COMPARISON OF BACTERIAL FLORA ON THE HANDS OF HEALTHCARE WORKERS AND NON-HEALTHCARE WORKERS

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ABSTRACT

The hands serve as reservoirs of microbial flora hence play an important role in infection transfer. This project work was carried out to demonstrate and compare the bacterial flora on the hands of health care workers (HCW) and non-health care workers (NHCW), their antibiotic susceptibility pattern and the efficacy of Smartans™ alcohol based hand sanitizer (ABHS) in hand hygiene. The samples were collected twice in sterile polyethylene bags before and after the use of hand sanitizer, plated on MacConkey agar, Nutrient agar and Blood agar after serial dilution within 5 hours of collection and incubated for 24 hours. Antibiotic susceptibility pattern of the isolates was tested. Probable organisms isolated include coliforms, *Staphylococcus* sp and *Pseudomonas* sp. A students t-test was used to analyze the mean heterotrophic count of the isolates ($p>0.05$). Amongst the isolates, *Escherichia coli* was the most prevalent in both HCW and NHCW. The least occurring species on NHCW was *Proteus* sp whereas there was no growth of *Enterobacter* sp recorded for HCW. Although exposure to environmental flora is important in developing a natural immune system, proper hand hygiene practice with the use of hand sanitizers is one of the ways to curb the prevalence of bacterial flora that could be of major clinical significance.

***Key words*: - N.A -**

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INTRODUCTION

Hands play a major role in infection transfer both in health care institutions and other settings (Aiella *et al.,* 2002).Hand hygiene is the most effective measure for interrupting microorganisms which cause infections within and outside the health care setting.In 2002, Centers for Disease control and prevention (CDC) reviewed the recommendation for hand hygiene to include the use of alcohol-based products for standard hand hygiene (Boyce and Pittet 2002). Several studies have compared the bacterial flora on the hands of patient care and non-patient care personnel and patients versus health individuals. Since the publishing of data addressing these issues in the last few decades, hand hygiene regimes within the hospital has changed dramatically. As lower level of hand hygiene are practiced outside the clinical setting, it was hypothesized that the hands of individuals in the non-health care systems would have higher overall bacterial count's and fewer antibiotic resistance organisms than in health care workers. Despite the increased attention directed to hand hygiene especially in clinical setting the threat of infectious disease in developing countries remains very high. Studies have shown that there are about 2-3 million deaths worldwide each year from diarrheal diseases, (WHO, 2000) many of which could have been prevented.

Hand washing with soap has been estimated to save a million lives a year (Wendt, 2001). Limited or non-existence of basic sanitary infrastructures presents an extra hurdle in developing countries. This has differentiated the approach to hand hygiene in developing and developed countries.However, a new public health campaign amongst others led by the World bank and the water sanitation programme in collaboration with many other partners, has been set up to address increased hand hygiene in some developing countries.Another retraining factor of inadequate compliance to hand hygiene recommendation in developing countries is the lack of scientific evidence to basic question such as: How should hand be washed, when should they be washed, which product should be used and for how long (Weeks, 1999). Most hand hygiene data concerning microorganism are for bacteria, viruses are also extremely important and as far more difficult to investigate.

The overall aim of hand hygiene studies is to provide evidence that adherence to hand hygiene practices result in a decrease in microbial load and infection.

AIM

This study is aimed at demonstrating the bacterial flora present on the hands of health care workers as compared to the hands of non-health care worker and the need for persistent hand hygiene.

OBJECTIVES

To evaluate the bacterial load on the hands of workers in the health care system and those in non-health care systems.

To study the antibiotic susceptibility pattern of the isolates,To determine the efficacy of

alcohol based hand sanitizers in antiseptic hand rub.

