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Diversity of Human Skin Microbiota in Healthcare Workers of South Punjab, Pakistan during COVID-19

Anosh Arshad Chaudhary¹, Iqra Arooj^{1*}, Amina Elahi², Saadia Ijaz¹, Humaira Yasmeen¹

1. Department of Microbiology and Molecular Genetics, The Women University, Multan, 60000, Pakistan.
2. University Institute of Medical Laboratory Technology, University of Lahore, Lahore, 55150, Pakistan.

*Corresponding Author's Email: iqra.6051@wum.edu.pk

ABSTRACT: *Human skin microflora plays important role in the functioning of skin and is modulated by several intrinsic and extrinsic factors including hygiene practices. During Covid-19 pandemic, focus has been particularly directed towards improving hygiene. We explored the skin microflora of healthcare workers in local population of Multan, Pakistan. A total of 44 samples of skin were collected from healthy workers along with the administration of questionnaire regarding hygiene practices. After isolation, bacteria were characterized by morphology, staining and biochemical tests. Majority of the workers was 20-30 years old females. Most of them reported to wear gloves during practice, wash hands 8-10 times a day, use sanitizer 8-10 times a week and took bath 12-15 times a month. Isolated microflora (n=110) included Staphylococcus aureus (62%), Escherichia coli (16%), Pseudomonas spp. (9%), Proteus spp. (5%), Enterobacter spp. (5%) and Klebsiella spp. (3%). Presence of pathogens, although in small numbers, emphasizes the necessity of disseminating knowledge regarding adoption and maintenance of hygienic practices, specifically among healthcare workers.*

Keyword: Skin microflora; Healthcare workers; Hygiene; Covid-19; Microbiome

INTRODUCTION

Skin is the largest organ of human body and it provides protection against the

external environment. Microorganisms inhabiting the skin are considered to be of great interest by the researchers as they play major role in shaping the

host's immunological responses towards environmental factors. There is a wide variety of microorganisms inhabiting our skin which make up natural microbiota. It is also called normal or residential flora of skin. Surprisingly, human skin has been proven to be unsuitable environment for bacterial growth (Bojar and Holland, 2002). Still many bacterial species successfully inhabit skin among which, commensals are the dominant resident species on skin (Barnard, 2017). This human microbiota plays major role in modulating the immune system by acting as a barrier against the foreign pathogens. In humans, immunological responses are frequently associated with the microorganisms living on skin (Skowron et al., 2021). Skin microbiota colonization is influenced by several factors related to an individual's lifestyle choices such as occupation, kind of clothes, use of antibiotics etc (Callewaert et al., 2020). Many opportunistic microorganisms can also live on the skin and they can cause severe infections of skin by evading the skin barrier (Bay et al., 2020). Bacteria capable of residing on skin are classified into four phyla including Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria.

Among these, the most dominant opportunistic microorganisms include *Staphylococcus* spp. (Peng and Biswas, 2020).

Several culture-based techniques and molecular methods are being used for detection of the skin micro flora predominantly including 16s rRNA sequencing which is the most reliable technique used for skin microflora detection. However, it is limited, to some extent, by no possibility of differentiating between the resistant and non-resistant strains of bacteria (Maroniche et al., 2017). By using traditional culturing techniques, *Staphylococcus* spp. and *Corynebacterium* spp. have been revealed as the most abundant bacterial species present on moist parts of skin (Emter and Natsch, 2008). Besides the bacterial species, fungal communities can also survive on skin but their population does not fluctuate with physiological changes in comparison with the bacterial communities (Wu et al., 2020). The antimicrobial resistance (AMR) acquired by the microflora residing at one place may be transferred to other places via human transportation. Many pathogenic microorganisms have been implicated in fatal infections owing to multidrug resistance (MDR)

genes. Those multidrug resistant microorganisms are referred to as superbugs due to their AMR in opposition to multiple drugs which were originally developed to fight them (Davies and Davies, 2010). MDR microorganisms thrive successfully in our surroundings and cause severe health issues to both human beings and animals.

