https://doi.org/10.29309/TPMJ/2025.32.09.3428

The Professional Medical Journal
www.theprofesional.com
2025, Volume, 32 Issue, 09

ORIGINAL ARTICLE

Hand washing measures and palmar bacterial floral susceptibility to antibiotics in a Tertiary Care Hospital, Peshawar.

Maria Khan[1], Saba Khan[2], Shifa Basharat[3], Syed Shahkar Ahmed Shah[4], Asfandyar Shah Roghani[5], Amina Gul[6]

Article Citation: Khan M, Khan S, Basharat S, Shah SSA, Roghani AS, Gul A. Hand washing measures and palmar bacterial floral susceptibility to antibiotics in a Tertiary Care Hospital, Peshawar. Professional Med J 2025; 32(09):1208-1215. https://doi.org/10.29309/TPMJ/2025.32.09.3428

ABSTRACT… Objective: To examine the practice of handwashing, the microbial pattern, and the susceptibility of microorganisms isolated from the palms of healthcare workers at a Specialized Cardiology hospital in Peshawar, Pakistan. **Study Design:** Cross-sectional Observational study. **Setting:** Peshawar Institute of Cardiology- Medical Teaching Institute (PIC-MTI). **Period:** 1st August till 31st September, 2023. **Methods:** Both qualitative and quantitative methods were used to retrieve data. A self-administered questionnaire was utilized to gather respondents' practice scale of handwashing. Culture-based and biochemical tests were carried out to identify bacterial isolates, and the Kirby-Bauer Disk diffusion method was used to determine the susceptibility pattern of bacteria. **Results:** The majority of the respondents were between the ages 25 and 58, with 63% being male. Almost all Healthcare personnel used an alcohol-based hand sanitizer, while only 45.3% of respondents admitted to always using soap to wash their hands. Presumptive identification of the organisms showed 43.1% of organisms as Staphylococcus epidermidis and 16.8% as Micrococcus spp. Only 5 isolates of Methicillin-resistant Staphylococcus aureus (MRSA) were recovered. None of the antibiotics was 100% effective. The sensitivity to chloramphenicol was high (83-91%), and more than 80% of the isolates showed resistance to Amoxicillin-clavulanate, tetracycline (37-73%), Fusidic acid (36%), and clindamycin (36%). Resistance to erythromycin was seen in 62% of organisms tested, and only 15% were resistant to Rifampicin. **Conclusion:** This study highlights the importance of proper hand washing awareness and monitoring among hospital staff. There were many instances of poor hand hygiene, which could accelerate the transmission of microbes through hand contact. Moreover, there was high resistance observed to the tested antibiotics.

Key words: Healthcare Personnel (HCP), Microorganisms, Methicillin Resistant Staphylococcus Aureus (MRSA), Resistance, Susceptibility, World Health Organization (WHO).

INTRODUCTION

Since the discovery of antibiotics, the issue of microorganisms becoming resistant to them has plagued humankind. Bacteria have evolved and developed resistance to antibiotics.[1] Antimicrobial resistance contributes to higher medical expenses, longer hospital stays, and a rise in mortality.

Without immediate action, the World Health Organization (WHO) warns that we are headed into pre-antibiotic period where ordinary diseases and mild infections can prove fatal.[2] This issue was readily resolved during the antibiotic era through the generation of novel antibiotics. However, due to the slow production of new antibiotics, the ever-evolving microbes outpace the development of new and effective antimicrobial agents.[3] millions of dollars and years are spent developing new antibiotics, which are then only effective for a few times before developing microbial resistance that reduces or completely stops the drug's effectiveness. As a result, pharmaceutical corporations have shifted their financial resources to more lucrative endeavors including the creation of medications for long-term ailments.[4] By limiting the incidence of disease transmission through hand carriage, we can cut back on the demand of antibiotics.

1. MBBS, M.Phil, FCPS, MHPE, Assistant Professor & Consultant Microbiologist, Peshawar Institute of Cardiology-MTI, Peshawar.
2. MBBS, FCPS, Experiential Registrar, Peshawar Institute of Cardiology-MTI, Peshawar.
3. MBBS, M.Phil, Assistant Professor, Rehman Medical College, Peshawar.
4. MBBS, FRCS, Dean, Peshawar Institute of Cardiology-MTI, Peshawar.
5. MBBS, M.Phil, DCP, District Pathologist, Women and Children Hospital, Charsadda.
6. MBBS, DCP, DHPE, Ph.D, PostDoc, Associate Professor Pathology, Khyber Medical College, Peshawar.