MATERIALS AND METHOD

Specimens were collected from 10 Health Care Workers from Federal Medical Center Umuahia and Michael Okpara University of Agriculture, Umudike school clinic and 10 Non-health care worker based on random selection.Samples were collected twice from each participant over a period of 2 weeks, making a total number of 40 samples.Before sampling, the participants were convinced/educated by word of mouth. Trying to convince the participants even after presenting a tangible proof that it strictly on academic grounds was hectic. The inadequate compliance led to setbacks and exclusion of certain parameters that would have improved my scope of study.Initially participants rinsed their hands with peptone water into a sterile polyethylene bag and on second collection, they rubbed their hands first with Smartans™ hand sanitizer before rinsing with peptone water. 50ml of peptone water was used. This solution served as both the transport media and in dispersing the macro colonies into single cells for quantitation. The entire hand was massaged in the bag for 30seconds and samples were taken to the lab within 5hrs for further processing.

BACTERIOLOGICAL ANALYSIS

0.1ml of sampling solution (undiluted, 1:10 dilutions) was plated on MacConkey agar, Blood agar and Nutrient agar.

All plates were incubated at 37^0C and observed daily for growth over 24 hours. Microbes grown on cultures were identified using various biochemical tests.

ANTIBIOTIC SUSCEPTIBILITY

Antibiotic susceptibility testing of the bacteria isolates was done by disc diffusion method using commercial paper antibiotics containing panel of 10 discs each. The concentration of each antibiotic used is listed in the table below

Gram positive Discs

ANTIBIOTICS	CODE	CONCENTRATION (mcg)
Ciproflox	CPX	100
Norfloxacin	NB	100
Gentamycin	CN	10
Amoxil	AMX	20
Streptomycin	S	30
Rifampicin	RD	20
Erythromycin	E	30
Chloramphenicol	CH	30
Ampiclox	APX	20
Levofloxacin	LEV	20

Gram negative Discs

ANTIBIOTICS	CODE	CONCENTRATION (mcg)
Travid	OFX	100
Reflacine	PEF	100
Ciproflox	CPX	100
Augumentin	AU	300
Gentamycin	CN	100
Streptomycin	S	30
Ceprox	CEP	10
Nalidixic acid	NA	30
Septrin	SXT	30
Ampicillin	PN	30

BIOCHEMICAL TESTING

The biochemical tests used in identification of the microorganisms include

Coagulase test: A drop of distilled water was placed on each ends of the slide and a colony of the test organisms was emulsified in each of the drops to make two thick suspensions.

A loopful of plasma was added to one of the suspension and mixed gently. Clumping was observed within 10 seconds.

Catalase test: 2ml of Hydrogen peroxide (H_2O_2) solution was poured into a test tube. Several colonies of the organism were collected using a sterile stick and immersed in the H_2O_2 solutions and observed for bubble formation.

Oxidase Test: A piece of filter paper was placed in a clean petri dish and 2 drop of freshly prepared oxidase reagent was removed using a stick and immersed on the filter papers. The color change was observed within few seconds. Blue-purple color formation indicates positive oxidase test.

Citrate Utilization Test: This was done by preparing Simon's citrate agar and inoculating the test organism in a test tube containing the agar. It was incubated for 24hrs and colour change was observed. A deep blue coloration indicated a positive test.

Indole Test: The test organism was inoculated in a test tube containing 3ml of peptone water and covered properly. The samples was incubated at 35-37^{0}C for up to 48h. 0.5ml of kovac's reagent was added and shaked gently. Color change at the surface layer was observed within 10 minutes.

Motility Test: Semi-solid nutrient agar media in test tubes was stabbed with the test organism and incubated at 37^{0}C for 24 hours. Filamentous growth outside the line of stabbing indicates motile organism.

Gram Technique

A smear was made and fixed on a grease free glass slide.The fixed smear was flooded with crystal violet stain for 60 seconds and washed off with clean running water.

Excess water was tipped off and the smear was again covered with lugol's iodine for 60 seconds. The stain was rinsed with clean water and the excess tipped off. The smear was decolorized with acetone alcohol for few seconds and rinsed immediately with clean water.

