–	–	–	–
	Cipro	53	48	48	53	56	52

(Continued)

Table 2. (*Continued*)

Hand sanitizer	Hand sanitizer concentration (%)	Test organisms					
		E. coli	*P. aeruginosa*	*S. typhi*	*E. feacalis*	*S. pneumoniae*	*S. aureus*
HS8	10	–	–	–	–	–	–
	20	–	–	–	–	–	–
	30	–	–	–	–	–	–
	40	–	–	–	–	–	–
	50	–	–	–	–	–	–
	Cipro	47	60	60	47	45	46
HS9	10	13	17	17	13	17	12
	20	15	17	17	15	17	12
	30	18	19	19	18	18	13
	40	19	20	20	19	24	19
	50	22	22	22	22	26	20
	Cipro	49	47	47	49	37	44
HS10	10	–	–	–	–	–	–
	20	–	–	–	–	–	–
	30	–	–	–	–	–	–
	40	–	–	–	–	–	–
	50	–	–	–	–	–	–
	Cipro	46	48	48	46	37	42

Notes: Key = (–) No inhibition; Cipro = Ciprofloxacin.

concentration of HS1 produced the same zone diameter of inhibition of *E. faecalis* as that of the higher 40% concentration.

HS2 was much more inhibitory to the growth of gram-negative bacteria with demonstrable zone diameters of growth inhibition that depended on the dose of applied HS. However, gram-positive pathogenic bacteria generally remained the resistant strains with only *S. aureus* showing sensitivity to the bactericidal action of HS2. Susceptibility of *S. aureus* to the growth inhibitory effect of HS2 was independent of the dose of applied HS. Two strains each of the gram-negative and the gram-positive bacteria were sensitive to the growth inhibitory effects of HS3, establishing, in each case, a bactericidal susceptibility that was dose-dependent. *P. aeruginosa* (gram-negative) and *S. pneumonia* (gram-positive) were the two bacteria that were not susceptible to bactericidal action of HS3.

With the exception of *E. faecalis*, all bacteria strains were sensitive to the growth inhibitory effects of HS5. Bacteria susceptibility to HS5 activities was shown to be dose-dependent. Meager growth inhibition of nearly all bacteria strains occurred only at the highest applied concentration (50%) of HS6. However, no bacteria growth inhibition, even at the highest applicable dose, was demonstrated by the gram-positive *E. faecalis*.

HS9 showed a concentration-dependent variation in the zone diameters of inhibitions for all bacteria strains. Zone diameters of inhibition were similar across the range of bacterial species with comparable numerical estimates for both gram-negative and gram-positive bacteria at each applied HS concentration. With the largest diameters of zone inhibition at each applied concentration, the gram-positive *S. pneumonia* demonstrated the maximum sensitivity to growth inhibitory effects of HS9. It is interesting to note that four ABHS (HS4, HS7, HS8, and HS10) displayed no inhibitory effect on all bacterial strains at all applied concentrations. Only three ABHS (HS1, HS3, and HS9) displayed activity against the gram-positive bacteria *E. faecalis*. Similarly only three ABHS (HS2, HS5,

and HS9) demonstrated a dose-dependent bactericidal activity against the gram-negative bacteria *P. aeruginosa.*

Maximum sensitivity to growth inhibitory effects was displayed by the gram-negative *P. aeruginosa* with demonstrable largest inhibitory zone diameters of any applied ABHS concentration. Among susceptible bacteria, the gram-positive *S. aureus* showed the least zone diameter of inhibition for each applied ABHS concentration. In fact, measurable inhibition of *S. aureus* was observed only at doses above 10%. Considerable variation in inhibitory zone diameters were displayed by the three gram-positive bacteria and by the three gram-negative bacteria. Among the three gram-positive bacteria, *S. aureus* was the most HS-sensitive showing susceptibility to the bactericidal activities of 5 of the 10 HS while *S. pneumonia* was the least sensitive with demonstrable susceptibility to only 2 HS activities. Among the gram-positive species, *S. aureus* was the most susceptible species while *S. pneumonia* remained, relatively, the most resistant strain, insensitive to the activities of the highest number of examined ABHS.

