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DETECTION OF EFFECTIVENESS OF HYGIENIC HAND WASHING

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Abstract-Health is the most valuable thing in life. Hand washing is one of the most important factors related to health. An evaluation of the effectiveness of hand washing for hygiene was undertaken to identify the microbial flora on the hand and establish their effects on microbiological quality. Examination of nine volunteer's hands before washing and after washing with routine soap (carbolic) using tap water found that there was unsatisfactory prevention for microbial contaminations. The isolated bacteria were Staphylococcus aureus, Staphylococcus epidermidis, Bacillus species, Corynebacterium species, Acinetobacter species, Micrococcus species and Streptococcus species. Nine samples from the same volunteers were taken after hand washing with disincentive soap (Dettol) and routine soap (carbolic) using sterial drinking water showed that there were no viable bacteria. Those nine samples taken after washing with dettol soap using tap water also showed no growth of bacteria. Total bacterial counts of almost all samples were approximately 10^6 CFU/ml before hand washing. However, total bacterial counts of sample 3,4 and 8 were higher than that of samples taken after hand washing with only tap water. It can, therefore, be concluded that hand washing is important for cleanliness and moreover, effective hand washing in use of proper soap and clean water contribute to the effective outcome.
Keywords- Hand washing, health, microbial contamination, soap and water.

1. INTRODUCTION

In daily life, cleanliness of hands is one of the important factors related to health. Many of us may not notice the importance of proper hand washing in several circumstances such as before handling food and eating. Transmission of diseases especially infections can occur through the five F's (fingers, food, formite, faeces and flies). Therefore, proper washing of fingers i.e., hands can prevent several infections and diseases.

In a healthy human or animal, the internal tissues are normally free of microorganisms. On the other hand, the surface tissues are constantly in contact with environmental organisms and become readily colonized by certain microbial species.

The normal flora of human is exceedingly complex and consists of more than 200 species of bacteria. The makeup of the normal flora depends upon various factors including genetics, age, sex, stress, nutrition and diet of individual. The normal flora of human consists of a few eukaryotic fungi and protists and some mrthanogenic Archaea that colonize the lower intestinal tract, but the bacteria are the most numerous and obvious microbial components of the normal flora [1].

A wide range of microbial pathogens is capable of infecting the gastrointestinal tract. They are acquried by the faecal-oral route, from faecallycontaminated food, fluids, or fingers and hand. Food borne illness is an ever-present threat that can be prevented with proper care and handling of food products. In the United States, food borne diseases occur between 24 and 81 million cases of each year. It is estimated that costing between $5 billion and $ 17 billion in medical care and last productivity [2].

Health is the most valuable thing in life. To maintain good hygiene, hands should always be washed after using toilet, changing a diaper or tending to someone who is sick, before eating, before handling or cooking food and after handling raw meat, fish or poultry.

Hand drying is as important as hand washing in preventing cross-contamination and the translocation of microorganisms since wet hands can transmit up to 500 time more bacteria as dry hands. Hand washing is the act of cleansing the hands with water or other liquid, with or without the use of soap or other detergents, for the purpose of removing soil or microorganisms. The main purpose of washing hands is to cleanse the hands of pathogens and chemicals which can cause personal harm or disease. The is especially important for people who handle food or work in the medical field. Washing our hands is one of the most effective ways to maintain health and prevent infection [3].

Conventionally, the use of soap and running water and the washing of all surfaces thoroughly, including under fingernails is seen as necessary. One should rub wet, soapy hands together outside the stream of running water for at least 20 seconds, before rinsing thoroughly and the drying with a clean or disposable towel. After drying a dry paper towel should be used to turn off water and open exit door. Moisturizing lotion is often recommended to keep the hands from drying out, should one's hands require washing more than a few times per day. People may catch diseases or pass diseases on to other people if they do not all keep their hands clean. Food prepared with dirty hands can carry diseases [4].

Therefore, to protect microbial culture, ourselves and the community, we also have to keep in mind, about proper hand washing in daily life. The aim of this research is to give public health knowledge about proper hand washing to the community.

2. METHODS

2.1 Isolation of Contaminated Bacteria from Volunteers' Hands

Firstly, samples were collected from nine different volunteer's hands by using five types of sampling. They are before hand washing without soap, after hand washing with carbolic soap and disincentive Dettol soap using tap water, and after hand washing without carbolic soap and Dettol soap using sterile water. And then, nutrient medium and sterile cotton swab were prepared for testing in this experiment. The portion of the palam and small fingers of volunteer's hands were swabbed and streaked on the sterile plates and then incubated at 35°C for overnight. After incubation, the isolated colonies were confirmed by using Gram's staining and microscopic examination.