Smear was covered with neutral stain for 2 minutes and washed with clean water. The back of the slide was cleaned and the slide was placed in a draining rack for the smear to air dry.

After which the smear was examined using a microscope first with X40 objectives and then with oil immersion objective.

DATA ANALYSIS

A students' t-test was conducted to compare counts of bacteria load on the hands of HCW & NHCW before using hand sanitizers.

RESULT

Specimens were collected twice from 20 participants. There was significant differences in the counts of bacterial load between the HCW and NHCW ($P > 0.05$).

The mean total log_{10} counts of organisms were 4.73 and 5.89 for HCW and NHCW respectively. A total of 128 organisms were isolated of which 60% (77) were gotten from NHCW and 40% (51) from HCW. Of the 77 isolates from NHCW, 22% (17) were Staphylococcus sp and total coliform count was 61% (47). Similarly 20% (10) of the 51 isolates from HCW were Staphylococcus spp and 51% (29) were coliforms.

Figure 1 shows the frequency of occurrence of the isolates. *E. coli* was the most prevalent in both HCW and NHCW. There was no *Entereobacter sp* among HCW. The percentage occurrence of *Proteus sp* was greater in HCW than NHCW.

The methods used to identify and characterize the isolates is shown in Table 1. All enterics isolated were motile but negative for coagulase test.

Tables 2 and 3 compares the prevalence of isolates in CFU/g from HCW and NHCW before and after the use of hand sanitizer. At dilutions of 10^{-4}, *E.coli* in NHCW recorded the highest CFU of 2.1 x 10^5. For all other organisms except *Proteus sp* NHCW had a higher percentage value. *Proteus sp* from HCW had 1.4 x 10^4Cfu compared to 0.8 x 10^5 recorded for NHCW. After the use of hand sanitizers, no significant growth was observed.

Table 4 shows the antibiotic susceptibility pattern of isolates from NHCW and the antibiotic susceptibility pattern of strains from HCW is presented in Table 5. There was high resistance of *staphylococcus sp* from both groups to ampiclox. All Gram negative isolates from NHCW were resistant to ampicillin. The Gram positive isolate from HCW were susceptible to ciproflox, levofloxacin, Rifamcin. Higher multidrug resistance was recorded among HCW.

Table 1: Identification and characteristics of isolated organisms

Colonial morphology	Catalase	Coagulase	Indole	Oxidase	Citrate	Motility	Gram reaction	Probable organism
Smooth pink colonies	+	-	+	-	-	+	-	*Escherichia coli*
White to colorless colonies	+	-	-	+	+	+	-	*Pseudomonas spp*
Creamy to yellow colonies	+	+	-	-	+	-	+	*Staphylococcus aureus*
Pink, moist sticky colonies	-	-	-	-	+	+	-	*Enterobacter spp*
Swarming colonies	-	-	+	-	-	+	-	*Proteus spp*
Creamy colonies	+	-	-	-	-	-	+	*Staphylococcus epidermidis*

Fig 1: Frequency of occurrence of isolates from hands of HCW and NHCW.

