RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Evaluation of the antibacterial activity of commonly used alcohol based hand sanitizers on common pathogenic bacteria.

KEYWORDS: Hand hygiene, hospital acquired infection, hand sanitizer

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ABSTRACT *Background: Hands are primary mode of transmission of microbes and infection. Hand hygiene is a simple and least expensive means of preventing hospital acquired infection. Companies producing hygiene products have offered a solution in form of sanitary, antimicrobial, alcohol based hand sanitizers to destroy these microbes.*

Aim: To investigating the antibacterial activity of commonly used alcohol based hand sanitizers on some selected common pathogenic bacteria.

Methods: Seven different sanitizers viz. A®, B®, C®, D®, E®, F®, and G® used for hand hygiene from seven different manufacturers were incorporated in this study. The test organisms used were common pathogenic bacteria responsible for hospital acquired infection. The antibacterial activity of hand sanitizers on pathogenic bacteria was carried out using the disc diffusion method.

Findings: Hand sanitizer brand E® was the most broad spectrum antibacterial agent, followed by brand F®. Brand G® was the most potent inhibiting most of the bacteria tested with very wide zone of inhibitions but the product showed poor activity against Ps. aeroginosa and S. marcescens. Other sanitizer brands A®, B® were effective only against a few bacteria with a limited scale, while the effectiveness of C®, D® were poorest as indicated by minimal or no activity against the tested microorganisms.

Conclusion: Manufacturers need to maintain strict quality control of their products in order to avoid breach of trust in promotion of hand hygiene. Regulatory authorities should enforce stringent quality control measures during production and routine inspections to ensure the efficacy of these products.

Introduction:

Hands are primary mode of transmission of microbes and have been considered to be the most important cause of hospital acquired infection (HAI) [1, 2]. The Center for Disease Control and Prevention (CDC) estimates that approximately 2 million people acquire HAI each year and that approximately 90,000 of these patients die as a result of their infections [3]. The CDC, WHO and many other experts promote hand hygiene as the single most important measure in the prevention of HAI [4, 5].

Companies producing hygiene products have offered a solution in form of sanitary, antimicrobial, alcohol based hand sanitizers to destroy microbes responsible for HAI. Due to its antimicrobial properties alcohol is used as the main antibacterial component of most waterless antiseptic agents [6]. Alcohols have a nonspecific mode of action, consisting mainly of denaturation and coagulation of proteins [7]. Cells are lysed [8, 9], and the cellular metabolism is disrupted [10].

Hand sanitizers are alcohol-containing preparations designed for application to the hands for reducing the number of viable microorganisms [5]. Various preparations of hand sanitizers are available including gel, foam and liquid solutions. Active ingredients of hand sanitizers include isopropanol, ethanol, n-propanol or providone-iodine while the inactive ingredients usually include a thickening agent (such as polyacrylic acid for gels), humectants (such as glycerin for liquid rubs) or propylene glycol and essential oils of plants.

Objective:

To investigating the antibacterial activity of commonly used alcohol based hand sanitizers on some selected common pathogenic bacteria was the aim of this study.

Material and Methods:

Test organisms

The test organisms used were ATCC strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa* and clinical isolates of *Staphylococcus aureus , MRSA, S. epidermidis, E. faecalis, Escherichia coli, K. pneumonia, Citrobacter freundii, Pseudomonas aeroginosa, Salmonella* Typhi , *Proteus mirabilis, Proteus vulgaris Acinetobacter baumannii, Acinetobacter lwoffii ,S. marcescens.*

Hand sanitizers

Seven different sanitizers used for hand hygiene from seven different manufacturers were included in this study. These sanitizers included the following: A®, B®, C®, D®, E®, F®, and G®.

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Table 1: Chemical composition of different sanitizer screened for antibacterial activity.