Particularly exposed in this regard are the healthcare workers, who are at great risk of encountering the pathogens in their daily routine (Cho and Blaser, 2012). Those infections which occur during the healthcare are specifically developed either in hospital or another healthcare facility within 48 hours of admission. It is also possible that they may occur at home within 30 days after receiving the healthcare. In all circumstances, these are known as healthcare associated infections (HAIs) (Revelas, 2012). The Agency for Health Care Quality and Research concluded that HAIs are the major and common complications faced in hospital care and are included among the top 10 causes of mortality in USA (Haque et al., 2018). Healthcare sector is, therefore, facing huge threat in the form of hospital acquired infections. During COVID-19 epidemic, because of contact with

infected patients and contaminated equipment, the health care workers were on the front line of vulnerability towards getting the infection. MDR pathogens have been widely distributed in various ecological niches in the hospital environment, as reported previously (Cruz-López et al., 2023). Among commonly found skin microbiota of healthcare workers isolated from their cell phones, resistance has been reported against the most commonly used antibiotics to treat skin infections highlighting the prevalence of MDR pathogens among skin microflora of these workers (Banawas et al., 2018).

Some hygiene conditions such as regular hand washing and using the sanitizer haven been recommended and employed for the purpose of prevention (Bowdle and Munoz-Price, 2020). It has been proven by many research groups that improving hand hygiene conditions can lessen the chances of developing HAIs. During the COVID-19 epidemic, hand hygiene conditions have been reported to be associated with decreased rate of HAIs (Roshan et al., 2020). Healthcare workers must consider maintaining the hand hygiene conditions as an important responsibility because they are well-taught about the risks of causing infections if they do not take it

seriously. The present study was conducted to analyze the composition of skin microflora of healthy healthcare workers and to ascertain the hygiene practices adopted by them.

Methodology

Data Collection

This perspective study involved 44 skin samples of healthy healthcare workers belonging to urban as well as rural population of Pakistan employing non-random sampling technique. The samples were taken from the Bakhtawar Amin hospital, Multan and Nishtar Hospital, Multan. They were processed in the Microbiology Laboratory of The Women University, Multan. All healthcare workers were administered a questionnaire to fill and provide consent to participate in this study. Informed written consent was taken from individuals whose sample were taken and they were guided to fill these questionnaires. All the samples were distributed in three different age groups including; group 1 of 20-30 years old, group 2 of 30-40 years, group 3 of 40-50 years old persons. Males and females were equally distributed among these three categories of age. All the processes involving collection and

processing of samples were in compliance with the declaration of Helsinki.

Questionnaire Administration

Questionnaire included questions relevant to their age, gender, occupation and the hygiene practices that are being followed during the duty hours. As described above, respondents were randomly selected to view the consent scenarios and were asked “Would you consent to share your samples and information with researchers in such manner”. After viewing the consent scenario, they said they would share samples and data for this study. This suggests that considering some of the potential risks and benefits of participation may inform and influence people’s decision to take part in the study.

Sample Processing

The standard Nutrient agar medium was prepared and poured in the autoclaved petri plates. All skin samples were processed on Nutrient agar plates by swabbing technique and incubated at 37°C for 24 hours. After 24 hours, diversity in the skin microflora was recorded by observing the growth of

different colonies on media plates. Morphological identification of isolated bacterial strains was done by following the colony morphology chart that includes the color, shape, elevation, texture and margin of the colonies. The selected isolates were then purified by quadrant streaking method. Gram staining was subsequently done to differentiate between gram-positive and gram-negative isolates on the basis of their staining characteristics. Gram-positive bacteria retain the primary dye crystal violet that stains their walls purple and gram-negative bacteria retain the secondary dye safranin that stains the bacteria pink. Results of microscopy were recorded subsequently.