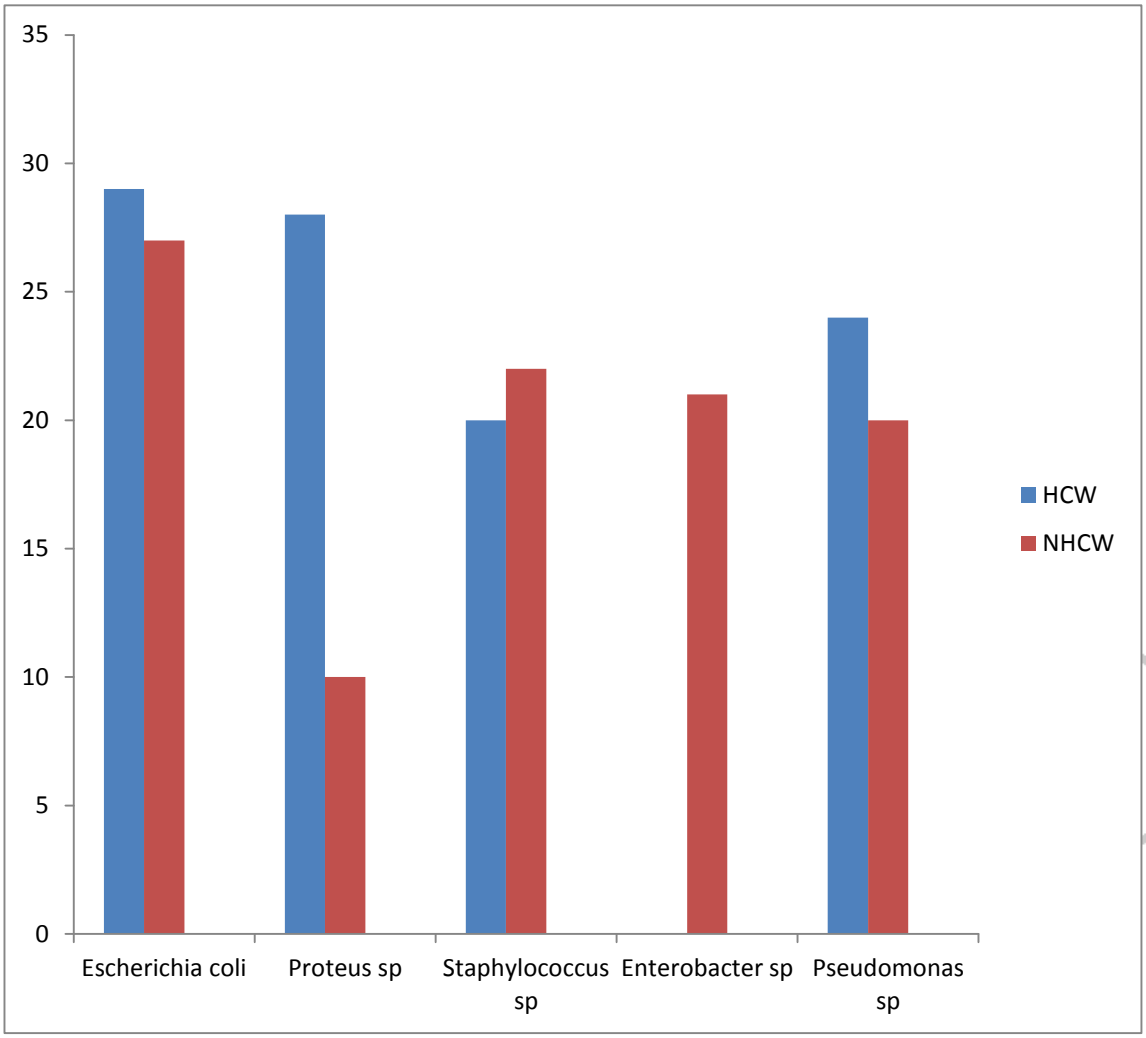

Table 2: Prevalence of isolates in CFU/g before the use of hand sanitizer.

ORGANISM	CFU/g	
	HCW	NHCW
Staphylococcus spp	1.0×10^4	1.7×10^5
Pseudomonas spp	1.2×10^4	1.5×10^5
Proteus spp	1.4×10^4	0.8×10^5
Enterobacter spp	NG	1.6×10^5
Escherichia coli	1.5×10^4	2.1×10^5

NG – No Growth

Table 3: Prevalence of isolates in CFU/g after the use of hand sanitizer.