Within the gram-negative panel, *E. coli* and *S. typhi* showed the highest susceptibility to HS activities while *P. aeruginosa* displayed the least susceptibility. Collectively, individual strains of both gram-positive and gram-negative bacteria displayed anomalous susceptibility to the bactericidal actions of the collective HS. Whether or not the growth inhibitory property or bactericidal activity of HS toward gram-positive bacteria were higher or lower than towards gram-negative bacteria could not be deciphered from the results with reasonable certainty. The dependence of the zone diameters of inhibitions on concentrations of HS2, HS3, HS5, and HS9 for some bacteria strains suggests that bactericidal efficacy increases with increasing HS concentration. Besides HS9, no other HS exhibited a broad-spectrum antimicrobial efficacy against every bacteria strain in the panel. In all cases, zone diameters of inhibition triggered by ABHS were lower by at least a twofold difference when compared with that elicited by the positive control substance Ciprofloxacin (Cipro in Table 2).

3.3. Broth dilution assay

Relative bacteria susceptibilities to the bactericidal activities of HS was additionally assessed through broth dilution assays that utilized the minimum inhibitory concentration (MIC) as relative estimates of bactericidal efficacies. MIC was taken as the lowest concentration of the assayed HS that triggered complete and visible growth inhibition of the specific bacterium under investigation. MIC determinations for each of the 10 ABHS was made against the 6 pathogenic bacteria (3 gram-positive and 3 gram-negative strains) and that afforded a total of 60 MIC values from all the possible combinations of ABHS versus pathogenic bacteria. Tables 3 and 4 lists the MIC values for all 10 ABHS against the panel of 6 bacteria pathogens.

Inhibitory concentration for HS1 was 10% for the two gram-negatives *E. coli*, and *S. typhi* and for the two gram-positives *S. pneumonia* and *S. aureus*. Together, the gram-negative *P. aeruginosa* and the gram-positive *E. faecalis* had inhibitory concentrations that was lower (half as much as the others) at (5%). On the whole, growth inhibitory concentrations for HS2 were higher than that for HS1. The gram-negative *P. aeruginosa* and the gram-positive *S. pneumonia* had inhibitory concentrations that were three times lower (10%) than that of all the other bacteria strains (30%). For HS3, higher MICs were recorded for gram-negative bacteria than for gram-positive bacteria. The gram-negatives *E. coli* and *S. typhi* recorded MICs of 20% while the gram-positives *E. faecalis* and *S. pneumonia* recorded MICs of 10%.

Besides the gram-negative *E. coli* that displayed an MIC of 20%, lower concentrations of HS4 (5–10%) were sufficient to prevent bacterial growth of all the other strains. An MIC of 5% against the gram-negative *P. aeruginosa* and the gram-positive *S. pneumonia* is recorded along with a 10% inhibitory concentration for *S. typhi*, *E. faecalis* and *S. aureus*. For HS5, MICs were mostly low and ranged from 5 to 10%. Only *E. faecalis*, a gram-positive bacteria, showed an inhibitory concentration of 10%. All other bacteria strains including the two other gram-positives and the three gram-negatives all recorded MICs of 5%.