Biochemical Characterization

Following the Bergey's Manual of Systematic Bacteriology, identification of isolated bacteria was done with the help of biochemical tests including catalase test, oxidase test, triple sugar iron (TSI) test, citrate test, coagulase test and indole test. In order to perform all of these tests, 18-24 hours fresh culture was used as prescribed. Specifically, for gram negative bacteria, oxidase, glucose fermentation and

indole tests were performed. On the other hand, for gram positive bacteria, catalase, TSI and coagulase tests were performed. After performing these tests, different species isolated from skin in this study were identified. The bacterial diversity of skin microflora was expressed as relative abundance (%) among the skin microflora community.

Results

Sample Characteristics

A total of 44 swab samples from skin of paramedical staff working in hospitals and microbiology labs were collected for conducting this study along with administration of a questionnaire which included questions related to age, gender, and occupation as well as about the hygiene conditions and practices during the Covid-19 pandemic. Majority of the samples taken belonged to group 1 which included 20-30 years old individuals followed by group 2 and 3 (Fig. 1). They were mostly lab workers followed by clinical assistants and doctors. A vast majority (73%) of them were females. The least prevalent were the pharmacists and only few were males.

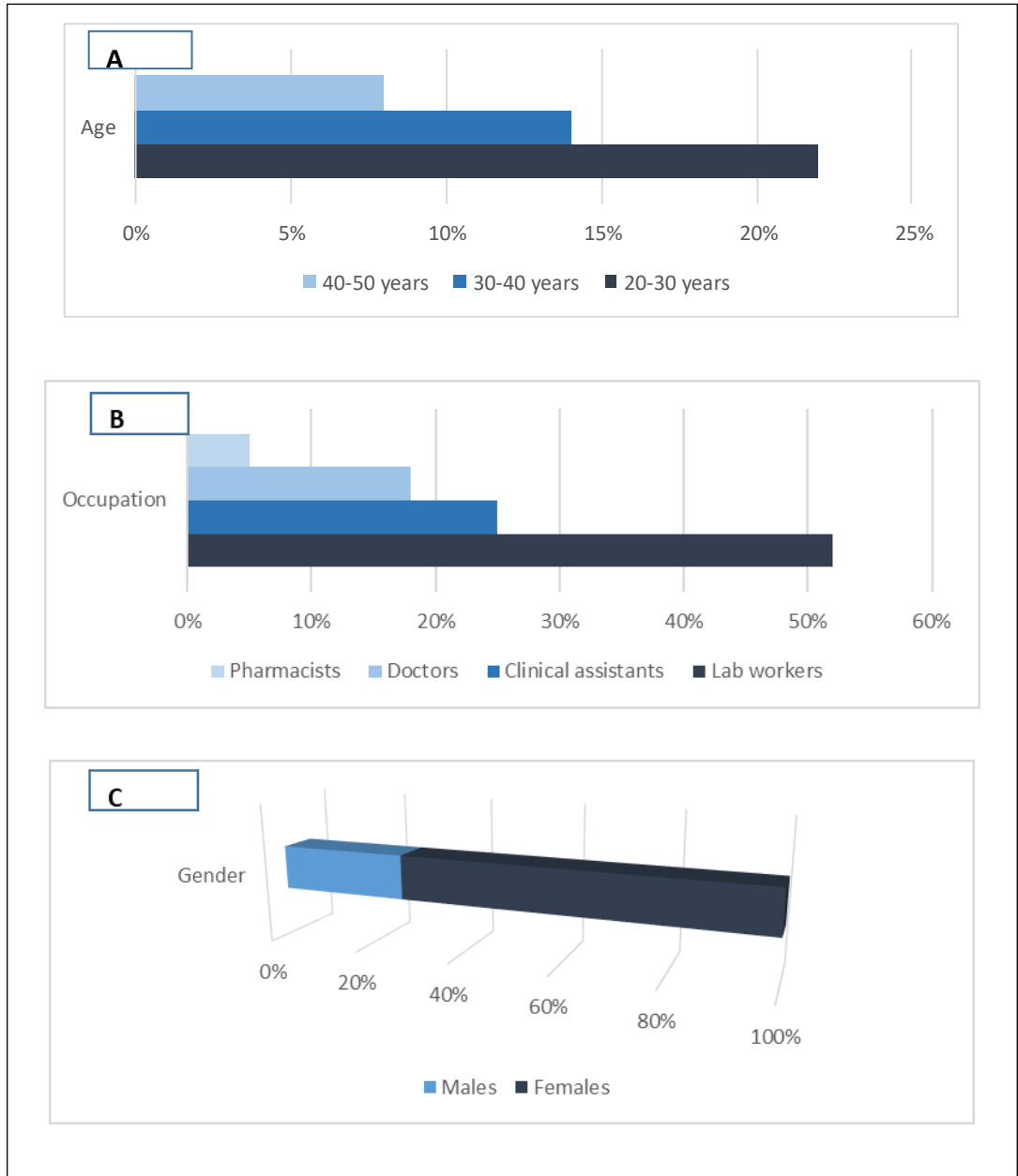