ORGANISM	CFU/g	
	HCW	NHCW
Staphylococcus spp	NS	NS
Pseudomonas spp	NS	NS
Proteus spp	NS	NS
Enterobacter spp	NS	NS
Escherichia coli	NS	NS

NS – Not Significant

Table 4: Antibiotic susceptibility of isolates from hands of NHCW

Code	Staphylococcus sp N=17 (100%)			Escherichia coli N=21 (100%)			Proteus sp N=8 (100%)			Enterobacter sp N=16 (100%)			Pseudomonas sp N=15 (100%)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
PN	-	-	-	0(0)	0(0)	21(100)	0(0)	0(0)	8(100)	0(0)	0(0)	16(100)	0(0)	0(0)	15(100)
CH	6(35)	7(41)	4(24)	-	-	-	-	-	-	-	-	-	-	-	-
CEP	-	-	-	11(52)	10(48)	0(0)	6(75)	2(25)	0(0)	0(0)	2(13)	14(87)	12(80)	2(13)	1(7)
CPX	13(77)	4(24)	0(0)	20(95)	1(5)	0(0)	8(100)	0(0)	0(0)	16(100)	0(0)	0(0)	15(100)	0(0)	0(0)
OFX	-	-	-	16(76)	4(19)	1(5)	8(100)	0(0)	0(0)	15(94)	1(6)	0(0)	15(100)	0(0)	0(0)
E	0(0)	11(65)	6(35)	-	-	-	-	-	-	-	-	-	-	-	-
NA	-	-	-	0(0)	1(5)	20(95)	0(0)	1(13)	7(87)	13(81)	0(0)	3(19)	1(7)	2(13)	12(80)
LEV	17(100)	0(0)	0(0)	-	-	-	-	-	-	-	-	-	-	-	-
PEF	-	-	-	11(52)	9(43)	1(5)	5(62)	3(38)	0(0)	9(56)	2(13)	5(51)	15(100)	0(0)	0(0)
CN	15(82)	2(12)	0(0)	13(62	8(38)	0(0)	6(65)	2(25	0(0)	9(56)	7(44	0(0)	10(67)	4(26)	1(7)

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AU	-	-	-	6(29)	15(71)	0(0)	1(13)	5(62)	2(25)	11(69)	5(31)	0(0)	10(67)	5(33)	0(0)
APX	0(0)	0(0)	17(100)	-	-	-	-	-	-	-	-	-	-	-	-
RD	9(53)	7(41)	1(6)	-	-	-	-	-	-	-	-	-	-	-	-
AML	10(59)	0(0)	7(41)	-	-	-	-	-	-	-	-	-	-	-	-
SXT	-	-	-	0(0)	4(19)	17(81)	2(25)	5(62)	1(13)	0(0)	0(0)	16(100)	4(26)	10(67)	1(7)
S	0(0)	6(35)	11(65)	10(48)	10(48)	1(5)	3(38)	5(62)	0(0)	0(0)	2(13)	14(87)	3(20)	12(80)	0(0)
NB	2(12)	3(18)	12(71)	-	-	-	-	-	-	-	-	-	-	-	-

CH – chloramphenicol, CPX – ciproflox, E – erythromycin, LEV – levefloxacin, APX – ampiclox, RD – rifampicin, AML – amoxil, S – streptomycin, NB – Nor floxacin, PN – ampicillin, CEP – ceporex, OFX – tarivid, NA – nalidixic acid, PEF -reflacine, CN – gentamycin, AU – augmentin, CPX – ciproflox, SXT – septrin.
S* – Sensitive, I* – Inhibitory, R* – Resistance