Table 3. Minimum inhibitory concentration (MIC) in broth dilution assay

Hand sanitizer	Organisms	Concentration (%)							
		50	40	30	20	10	5	2.5	1.25
HS1	*E. coli*	−	−	−	−	−	+	+	+
	P. aeruginosa	−	−	−	−	−	−	+	+
	S. typhi	−	−	−	−	−	+	+	+
	E. feacalis	−	−	−	−	−	−	+	+
	S. pneumoniae	−	−	−	−	−	+	+	+
	S. aureus	−	−	−	−	−	+	+	+
HS2	*E. coli*	−	−	−	+	+	+	+	+
	P. aeruginosa	−	−	−	−	−	+	+	+
	S. typhi	−	−	−	+	+	+	+	+
	E. feacalis	−	−	−	+	+	+	+	+
	S. pneumoniae	−	−	−	−	−	+	+	+
	S. aureus	−	−	−	+	+	+	+	+
HS3	*E. coli*	−	−	−	−	+	+	+	+
	P. aeruginosa	−	−	−	−	−	+	+	+
	S. typhi	−	−	−	−	+	+	+	+
	E. feacalis	−	−	−	−	−	+	+	+
	S. pneumoniae	−	−	−	−	−	+	+	+
	S. aureus	−	−	−	−	+	+	+	+
HS4	*E. coli*	−	−	−	−	+	+	+	+
	P. aeruginosa	−	−	−	−	−	−	+	+
	S. typhi	−	−	−	−	−	+	+	+
	E. feacalis	−	−	−	−	−	+	+	+
	S. pneumoniae	−	−	−	−	−	−	+	+
	S. aureus	−	−	−	−	−	+	+	+
HS5	*E. coli*	−	−	−	−	−	−	+	+
	P. aeruginosa	−	−	−	−	−	−	+	+
	S. typhi	−	−	−	−	−	−	+	+
	E. feacalis	−	−	−	−	−	+	+	+
	S. pneumoniae	−	−	−	−	−	−	+	+
	S. aureus	−	−	−	−	−	−	+	+
HS6	*E. coli*	−	−	−	+	+	+	+	+
	P. aeruginosa	−	−	−	−	−	+	+	+
	S. typhi	−	−	−	+	+	+	+	+
	E. feacalis	−	−	−	−	−	−	+	+
	S. pneumoniae	−	−	−	−	+	+	+	+
	S. aureus	−	−	−	+	+	+	+	+
HS7	*E. coli*	−	−	−	+	+	+	+	+
	P. aeruginosa	−	−	−	−	+	+	+	+
	S. typhi	−	−	+	+	+	+	+	+
	E. feacalis	−	−	−	+	+	+	+	+
	S. pneumoniae	−	−	−	−	+	+	+	+
	S. aureus	−	−	−	−	+	+	+	+

(Continued)

Table 3. (*Continued*)

Hand sanitizer	Organisms	Concentration (%)							
		50	40	30	20	10	5	2.5	1.25
HS8	*E. coli*	–	–	–	–	–	+	+	+
	P. aeruginosa	–	–	–	–	–	–	+	+
	S. typhi	–	–	–	–	–	–	+	+
	E. feacalis	–	–	–	–	–	+	+	+
	S. pneumoniae	–	–	–	–	–	–	+	+
	S. aureus	–	–	–	–	–	–	+	+
HS9	*E. coli*	–	–	–	–	–	+	+	+
	P. aeruginosa	–	–	–	–	–	–	+	+
	S. typhi	–	–	–	–	–	–	+	+
	E. feacalis	–	–	–	–	–	–	+	+
	S. pneumoniae	–	–	–	–	–	–	+	+
	S. aureus	–	–	–	–	–	+	+	+
HS10	*E. coli*	–	–	+	+	+	+	+	+
	P. aeruginosa	–	–	–	+	+	+	+	+
	S. typhi	–	–	–	+	+	+	+	+
	E. feacalis	–	–	–	+	+	+	+	+
	S. pneumoniae	–	–	–	+	+	+	+	+
	S. aureus	–	–	–	+	+	+	+	+

Notes: Key = (–) No growth; (+) Growth.