Serial no	Hand sanitizer name	Composition
1.	A®	2- Propanol 1- Propanol Mecetronium Ethyl sulphate
2	B®	2- Propanol 1-Propanol Mecetronium Ethyl sulphate
3	C®	2-Propanol (isopropyl alcohol) 70%v/v with Moisturizer 2-Propanol.....70 % v/v
4	D®	2- Propanol 1- Propanol Ethyl-hexadecyl-dimethyl ammonium-ethylsulphate Mecetronium Ethyl sulphate
5	E®	2-Propanol-70 % v/v, Chlorhexidine Gluconate Solution IP-2.5 % v/v, (equivalent to Chlorhexidine Gluconate- 0.5% w/v)
6	F®	Ethanol 70% Chlorhexidine 0.5%
7	G®	Ethyl alcohol 75 %+ Triclosan 0.5%

Determination of in-use potency of hand sanitizer

The modified method of David was used to determine the in use potency of the hand sanitizers [11]. Twenty one individuals were randomly selected for the study and verbal informed consent was obtained from all participating subjects prior to the conduct of the experiment. The subjects chosen were without clinical evidence of dermatoses, dermal abrasion, trauma and infection. Surface samples were obtained by swabbing each individual hand (areas of 4 cm^2) moistened with sterile distilled water only and after using 3 ml of sanitizer respectively. The samples obtained was inoculated onto blood agar plates, incubated at 30°C for 48 h and examined for growth. This temperature was chosen for incubation to ensure detection of both normal aerobic skin microflora and slow growing bacteria from the environment that required a lower incubation temperature. Percentage reduction in the bacterial load was calculated as % R = [(BBW –BAW)/BBW] × 100; where BAW is bacterial load after sanitizer use and BBW is bacterial load beforehand wash.

Assessment of antibacterial potency of sanitizers

Briefly, inoculums were prepared by transferring five distinct colonies from freshly revived stock cultures and suspended in a tube of sterile normal saline (NaCl 0.85%). The resulting suspension was vortexed and turbidity adjusted to match with 0.5 McFarland standard of Barium Sulphate solution [12]. The antibacterial activity of alcohol based hand sanitizers on pathogenic bacteria was carried out using the disc diffusion method in accordance with CLSI document [13]. Mueller-Hinton Agar plates (90mm in diameter) at a depth of 4.0 mm were used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. Sterile discs(prepared in-house) moistened with constant volume of different sanitizers were placed onto the surfaces of the inoculated plates, and the plates were incubated in air at 37°C and read at 24 h. Sterile disc without sanitizer was used as control. Zone diameter is measured to the nearest whole millimeter at the point at which there was prominent reduction of the growth.

Results:

According to the zone of inhibition formed resulting from each sanitizer against different bacterial isolates, findings in table 2 showed that hand sanitizer brand E® was the most broad spectrum antibacterial agent, followed by brand F®, although F® did not showed any activity against *K. pneumonia*.

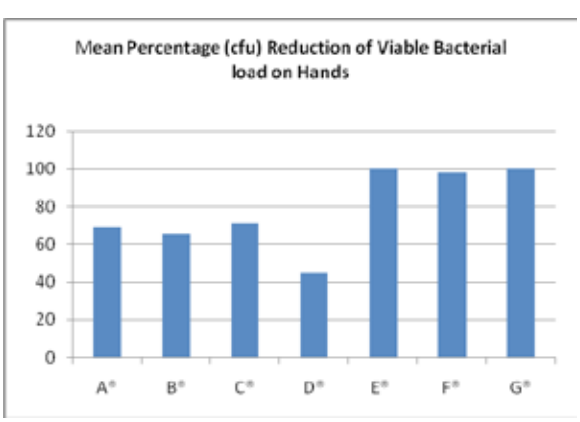
Comparing the antibacterial properties, brand G® was the most potent inhibiting most of the bacteria tested with very wide zone of inhibitions but the product showed poor activity against *Ps. aeroginosa* and *S. marcescens.* Other sanitizer brands A®, B® were effective only against a few bacteria with a limited scale, while the effectiveness of C®, D® were poorest as indicated by minimal or no activity against the tested microorganisms.

Table 2: Zone of inhibition for sanitizers against different bacterial isolates.

Isolates	Hand sanitizers/zones of inhibition (mm)						
	A®	B®	C®	D®	E®	F®	G®
S. aureus	10	10	8	8	20	23	58
S. aureus ATCC 259213	11	11	7	7	22	22	58
MRSA	9	10	7	7	22	24	56
S. epidermidis	9	9	7	7	23	24	50
E. faecalis,	9	10	6	7	16	17	26
Escherichia coli	9	11	8	7	18	18	33
Escherichia coli ATCC 25922	10	10	7	8	17	18	32
C. freundii	9	10	8	7	17	19	30
K. pneumonia	7	10	6	6	14	6	34
S. typhi,	7	8	9	6	21	20	38
P. mirabilis	10	8	6	6	16	15	25
P. vulgaris	8	8	6	6	17	15	23
Ps. aeroginosa	6	7	8	6	14	13	8
Ps. aeroginosa ATCC 15442	6	7	7	6	15	15	8
A. baumannii	6	6	9	6	12	16	27
A. lwoffii	7	6	8	6	11	14	24
S. marcescens.	9	9	8	6	14	11	8

Figure 1 shows the mean percentage reduction of viable bacterial load on hands of subjects after applying hand sanitizers. Sanitizer brand E® and G® reduced the bacterial load 100 percent followed by F® 98 percent. The percent reduction of bacterial load of D® was least 45 percent.