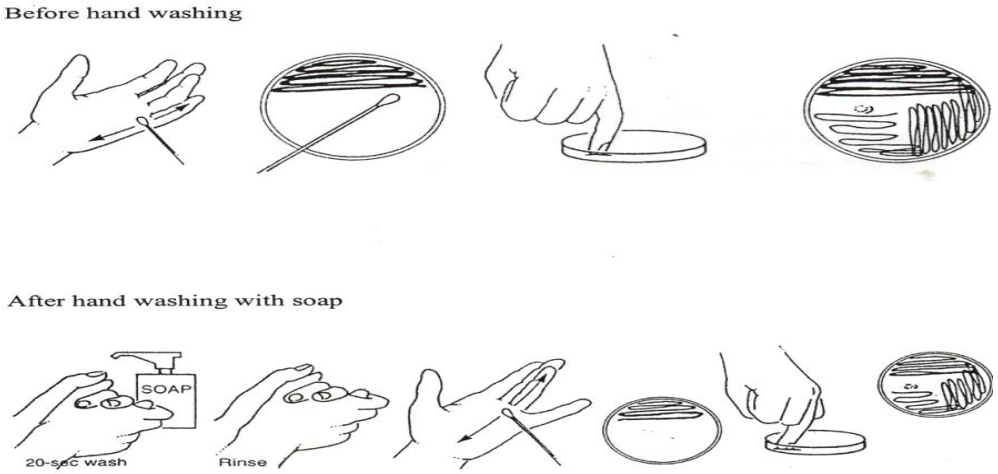


Fig. 2.1 Testing the Effectiveness of Hand Washing (Source: Barnett,1977)

2.2 Plate Count

Nutrient agar were prepared according to direction and dried at 40°C for 10 minutes. 1ml each of the samples was inoculated into sterile test tubes containing 9ml normal saline solution. Then 10-fold serial dilutions were made in normal saline using 1ml of sample. Diluted ranges from 10^{-7} to 10^{-8}. 1ml of each dilution was placed in sterile petridishes containing 15ml of nutrient agar. After 24 hours, colony counts were made on each plate. The average number perr plate was calculated and multiplied by dilution factor to obtain total bacteria count per ml of sample [4].

Total bacterial count = Number of colonies on selected plate x Selected dilution factor

3. IDENTIFICATION OF ISOLATED BACTERIA

3.1 Gram's STAINING

Each and every type of colony from agar plates was stained with Gram's solution and examined under oil immersion lens of the microscope to study gram reaction and morphology.

3.2 Motility Test

The 10ml amount of semi-solid agar media was dispensed in test-tubes and left to set in the vertical position. Incubated with a straight wire, making a single stab down the center of the tube to about half the depth of the medium. Incubated under the conditions favoring motility. The motile bacteria were swarmed and given a diffuse spreading growth that easily recognized by the naked eyes. The non-motile bacteria were given growths that were confirmed to the stab-line, had sharply defined margins and left the surrounding medium clearly transparent.

3.3 Biochemical Characteristics of Isolated Bacteria

Biochemical tests for isolated bacteria were carried out according to Cruickshank 1968, 1975 and Collins 1995.

3.3.1 Test for Catalase Production

At 18-24 hours agar plate containing a pure culture growth of the organism was tested with an inoculating needle. Center of a well-isolated colony was transferred to the surface of a glass slide. One drop of 3% hydrogrn peroxide solution was added, production of gas bubbles was indicated as catalase positive.

3.3.2 Methyl Red Test

The MR-VP broth was inoculated with a pure culture of the test organism. It was incubated at 35°C for 48 hours. At the end of this time, 5 drops of the methyl red reagent was added directly to the broth. The

development of a stable red color in the medium was indicated as a positive test. An intermediate orange color between yellow and red was indicated as a negative test.

3.3.3 Voges-Proskauer Test

The broth was inoculated according to MR test. 1ml of 40% potassium hydroxide and 3ml of 5% solution of α-naphthol in absolute ethanol were added. A positive rection was indicated by the development of a pink color in 2-5 min, becoming crimson in 30 mins.

3.3.4 Indole Test

The medium was inoculated with a pure culture organism. It was incubated at 35°C for 48 hours. Then, 0.5 ml of KovacS reagent was added. A red colour in the alchol layer was indicative of the presence of indole and a positive test.

3.3.5 Nitrate Reduction Test

The nittrate medium was inoculated with a loopful of the test organism isolated in pure culture on agar medium and incubated at 35°C for 48 hours. At the end of incubation, 0.1ml of α- naphthylamine in acetic acid was added to the test culture. The development of a red color within 30 seconds after adding the reagents, indicated the presence of nitrate and hence the ability of the organisms to reduce nitrate.