Table 4. Summary of the individual MIC values of ABHS for each bacteria specimen

Organisms	Concentration of samples (%)									
	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10
E. coli	10	30	20	20	5	30	30	10	10	40
P. aeruginosa	5	10	10	5	5	10	20	5	5	30
S. typhi	10	30	20	10	5	30	40	5	5	30
E. feacalis	5	30	10	10	10	5	30	10	5	30
S. pneumoniae	10	10	10	5	5	20	20	5	5	30
S. aureus	10	30	20	10	5	30	20	5	10	30

The data suggest that HS6 may be less effective with two gram-negative bacteria *E. coli* and *S. typhi* and with one gram-positive *S. aureus* as demonstrated in their relatively high MIC values of 30%. The gram-positives *E. faecalis* and *S. pneumonia* are singularly inhibited at 5 and 20%, respectively, while the gram-negative *P. aeruginosa* is singularly inhibited at 10%.

MICs for HS7 were 20% against both the gram-negative *P. aeruginosa* and the gram-positive *S. pneumonia* and 30% against three other strains that included *E. coli*, *E. faecalis*, and *S. aureus*. MIC against the gram-negative *S. typhi* is recorded at a higher concentration of 40%. Four of the six bacteria strains including the gram-negatives *P. aeruginosa* and *S. typhi* and the gram-positives *S. pneumonia* and *S. aureus* displayed higher susceptibility to the effects of HS8 recording in each case a 5% MIC while the remaining two strains (the gram-negative *E. coli* and the gram-positive *E. faecalis*) were of moderate susceptibility each recording a twofold increase in MIC at 10%.

Table 5. MIC values for the positive control—the standard drugs Ciprofloxacin

Test organisms	Concentration (%)					
	0.01	0.005	0.0025	0.00125	0.000625	0.0003125
E. coli	–	–	–	–	–	+
P. aeruginosa	–	–	–	–	–	+
S. typhi	–	–	–	–	–	+
E. feacalis	–	–	–	–	–	+
S. pneumoniae	–	–	–	–	–	+
S. aureus	–	–	–	–	–	+

Notes: Key = (–) No growth; (+) Growth.

Bacterial growth inhibition was observed at low HS9 concentrations with four of the six strains (two gram-negatives *P. aeruginosa*, *S. typhi* and two gram-positives *E. faecalis*, *S. pneumonia*) recording MICs of 5% while that of the remaining two strains (*E. coli* and *S. aureus*) recorded MICs at a higher 10% concentration. Bacteria susceptibility to the growth-inhibitory effects of HS10 were low as recorded MICs showed large increases over the best 5–10% range observed for some ABHS. With the exception of the gram-negative *E. coli* that had an MIC of 40%, all of the remaining five bacteria strains including *P. aeruginosa*, *S. typhi*, *E. faecalis*, *S. pneumonia*, and *S. aureus* had an inhibitory concentration of 30%.

ABHS MICs for most bacteria strains was found to be within the 20–30% range but lower MICs of 5–10% was also observed for some HS. HS8 and HS9 exhibited strongest relative bactericidal activities as demonstrated by the consistently lower MIC for all bacteria strains. HS8 and HS9 triggered similar patterns of bacteria susceptibility in the broth dilution assay. Similar to the overall results in agar diffusion assay, HS9 again displayed much broader spectrum of bactericidal action than all the other HS. MIC's elicited by the positive control drug (Ciprofloxacin) were by comparison relatively low (Table 5); in fact, MIC values were lower than 0.01%.

3.4. Viable bacterial count reduction assay

Reduction in viable bacterial count after treatment of non-artificially contaminated hands with full strength HS was used as an additional evaluator of ABHS bactericidal efficacy. In all cases and for all ABHS brands, bacteria recovered from ABHS-treated hands were less in quantitative numbers than that recovered from untreated hands. Table 6 shows the considerable brand-specific variation in recorded differences in viable bacteria counts from post ABHS-treated hands.