Table 5: Antibiotic susceptibility of isolates from hands of HCW

Code	Staphylococcus sp			Escherichia coli			Pseudomonas sp			Proteus sp		
	N=10		(100%)	N=15		(100%)	N=12		(100%)	N= 14		(100%)
	S	I	R	S	I	R	S	I	R	S	I	R
PN	-	-	-	0(0)	15(100)	0(0)	0(0)	0(0)	12(100)	0(0)	1(7)	13(93)
CH	0(0)	1(10)	9(90)	-	-	-	-	-	-	-	-	-
CEP	-	-	-	1(7)	4(26)	10(67)	7(58)	5(42)	0(0)	2(14)	5(36)	7(50)
CPX	5(50)	5(50)	0(0)	9(60)	6(40)	0(0)	12(100)	0(0)	0(0)	9(64)	5(36)	0(0)
OFX	-	-	-	12(80)	2(13)	1(7)	9(75)	3(25)	0(0)	8(57)	6(43)	0(0)
E	0(0)	6(60)	4(40)	-	-	-	-	-	-	-	-	-
NA	-	-	-	1(7)	5(33)	9(60)	1(8)	2(17)	9(75)	1(7)	3(21)	10(71)
LEV	7(70)	3(30)	0(0)	-	-	-	-	-	-	-	-	-
PEF	-	-	-	13(87)	2(13)	0(0)	6(50)	5(42)	1(8)	14(100)	0(0)	0(0)
CN	1(10)	3(30)	6(60)	4(26)	1(7)	10(67)	1(8)	3(25)	8(67)	1(7)	1(7)	12(86)
AU	-	-	-	0(0)	3(20)	12(80)	0(0)	4(33)	8(67)	0(0)	9(64)	5(36)
APX	0(0)	1(10)	9(90)	-	-	-	-	-	-	-	-	-
RD	7(70)	3(30)	0(0)	-	-	-	-	-	-	-	-	-
AML	2(20)	0(0)	8(80)	-	-	-	-	-	-	-	-	-
SXT	-	-	-	9(60)	5(33)	1(7)	0(0)	12(100)	0(0)	8(67)	5(36)	1(7)

S	1(10)	2(20)	7(70)	1(7)	4(26)	10(67)	2(17)	2(17)	8(67)	3(21)	4(29)	7(50)
NB	0(0)	0(0)	1(100)	-	-	-	-	-	-	-	-	-

CH – chloramphenicol, CPX – ciproflox, E – erythromycin, LEV – levofloxacin, APX – ampiclox, RD – rifampicin, AML – amoxil, S – streptomycin, NB – Nor floxacin, PN – ampicillin, CEP – ceporex, OFX – tarivid, NA – nalidixic acid, PEF -reflacine, CN – gentamycin, AU – augmentin, CPX – ciproflox, SXT – septrin.
S* – Sensitive, I* – Inhibitory, R* – Resistant

DISCUSSION

The results from the research carried out suggest that the type and number of bacteria on the hands of HCW varies with that of NHCW. The bacterial load in terms of Total Heterotrophic Count obtained for the two study groups are significantly different. The species found on hands of NHCW were *Escherichia coli*, *Enterobacter spp*, *Proteus sp*, *Pseudomonas sp* and *Staphylococcus spp*. And on the hands of HCW; *Staphylococcus sp*, *Proteus sp*, *Pseudomonas pp*, *Escherichia coli*. A few studies have shown lower counts of bacteria on the hands of nurses versus on the hands of individuals with no hospital association. (Larson, 1971). In this study, was no growth observed on the hands of 2 HCW. A total of 128 organisms were isolated of which 60% (77) were gotten from NHCW and 40% (51) from HCW. Of the 77 isolates from NHCW, 22% (17) were Staphylococcus spp and total coliform count was 61% (47). Similarly 20% (10) of the 51 isolates from HCW were *Staphylococcus sp* and 51% (29) were coliforms.

Other studies have compared the hand flora of healthcare personnel with that of non-patient-care staff or other control groups, but were made several decades ago (Cespedes *et al*,. 2002).A few studies have shown lower counts of bacteria on the hands of nurses versus on the hands of individuals with no hospital association. For example, in the study by McBride *et al*. (1972) highest bacterial counts were found on hands of housewives. However the results are surprisingly consistent with this study. One study comparing hospital staff with community controls, with no association to the hospital environment, reported a higher proportion of control subjects carrying (GNB) Gram negative bacteria (Larson, 1981).A similar pattern was also found when comparing the most prevalent GNB, with the exception of *Proteus sp*. The differences in GNB is attributed to variations in exposure to bacterial populations. A study by Guenther *et al.*, (1981) reported that GNB were more frequently found on the hands of nurses when they had just arrived to work from home versus later on in their working shift. For most antibiotics tested, HCW had a greater proportion of antibiotic-resistant strains on their hands compared to NHCW. Studies have reported a similar trend comparing medical personnel and non-medical personnel and/or community controls (Cespedes *et al.*, 2002).