Figure 1: Baseline Characteristics of the Sample Population including A) Age, B) Occupation and C) Gender

Hygiene Practices

In addition to this information, other questions regarding their hygiene practices were asked such as the

frequency of washing hands, frequency of bathing, frequency of using sanitizers and whether or not they were wearing gloves during the working hours

(**Figure 2**). They reported a change in hygiene practices during pandemic era with regard to the frequency of all factors. For instance, there was a high frequency of washing hands which was at least 8-10 times per day, exactly. There was high rate of bathing i.e. 12-15 times per month at the minimum. The

use of sanitizer per week was not as high as it should have been or as expected. It was 8-10 times per week. In addition, 73% of the healthcare workers used to wear gloves while serving the community whereas, the remaining 27% did not.

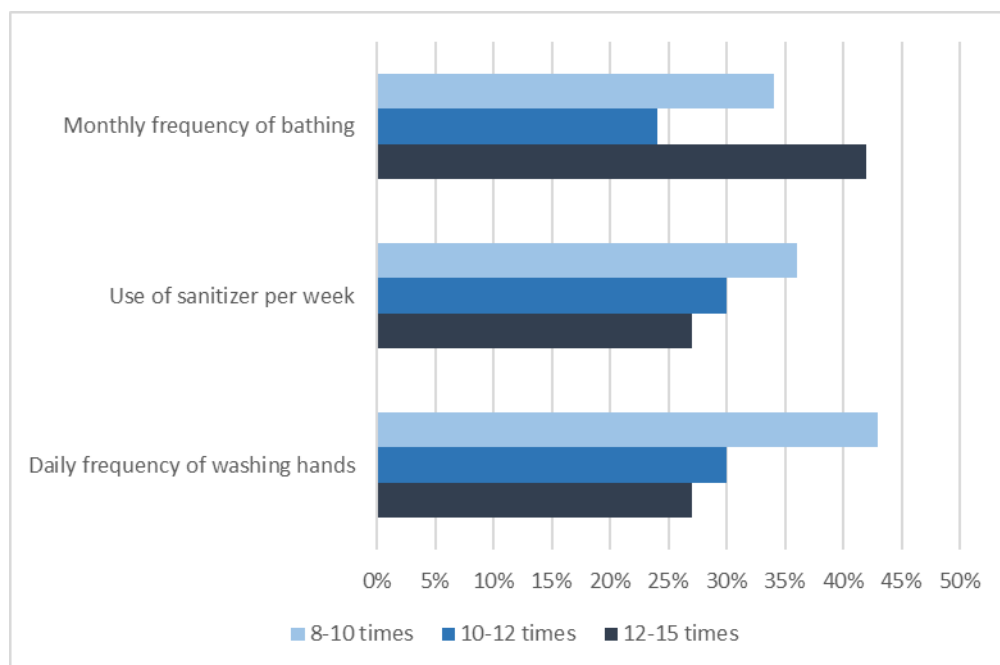


Figure 2: Basic hygiene practices during Covid-19 among healthcare workers

Gram Staining

Individual cells of isolated strains of bacteria were studied by gram staining technique and the data was recorded. Gram staining helped in distinguishing 110 isolated strains into two groups of

gram positive and gram negative bacteria. It was revealed that majority of the isolated bacteria were gram-positive. In total, there were 67 gram positive and 43 gram negative isolates. The results are mentioned as percentage in Fig. 3.