Bacteria count from hands showed little decrease (by only 13 counts) after application of HS1. HS2 was a more effective bactericide than HS1 reducing by 16 counts more hand bacteria than HS1. Hand bacteria counts from HS3-treated hands showed a decrease of 17 counts, a number that is identical to that produced by HS1. HS4 produced a relatively small reductions in viable bacteria (11 counts) when compared with reductions obtained with HS1.

Table 6. Viable bacterial count reduction on hands of volunteers

Samples	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10
Subjects	A	B	C	D	E	F	G	H	I	J
Bacteria Count**	13	29	17	11	6	22	26	8	38	31

*Loss of viable bacteria estimated from the quantitative difference in bacteria counts between "untreated" and "treated" hands.

HS5 recorded just a meager reduction of six in viable bacteria count on treated hands. Observed decrease in bacteria counts resulting from HS5 treatment was almost less than twofold that of HS1. Recovered counts of hand bacteria, after treatment with HS6, decreased by 22 (a number which is an almost twofold decrease relative to that of HS1). HS7 triggered a decrease in the total bacterial count by 26, a comparable number to that elicited by HS6.

In fact, bacteria count from hands showed little decrease after application of HS8. With only eight as the reduction in viable bacteria on treated hands, HS8's application yielded a low quantitative number that is comparable to that of HS5. HS9 gave the highest recorded reduction (a 39 counts decrease) in viable bacteria counts from treated hands. In relative terms, hand bacteria counts from HS9 treatment dropped by as much as a threefold difference to that of HS1. HS10 gave considerably decreased bacteria counts of 31 after application, a recorded number that is comparable to that produced by HS9.

4. Discussion

Three patented biochemical assays: agar well diffusion, broth dilution, and viable bacteria count reduction were used in combination to assess the relative bactericidal efficacy of 10 widely used ABHS in Ghana. High ABHS bactericidal efficacy was derived, implicitly, from relatively large inhibitory zones diameters in agar diffusion assay; from lower quantitative values of MIC in broth dilution assay, and from lower relative counts of recovered viable bacteria from HS-treated hands. By contrast, relatively small zone diameters of inhibition, higher quantitative values of MIC, and higher relative numbers of recovered viable bacteria counts connoted, in relative terms, low ABHS bactericidal efficacy.

Bacteria species showed variable susceptibility to HS bactericidal action. The demonstrated numerical differences in the growth inhibitory zone diameters for all bacterial species in the agar diffusion assay and from the MICs in broth dilution assay show that bactericidal efficacy were both formulation brand- and bacteria strain-dependent. However, neither the gram-positive strains nor the gram-negative pathogenic bacteria showed a clear discernable pattern of preferential susceptibilities to the bactericidal activities of any of the 10 hand sanitizers. The lack of growth inhibitory effect displayed by HS4, HS7, HS8, and HS10 in agar diffusion assay is likely attributable to the high viscosity of these branded ABHS gel formulation that hindered the formulation's ability to diffuse efficiently, from the point of application, through the agar matrix. HS9 exhibited the broadest antibacterial spectrum among examined brands as shown in its relatively strong bactericidal activities against all six bacteria specimens in agar diffusion (highest relatively numbers of zone diameters of inhibition) and in broth dilution assays (lowest relative quantitative values of MICs).

The observation that all estimated MICs were above 2.5% (all values ranged from 5 to 40%) suggests low to moderate bactericidal efficacy relative to that of Ciprofloxacin, the positive control. In utilizing relative decreases in bacteria counts from post-HS treated hands, the viable bacterial count reduction assay became an important additional proxy for the assessment of bactericidal efficacy under normal ABHS use conditions. The general decrease in bacterial load, with fewer colony-forming units, on hands after application of each ABHS, show that all available brands possess measurable bactericidal effectiveness. Brand-specific decreases in viable bacteria colonies on ABHS-treated hands that ranged from 6 to 38 counts largely complemented the results of bactericidal efficacies that was assessed via agar diffusion and broth dilution assays. Consistently, ABHS brands that demonstrated relatively good bactericidal efficacy with agar diffusion and broth dilution recorded lower viable bacteria counts relative to pre-treatment counts on hands. Relative reductions in bacteria counts on treated hands was much greater for HS9 than for all the other brands. HS9 also provided the highest correlation between inhibition zone diameters and MICs and viable bacteria counts.