All Gram positive bacteria isolated from both HCW and NHCW were susceptible to Ciprofloxacin, Levofloxacin and Rifampicin but resistant to Norfloxacin.

All Gram negative bacteria isolated from the two study groups were susceptible to Ciproflox and Tarivid.

There was significantly greater proportion of Ampicillin, Ceporex, Nalidixic acid, Gentamycin, Augumentin and Streptomycin resistant *E. coli* from the hands of HCW versus NHCW. There was no

significant growth observed after the use of ABHS from both HCW and NHCW.

It is clear that working in a healthcare setting influences the bacteria flora of the hands with its merits (i.e. lower bacterial counts) and demerits (i.e. higher proportion of antibiotic resistance). The hands of NHCW can also serve as reservoir for strains resistant to certain antibiotics of clinical significance. This from previous studies has been attributed to indiscriminate consumption of antibiotics rather than patient contact with regards to HCW (Larson *et al.*, 2003). Also the use of ABHS in hand rubs proved effective and is recommended for use on hands without visible dirt. (Girou, 2002). Some other studies have compared the use of soap and water and other hand hygiene regime but has recorded significant growth (Ehrenkranz, 1991). It is therefore suggested that soap and water should be used for removal of large clumps of dirt but it is not a guarantee for clean safe hands.

CONCLUSION

In conclusion, the hand flora present on HCW can be causative agents of serious nosocomial infections owing to their high multidrug resistance patterns. The entire exclusion of microbial flora from the environment and subsequently on hands is not feasible hence the promotion of efficient hand hygiene. This promotion of hand hygiene cannot be confined to a healthcare setting alone because there are many issues concerning all aspects of hand hygiene which remain unresolved. While hand hygiene practices are simple, compliance with hand hygiene is about human behavior and altering human behavior is complex and constitutes an enormous challenge. This is reflected in the lack of success so far. Hand rubs with an alcohol base have recently been recommended as being more effective in reducing hand contamination compared with hand washing with an antiseptic soap, where hands are not macroscopically contaminated. Their use has been recommended for years because of their increased convenience compared with hand washing and they have become widely promoted in hand hygiene practice in clinical settings . They have a wide antimicrobial spectrum, they act rapidly, they spread easily without friction which damages skin, they evaporate rapidly, there is no need for a sink or drying facilities and they save time when compared with conventional handwashing. There is also evidencethat HCWs are more likely to use them than to wash hands with soap and water.In a healthcare setting they may also be cost effective in terms of the number of nosocomial infections prevented, though further analyses are necessary to substantiate this.Exposure to environmental flora is also important in the development of a normal immune system.

In the domestic setting the message regarding hand hygiene practices should be focused on interrupting the transfer of microorganisms and the spread of infection rather than just killing microorganisms per se. In the high-risk

health care setting, then the need to reduce the overall microbial load in the hospital environment becomes important.

Active measures should be taken by Health agencies and support groups to educate the public on the harm of self-medication especially with the use of antibiotics.

There is a wide variation in individual understanding and ideas which are due to differences in cultural, religious and socio-cultural beliefs hence prospective researchers on related project works should be aware of the impending challenge and face it courageously.

There should be the creation of a culture promoting hand hygiene at all levels of society to provide a foundation on which to establish a structure promoting compliance. It is impossible to make global recommendations regarding hand hygiene practices because what works in one culture may not work in another and all recommendations must take geographical and cultural factors into account.Standardized protocols and definitions are required both for laboratory investigations of hand hygiene preparations and for the study of hand hygiene behavior. Provision of hand sanitizers at work places, homes, cars, etc. will help promote effective hand hygiene practice.

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