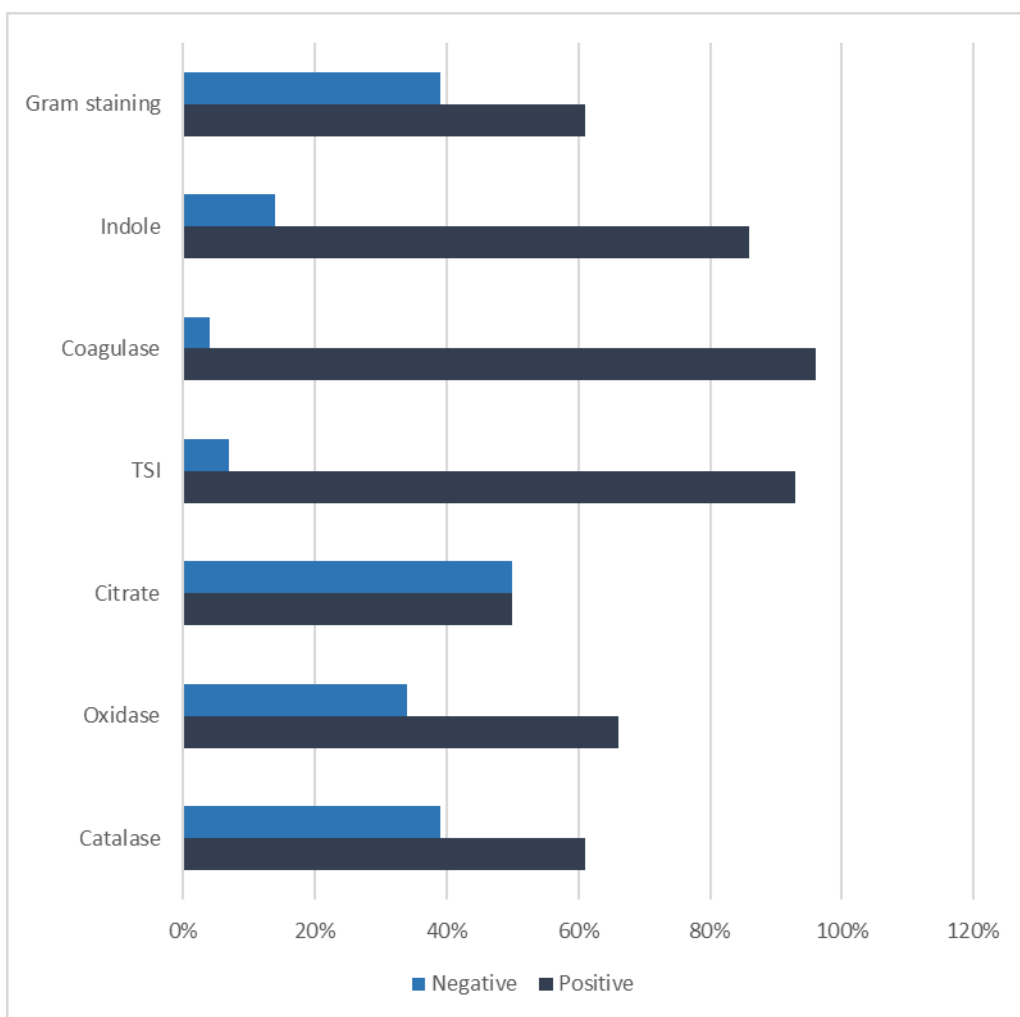


Figure 3: Results of Biochemical tests and Gram Staining for Skin Microflora of Healthcare Workers

Biochemical Characterization

Various biochemical tests were performed in order to identify the bacterial isolates up to species level in accordance with the scheme provided by Bergey’s manual of Systematic Bacteriology.