Figure 1: Mean percentage reduction of viable bacterial load on hands of subjects after applying hand sanitizers.



Discussion:

Human skin provides nutrients and suitable growth conditions for most pathogens as well as opportunistic bacterial pathogens and these bacteria evidently have the ability to be resistant to most of the cleaning regimen, thus contributing to their persistence in an ecosystem [14]. Hand hygiene is a simple and least expensive means of preventing hospital acquired infections specially derived from environmental surfaces, [15]. Alcohol based hand sanitizer is one of the components of hand hygiene, and is recommended

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

to reduce infections in healthcare settings. Food and Drug Administration (FDA) recommends a concentration of 60% to 95% ethanol or isopropanol, the concentration range is of greatest germicidal efficacy, OIC [16]. The use of hand sanitizers is gaining popularity both among medical and non medical personnel.

The results of this study shows that hand sanitizer reduced the bacterial load on the hands of the subjects to a varying degree. Complete biocidal activity was shown only by brands E® and G® although claimed by all. This is probably because that the reduction in the bacterial load is not just because of alcohol but also other ingredients incorporated. The active component of E®, F® is Chlorhexidine 0.5% w/v in alcohol base. Chlorhexidine is a cationic biguanide [17] and has good activity against most vegetative bacteria. The main target is the bacterial cytoplasmic membrane [18, 19]. After chlorhexidine has caused extensive damage to the cytoplasmic inner membrane, precipitation or coagulation of protein and nucleic acids occurs [20]. Although chlorhexidine has been described to be less effective in vitro against various nosocomial pathogens to other active agents like benzalkonium chloride [21].

The results of this study also throw light on the antibacterial activity of the hand sanitizers that are commonly used in the study area. Of the seven sanitizer investigated in this study, E® was the most broad spectrum antibacterial agent inhibition of the growth of the tested bacteria followed by F® and G®. Brand G® was the most potent sanitizer inhibiting most of the bacteria tested with wide zone of inhibitions although did not have activity against *P. aeroginosa* and *S. marcescens.* The active component of G® is 0.5% Triclosan, phenol derivatives (diphenoxyethyl ether). It blocks lipid synthesis by inhibition of the enzyme enoyl-acyl carrier protein reductase, which plays an essential role in lipid synthesis [22]. The reasons of failure of activity against *P. aeruginosa* and various other bacterial species is because of mutation and overexpression of the fabI gene—which encodes the enoyl-acyl carrier protein reductase—and thus are able to abolish the blockage of lipid synthesis caused by triclosan [23, 24].

Some of the sanitizers examined did not inhibit the growth of the tested bacteria probably as a result of low concentrations or lack of biocides in them and or noncompliance to stringent condition (good manufacturing practices). The lack of bactericidal activity observed among some of the products could also be due to poor or prolonged storage of the products which could lead to increased temperature causing evaporation of the active ingredient.

Conclusion

As we observed only one of the products inhibited growth of all the test organisms *in vitro* despite claimed broad spectrum antimicrobial activity by all. The claim of reducing bacteria by 99.9% by all the seven manufacturers, but only two products could be observed with desired efficacy of 100%. From these findings, we can conclude that there is strong need to confirm the concentrations of biocides and their activity before recommending for consumers. Manufacturers need to maintain strict quality control of their products in order to avoid breach of trust in promotion of hand hygiene. Regulatory authorities should enforce stringent quality control measures during production and routine inspections to ensure the efficacy of these products. Lastly, consumers should not blindly trust manufacturer's claims and must be beware of the existence of substandard products available in the market.

Acknowledgement: The authors are heartily thankful to Mrs. Vasvee Student PGDHC Department of journalism and mass communication, BHU, Varanasi, for her assistance in preparing the manuscript.

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