3.3.6 Gelatin Stab Liquefaction Test

A nutrient broth provided with 15% gelatin was sterilized by autoclaving. The sterilized medium was inoculated with organism under investigation and incubated at 37°C for 48 hours. A control gelatin tubes were then kept in a refrigerator for about 2 hours, and liquefaction of the inoculated stab was compared with that of the uninoculated one.

3.3.7 Triple Sugar Iron (TSI) Agar Test

The test is used for fermentation of lactose, sucrose, and glucose producing acid and gas by the phenol red indicator. H_2S production can be detected. Color changes in the medium in the butt and slant show acid production. All yellow means lactose/ sucrose/ glucose are fermented. All red means no sugar fermentation. Red slant, yellow butt means glucose is fermented. Black butt means H_2S production. Incubated at 37°C for 18-24 hours.

3.3.8 Citrate Utilization Test

The medium was poured into a tube on a slant. A well isolated colony was picked from the surface a primary solution medium and inoculated as a single streak on the slant surface of the citrate agar tube. The tube was incubated at 35°C for 48 hours. A positive test was represented by the development of a deep blue colour within 48 hours that the test organism had been able to utilize the citrate contained in the medium with the production of alkaline products.

3.3.9 Starch Hydrolysis Test

The medium was poured into a sterile petridish. The medium was incubated at 35°C for 48 hours. Then the plate was flooded with the Lugol's iodine and appearance of clear zone around the steak line indicated the starch hydrolysis.

4. RESULTS

In this study for bacteriological examination, total bacterial count CFU/ml of 9 samples befor hand washing and after washing with only tap water and after washing wtih carbolic soap with tap water are shown in Table 1. In total bacterial count CFU/ml of 9 samples after hand washing with Dettol using tap water, after washing with carbolic soap using sterile water and after washing wtih Dettol using sterile water experiment, there cannot be detected bacterial contamination.

Table-4.1 Total Bacterial Count CFU/ ml of 9 Samples

Sample No.	Total bacterial count (before hand washing)	Total bacterial count (after hand washing with only tap water)	Total bacterial count (after hand washing with carbolic soap using tap water)
1	3×10^8	4×10^6	6×10^4
2	3×10^8	3×10^6	2×10^5
3	2×10^8	2×10^6	$2 \times 10^{7*}$
4	3×10^8	3×10^6	$2.5 \times 10^{8*}$
5	4×10^8	3×10^7	4×10^5

6	5 x 10^8	6 x 10^6	4 x 10^5
7	2 x 10^8	2.5 x 10^6	6 x 10^5
8	3 x 10^8	4 x 10^6	2 x 10^{7*}
9	2 x 10^8	3 x 10^6	6 x 10^4

***= Increased counts**

Table 4.2 Morphological, Cultural and Biochemical Characteristics of Isolated Bacteria

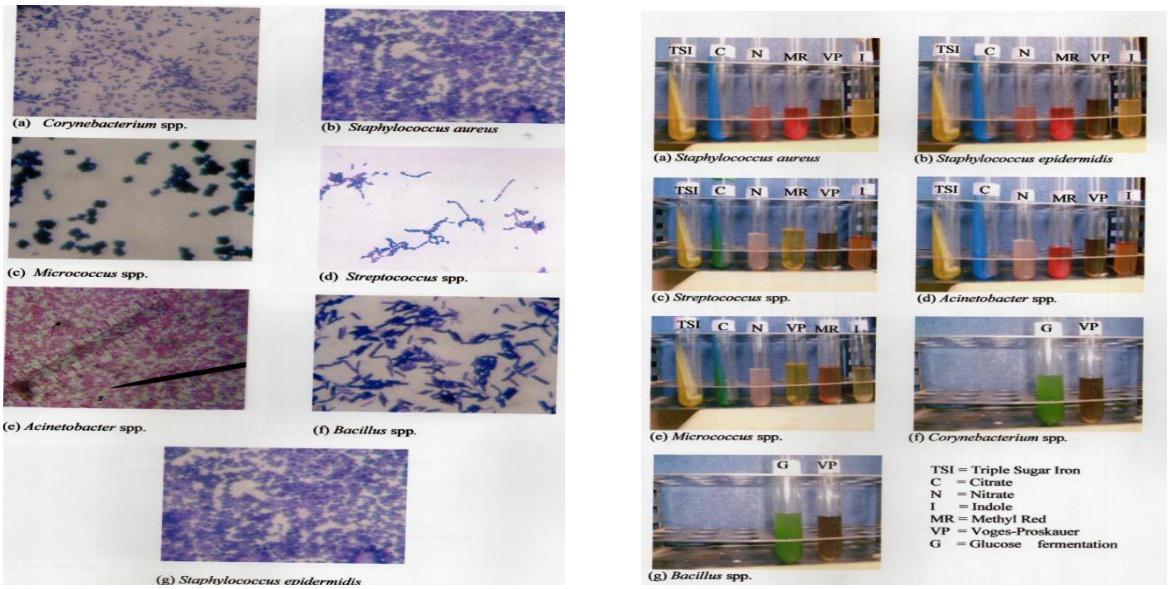
	S. aureus	**S. epidermidis**	**Streptococcus spp.**	**Acinetobacter spp.**	**Micrococcus spp.**
Gram staining	+	+	+	-	+
Morphology	Cocci arranged in grape like clusters	Cocci arranged in grape like clusters	Cocci arranged in bead like chain	Coccobacilli in pairs	Spheres in pairs, four and small clusters made up of uniform size
Colonial morphology	Golden-yellow colonies	Large, irregular flat of white colonies	Yellow colonies	Small white colonies	Colonies are convex with an entire margin and appear yellow
Motility	-	-	-	-	-
Catalase	+	+	-	+	+
TSI	+	+	+	+	+
Nitrate	+	+	-	-	-
Citrate	+	+	+	+	-
Indole	-	-	+	+	-
Gelatin	+	+	+	+	+
Starch	-	-	-	-	-
MR	+	+	-	+	+
VP	+	+	+	+	-