Biochemical characteristics of the isolated bacterial strains were observed and the data was

recorded. 94 isolates were Indole positive and 16 isolates were Indole negative; 105 isolates were coagulase positive and 5 isolates were coagulase negative; 102 isolates were TSI positive and 8 isolates were TSI negative; 72 isolates were oxidase positive and 38 isolates were oxidase negative; and, 67 isolates were catalase positive and 43

isolates were catalase negative. For citrate utilization test, half (55) of the isolates produced positive and remaining half (55) gave negative test result. The results are mentioned as percentage in **Figure 3**.

Prevalence of Skin Microflora

To understand the diversity of skin microflora that prevails among healthcare workers working in clinical environments, a comparative study was done among healthy healthcare workers

serving in the labs and hospitals. Out of a total of 110 bacterial strains which were isolated and identified based on the results obtained from different biochemical tests, 68 species were of *Staphylococcus aureus*, 17 species were of *Escherichia coli*, 9 species were of the genus *Pseudomonas*, 5 species were of the genus *Proteus*, 5 species were of the genus *Enterobacter* and, 3 species were of the genus *Klebsiella*. The results are mentioned as percentages in **Figure 4**.

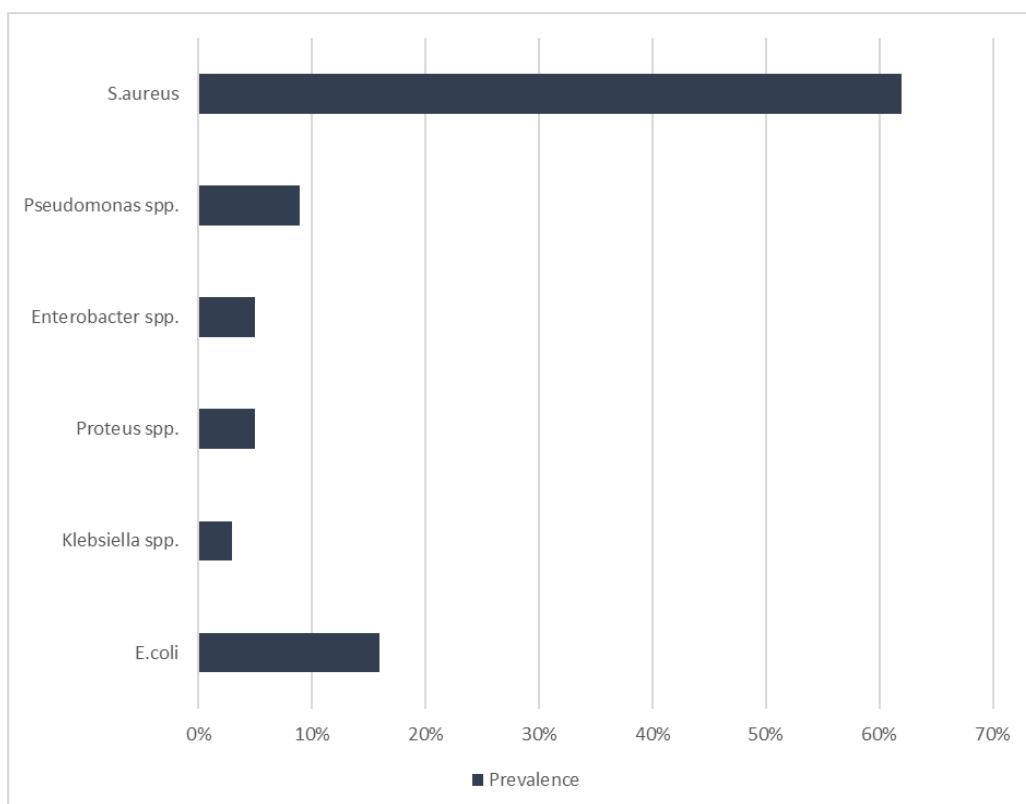


Figure 4: Composition of Skin Microflora of Healthcare Workers

Discussion

Skin is considered as an ecosystem of large number of microbial communities

which play a critical role in maintaining health and immune regulatory functions of the skin (Hoffmann, 2017). During COVID-19 pandemic the health care workers working in hospitals and diagnostic labs were at great risk of getting health-care associated infections (HAIs). The current study is the novel one in the aspect that it investigated the culturable skin microflora of the health workers during COVID-19 pandemic who were following WHO recommended hand hygiene practices which included; hand washing and hand sanitizing. The findings suggested that the frequency of the pathogenic microorganisms was significantly reduced. This is possibly due to strict compliance to guidelines pertinent to periodic hand washing with antibacterial soaps, frequent use of hand sanitizers and bathing after dealing with the infected patients (Christopher et al., 2020; Daye et al., 2020; Esther et al., 2022). The regime had been followed because several studies have shown that, by following specifically the hand hygiene rules, chances of infection can be reduced at a greater level since unhygienic conditions may lead to several diseases (McDonald et al., 2021). Various studies have shown that subungual parts of hands include large

number of gram-positive pathogenic bacteria, mostly *Corynebacterium*, *Staphylococci* and yeast (Nieradko-Iwanicka, 2020). Paramedical staff have high number of pathogens in their subungual areas, and because of this they are asked strictly to follow the hand hygiene strategies. The skin samples collected from the healthcare workers working in the hospitals and diagnostic labs included both gram-positive and gram-negative bacterial species of *Klebsiella* (3%), *Proteus* (5%), *Enterobacter* (5%), *Pseudomonas* (9%), and *E. coli* (16%) while the most abundant strain isolated was *S. aureus* (62%). Similar results were found out in a randomized clinical trial on university students after handwashing with water, plain soap and alcohol-based hand sanitizers (Zefenkey, 2021).