While underlying differences in bactericidal efficacies of ABHS accounts primarily for the differences in post-treatment hand bacteria counts, other factors, unrelated to direct bactericidal efficacies, might also contribute to the observed brand-specific differences in post-treatment levels of

viable bacteria counts. Minimum time required for the initiation of bactericidal action may also by brand-dependent with some brands potentially requiring longer times (than the two minutes contact time) to trigger its complete repertoire of bactericidal activities. Although, total microbial load on hands of volunteers might differ, its association with observed bactericidal efficacy of ABHS was not explored. Taken together, lack of stringent control on pertinent operational factors could exert a combination of unknown effects that might skew the results of the viable bacteria reduction count assay and distort its association with the assessed bactericidal efficacy of branded HS. Nevertheless, the reduction in viable bacteria count following ABHS treatment of non-artificially contaminated hands provides an additional supporting assay for the comprehensive assessment of the relative ABHS bactericidal efficacies.

ABHS dose for effective bactericidal action might differ in a brand-specific manner raising the possibility that increasing the amount of applied ABHS may increase the bactericidal efficacy of some examined ABHS formulations. In other words, doses larger than the utilized 3 mL might be required by some ABHS to trigger a more potent and efficacious bactericidal action. It is likely that satisfactory bactericidal activity on all examined bacteria species can be achieved with higher concentrations (up to 80%) of ABHS. But utilization of higher doses will likely mean that higher than normal volumes of ABHS will have to be applied on hands for just a minimal bactericidal efficacy. In that sense, normal brief exposure of hands to small quantitative amounts of ABHS may be inadequate for effective hand disinfection.

No direct correlation was established between relative bactericidal efficacies and the presence or absence of specific chemical constituent within the ABHS formulation. Given that all sampled ABHS contain alcohols, differences in bactericidal efficacy may originate either from the relative proportions of the alcoholic content or from the bactericidal potency of the other supporting chemical additives. Since all brands contain alcohol, the inability of some brands to act as satisfactory bactericides may be attributed to antagonistic interaction between the chemical additives and the bactericidal activity of alcohol. Chemical constituent types and dose of non-alcoholic additives differ by brand and this observation lends credence to the idea that distinct mechanistic modes of bactericidal actions might be in operation on a brand-specific basis. As details of the functions of the non-alcoholic additives and constituents accumulate, and as new bactericidal substances emerge, brand differences in the chemical constituents of ABHS will persist and so will differences in bactericidal mechanism and, by extension, differences in bactericidal efficacies of market available brands. Ghana's Food and Drug Authority may legislate bactericidal efficacy assessment of ABHS as a regulatory tool before the issuance of licenses to providers for the commencement of marketing.

Study results could not be compared to other collaborative studies for the assessment of hand sanitizer efficacy *in vitro* due primarily to the differences in formulations brands and to the variations in utilized experimental methodology. A lack of publications regarding the use of agar diffusion or of broth dilution for the assessment of the efficacy of HS also precluded direct comparisons of study results with other reports (Sickhert-Bennett et al., 2005).

Future additional studies can: assess the contributory role of other chemical additives and constituents, to the demonstrated bactericidal activities conferred by the alcoholic content of ABHS; examine the relative antibacterial efficacy of available ABHS with higher applied doses of ABHS and perform the viable bacteria counts assay with a longer post application wait time (longer than two min). Such studies could be expanded to encompass comparison of the efficacy of soap- and water-based handwashing with that of ABHS under a variety of normal activity-related conditions to assist in the determination of whether co-utilization of both approaches represent a more efficacious hand disinfection (that is ABHS use followed by soap- and water-based handwashing or vice versa). The lack of identification of the specific bacteria strain in a follow-up cultures to the viable bacteria count reduction is a prominent limitation of the study. Follow-up studies on the viable bacteria count reduction assay can identify the bacteria strains in each recovered colony to aid in the assessment of the bacteria strains that are preferentially targeted by each brand of ABHS. ABHS efficacy studies should

also be extended to include the assessment of microbicidal effect on fungi and other pathogenic microbes such as actinomycetes that are commonly present in prevalent hand microbial pools.