(+) = Positive result
(-) = Negative result

Table4. 3. Morphological, Cultural and Biochemical Characteristics of Isolated Bacteria

	Corynebacterium spp.	**Bacillus spp.**
Gram staining	+	+
Morphology	Club-shaped straight to slightly curved pleomorphic rods, non-spore forming	Rod-shaped endospore-forming bacteria
Colonial morphology	Usually convex, semi-opaque with white colour	Opaque colonies with white colour
Motility	-	+
Catalase	+	+
H_2S	-	-
VP	-	-
Glucose (acid)	-	-

(+) = Positive result, (-) = Negative result



(a) **(b)**

Fig. 4.1 (a) Microscopic Morphology of Isolated Bacteria (Oil immersion 100x), Biochemical Tests for Isolated Bacteria

Table4.4 Comparison of Isolated Bacteria before and after Hand Washing

Sample No.	Before hand washing	after hand washing with carbolic soap using tap water	after hand washing with Dettol soap using tap water	after hand washing with carbolic soap using sterile water
1	S.epidermidis, S.aureus	S.epidermidis, S.aureus, Corynebacterium spp.	-	-
2	S.epidermidis, S.aureus, Corynebacterium spp., Bacillus spp	Bacillus spp., Corynebacterium spp.	-	-
3	S.epidermidis, S.aureus, Corynebacterium spp., Bacillus spp	Micrococcus spp., Corynebacterium spp.	-	-
4	S.epidermidis, S.aureus, Corynebacterium spp., Streptococcus spp	Bacillus spp., Corynebacterium spp.	-	-
5	S.epidermidis, S.aureus	Micrococcus spp., Acinetobacter spp.	-	-
6	S.epidermidis, S.aureus, Corynebacterium spp., Acinetobacter spp.	S.epidermidis, S.aureus, Corynebacterium spp., Bacillus spp	-	-
7	S.epidermidis, S.aureus, Streptococcus spp	Micrococcus spp., Corynebacterium spp., Bacillus spp.	-	-
8	S.epidermidis, S.aureus, Corynebacterium spp., Bacillus spp, Acinetobacter spp.	S.epidermidis, S.aureus, Bacillus spp.	-	-
9	Bacillus spp, Corynebacterium spp., Streptococcus spp.	Micrococcus spp., S.epidermidis , Bacillus spp.	-	-

(-) = Not detected

CONCLUSION

This study evaluated the effectiveness of hand washing by identifing the microbial flora on the hand and establishing their effects on microbiological quality. Examination of samples from nine volunteer's hands before washing and after washing with routine soap (carbolic) using tap water found that there were unsatisfactory

prevention for microbial contamination. The bacteria detected were S. aureus, S.epidermis, Bacullus spp., Corynebacterium spp., Acinetobacter spp., Micrococcus spp., and Streptococcus spp. These bacteria were isolated and identified according to their morphological, cultural and biochemical characteristics. Total bacterial counts are approximately 10^6 CFU/ml before hand washing and after hand washing in ordinary way.

Those nine samples taken after hand washing with routine soap (carbolic) and disinfective soap (Dettol) using sterile drinking water showed no viable bacteria. The samples that were taken after hand washing with disinfective soap (Dettol) using tap water also showed no growth of bacteria. Hence, the hygiene status was found to improve by using disinfective soap (Dettol).

In order to prevent microbial cultures, ourselves, the community, we also have to keep in mind, about proper hand washing in daily life. And then, the hand washing is important in reducing the spread of infection. We can, therefore, conclude that proper hand washing is important for cleanliness and moreover, effective hand washing i.e., proper use of soap and clean water contribute greatly to the effective outcome.

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