A reduction in number of bacterial isolates from skin of healthcare workers was observed when they followed the hand hygiene especially in those who were working in the diagnostic labs. Frequency of pathogenic bacteria isolated from volar forearm of paramedical staff which can cause fatal infections was 10% in our study. Furthermore, questionnaires about hand hygiene and other hygienic conditions were given to the healthcare workers

working in hospitals, diagnostic labs and pharmacies. According to the data, most of the individuals used 36% hand sanitizer 6 to 8 times, 37% used 4 to 6 times per day and 27% used 2 to 4 time per day. Among all of these self-hygiene regimes, frequencies of using hand sanitizers, which was 36% (6 to 8 times), was found out to be the most accurate to avoid contamination. In another study done on 24 primary schools in Dhaka, Bangladesh, it was shown that the incidence of influenza was 53% reduced in the group who cleansed their hands with hand sanitizers as compared with the controlled group (Biswas et al., 2019).

Hand washing was another hygiene condition asked in the questionnaire which concluded that 27% individuals washed their hands more than 12 times per day, 30% individuals 10 to 12 time a day, whereas 43% washed their hands 8 to 10 time a day. These frequencies proved that healthcare workers follow the hand hygiene conditions regularly to prevent themselves from infections during the COVID-19 pandemic. A similar study was also done on 896 Indonesian citizens over 18 years old which showed that 82.32% of females and 73.37% males reported handwashing practice 8 times or more

per day during COVID-19 pandemic (Dwipayanti et al., 2021).

Several reports have previously highlighted the effect of hygiene practices on prevalence of resistant pathogens, particularly MDR pathogens among microflora of humans during COVID-19. For instance, a study reported overall 35% decrease in the proportion of MDR bacteria, 41% decrease in MRSA, and 21% decrease in ESBLs in the post-COVID time as compared to pre-COVID pandemic (Cole & Barnard, 2021). Another study reported that during COVID-19, 4 times increase in the demand of hand sanitizers was observed and consequently, compliance to hand hygiene practices doubled from December, 2019 to April, 2020. Correspondingly, during the same time period, 50% decline in the prevalence of MDR pathogens among HAIs was noticed (Roshan et al., 2020).

The most important aspect of infection prevention is hand hygiene. It is the responsibility of healthcare centers to maintain the hand hygiene regulations. Paramedical staff, nurses, physicians and healthcare professionals throughout the world must get ready to inculcate the efficient, simple and fundamental practices of hand hygiene during their

daily routine for serving as a reference model for succeeding generations.

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Conflict of Interest

None

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