Correspondence Address:
Dr. Amina Gul
Department of Pathology
Khyber Medical College, Peshawar.
dr.aminagul@gmail.com

Article received on: 25/03/2025
Date of revision: 28/04/2025
Accepted for publication: 29/05/2025

The process of spreading a pathogen involves five steps: (i) presence of organisms on the skin and nearby inanimate surfaces; (ii) transfer of the organisms to the hands; (iii) ability of the organisms to be to survive on hands of the carrier; (iv) improper hand antisepsis; and (v) spread of the organisms are through contaminated hands of the carrier to an inanimate object or another person. When hand hygiene is implemented at any of the five steps, the number of bacteria carried on the hands will be significantly reduced.[5]

Individuals' handwashing routines, attitudes, and knowledge have been the subject of several studies. Duong et al.[6] shown that although most participants were aware of handwashing benefits to reduce the risk of spread of infection, handwashing on daily basis was practiced by only less than half of the participants. These findings revealed that proper handwashing practices would be encouraged in ongoing medical education. It has been determined that an awareness of the benefits of handwashing is insufficient in certain scenarios.

According to a Saudi Arabian survey, just 46% of participants believed that washing one's hands may prevent disease, while 34% of the study's correspondents considered that washing one's hands should simply be done to eliminate dirt.[7] The findings reported by Sultana et al.[8] demonstrate that provision of an adequate water supply and availability of soap within the university, could encourage the practice of handwashing.

It is necessary to evaluate the public's awareness of hand washing since hand carriage of microbes contribute significantly to the spread and selection of antibiotic-resistant bacteria. Microorganisms spread less when people are more conscious of their surroundings, and vice versa.

Determination of the level of hand washing awareness could be a significant step towards improving public health knowledge and sanitation. According to the WHO, "it has been recognized that inappropriate hand hygiene practices contributed significantly to disease outbreaks especially in hospital settings with consequent spread of multidrug-resistant organisms.[5] Therefore, it's critical to assess public awareness of the potential benefits of washing hands and to identify and characterize the microbiological community that lives on people's hands. The present study was designed to identify the microbiological spectrum, and the antimicrobial susceptibility pattern of microorganisms isolated from the palmar surface of healthcare personnel along with an assessment of the hand washing habits of the chosen healthcare workers of a tertiary cardiac care hospital in Peshawar, Pakistan.

METHODS

This cross-sectional study was carried out at the Peshawar Institute of Cardiology, Peshawar, Pakistan. Ethical approval (IRC/23/35, Dated: 11th July, 2023) was granted by the Institutional Review Board, Committee. This hospital is the only specialized tertiary institution for cardiac care in the Khyber Pakhtunkhwa province, in Pakistan. Both Male and female healthcare workers between 25 years to 58 years of age were included and subjected to microbiological testing after filling out a pre-designed questionnaire. Approximately 52 participants were enrolled, and a total of 60 pretested closed-ended questionnaires were administered to make an allowance for non-response. A written informed consent was obtained from the study participants after thoroughly explaining to them the purpose and overall requirements of the study. The purpose of the questionnaire and how to fill it out accurately was explained in detail to the HCPs. Confidentiality was assured for the HCPs to fill out the questionnaire without any bias, accurately. A sterile swab stick was initially moistened with a sterile 0.1% Tween 80 solution and then swabbed across the palmar surfaces of both hands of the HCPs. To ensure that the sample included the maximum surface area of each palm, the swab stick was rubbed in two perpendicular directions to cover the entire palmar surface. The swab sticks were then placed in universal bottles holding 2.5 ml of sterile 0.1% Tween 80 solution. The inoculated bottles were then shaken for 5 minutes using a wrist-action shaker.[9] The samples were initially streaked onto the surface of a Blood agar

plate, an enriched growth medium to isolate and cultivate all the bacteria. For selective isolation of Gram-positive bacteria (Staphylococcus and Micrococcaceae), Mannitol Salt agar was used. For isolation and differentiation of Gram-negative bacteria, samples were inoculated onto the MacConkey agar plate. All the culture media plates were incubated for 48 hours at 37^{0}C. After incubation, isolated colonies were picked up using a sterile swab and then sub-cultured on freshly prepared blood agar plates. The plates were again incubated for 48 hours at 37^{0}C. Bacterial isolates were identified based on specific colony characteristics, gram stain and various biochemical tests (catalase, coagulase, oxidase & Analytical profile index) as described earlier.[11] Muller-Hinton Agar (Oxoid Ltd, Hampshire, UK) was used for antibiotic susceptibility testing using Kirby-Bauer disk diffusion technique.[12] Tetracycline (30μg), Chloramphenicol (30μg), Novobiocin (5μg), Clindamycin (2μg), Cotrimoxazole (25μg), Rifampicin (5 μg), Fusidic acid (10 μg), Erythromycin (15 μg), Amoxicillin-clavulanate (30 μg) and ciprofloxacin (5 μg) were tested against bacterial isolates. Moreover, Methicillin Resistant Staphylococcus aureus (MRSA) strains were identified using a Cefoxitin (30 μg) disk. The results were obtained by measuring the zone of inhibition using CLSI guidelines 2024[13,14] Data was entered in Microsoft excel and analyzed using Statistical Package for Social Sciences (SPSS) Version 29.