5. Conclusions

Increasing availability of ABHS has led to a substantial increase in use of brands with uncertain bactericidal effectiveness. This study assessed the relative bactericidal efficacy of 10 different marketed ABHS using agar diffusion and broth dilution assays along with the viable bacteria count reduction assay. Agar diffusion and broth dilution assays were performed on a panel of six medically relevant bacteria specimens and viable bacteria count reduction assay was performed on non-artificially contaminated hands.

Bacteria susceptibilities to antibacterial effect varied across brand in a dose-dependent manner in both agar diffusion and broth dilution assays. But neither gram-positive bacteria strains nor gram-negative bacteria strains showed preferential susceptibility to the bactericidal activities of any specific brand of ABHS. Agar diffusion-based estimation of zone diameters of inhibition of bacterial growth varied considerably with both the brand of ABHS and with the strain of the investigated bacteria species. *P. aeruginosa* (gram-negative) and *S. pneumonia* (gram-positive), in general, were comparatively more resistant to ABHS activity in agar diffusion assay. *E. coli* (gram-negative) and *S. aureus* (gram-positive) exhibited a relatively marked sensitivity to ABHS activities in agar diffusion assays. Broth dilution-based MICs estimated against the same panel of test bacteria used for the assessment of inhibitory zone diameter in agar diffusion, yielded numerical values that suggested brand-specific antibacterial activity. Trends in relative bactericidal efficacy estimated with broth dilution MIC compared favorably with that estimated with agar diffusion. In general, high quantitative MIC values (30–40%) corresponded to small or no inhibitory zone diameters. Similarly, low ABHS MIC values (5–10%) was associated with a larger zones of inhibition.

Individual ABHS exhibited limited, strain-dependent activity against the tested bacteria strains. Results of the growth inhibitory activities based on the experimental outcome of the two independent assays (agar diffusion and broth dilution) favor HS9 as the most efficacious. HS9 was the only ABHS formulation that showed strong inhibitory effects on the growth of all bacteria strains in agar diffusion assays. And with the lowest MIC values in the series and the broadest exhibition of antibacterial spectrum in the broth dilution assay, HS9 was also considered the most effective formulation.

For all ABHS, pre-treatment levels of viable bacteria on hands of volunteers were higher than post-treatment counts. HS9 treatment resulted, significantly, in the highest reduction in bacteria count from treated hands. A consistency of data therefore favors HS9 as the most efficacious: highest inhibition zone diameter against all bacterial strains in agar diffusion assay, lowest MIC against all of the bacterial strains in broth dilution assay and lowest colony counts in viable bacteria tests. These data emphasize the need for regulatory authorities to monitor antibacterial susceptibility of all brands of ABHS as a necessary regulatory check against the wanton marketing of ineffective ABHS formulations. Future studies could extend the scope of microbial investigation to include fungi, actinomycetes, amoeba, and other prevalent specific microbes that are known to frequently contaminate hands.

Funding
The authors received no direct funding for this research.

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Citation information
Cite this article as: Relative bactericidal efficacies of a selection of alcohol-based hand sanitizers (ABHS) available in Ghana, John Kenneth Mensah, Gideon Dela Akorsu & Charles Anamoah, *Cogent Chemistry* (2017), 3: 1317317.

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***Cogent Chemistry* (ISSN: 2331-2009) is published by Cogent OA, part of Taylor & Francis Group.**