RESULTS

All of sixty (60) questionnaires administered were retrieved. Regarding the gender of the respondents, 63% of them were male. Mean age of respondents was 38.5±3 years (range: 25-52 years). With respect to their attitude to hand hygiene, most of the HCPs (45.3%) washed their hands frequently, while only 32.8% washed their hands twice daily. While 47.5% of respondents performed hand washing for 20 seconds to 1 minute, most of the respondents (52.5%) reported hand washing for less than 20 seconds. On average total time utilized for hand washing was 12.6±2.4 seconds. When inquired about the timing of their last hand hygiene practice, 72.4% reported washing their hands more than 30 minutes ago, 6.5% had done so in the last 15 minutes, 12.2% between 5-15 minutes ago and 8.9% practiced hand hygiene between 15-30 minutes ago. Most of the respondents (79.5%) revealed hand washing after toilet use, while only 25.8% reported washing their hands after eating. Hand hygiene was practiced by 56.7% of the respondents after leaving the hospital. While 44%of the respondents reported hand washing without using hot water, only 14.3% were using an alcohol-based hand sanitizer for cleaning their hands. About half of the HCPs (48.6%) Wiping the hands after washing was practiced by almost half of the HCPs (48.6%) and 35.7% of HCPs practiced drying their hands by shaking off the water droplets. Distribution of the microorganisms isolated from the palmar surfaces of the study population is shown in Figure-1. Among gram-positive bacteria, Staphylococcus epidermidis accounted for 43.10% of the microorganisms isolated from the palmar surfaces of HCPs. Micrococcus spp followed next accounting for 16.8% of the isolates; A total of 9.7% were Staphylococcus aureus, among which 30% were MRSA; 9.3% were Corynebacterium spp; 6.2% were Streptococcus spp; and 6% were from the Bacillus genus. A relatively small percentage was observed for Neisseria spp (3.5%), Klebsiella/ Enterobacter spp (2.3%), Pseudomonas spp (1.10%) Haemophilus spp (1.7%), with Proteus spp and Escherichia coli being the rarest isolates at 0.3%.

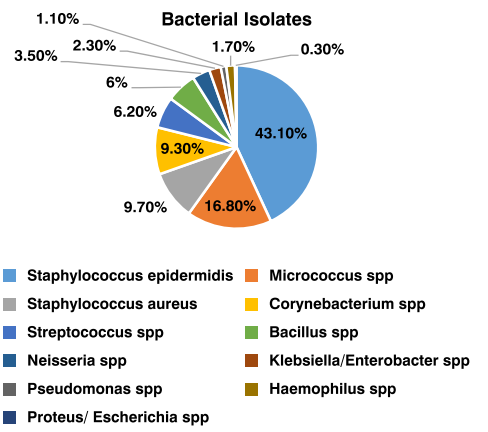

Figure-1. Percentage of bacteria isolated from palms of healthcare personnel

Table-I shows the susceptibility and resistance pattern of the isolated microorganisms. A

comparatively high susceptibility was noted for Chloramphenicol irrespective of the genus of bacterial species. About 85% of gram-positive isolates demonstrated susceptibility to Rifampicin, and over 60% were sensitive to Clindamycin, Novobiocin, and Tetracycline. Fusidic acid, Erythromycin and Ciprofloxacin showed the least sensitivity (<40%) to gram-positive isolates. Gram-negative bacteria demonstrated a sensitivity of 27% to tetracycline and 19% to Amoxicillin-clavulanate.

Antibiotics	Susceptible, n (%)	Resistant, n (%)
Gram-positive isolates		
Chloramphenicol	91	9
Clindamycin	64	36
Novobiocin	61	39
Tetracycline	63	37
Cotrimoxazole	55	45
Fusidic acid	46	54
Erythromycin	38	62
Rifampicin	85	15
Ciprofloxacin	33	67
Gram-negative isolates		
Chloramphenicol	83	17
Tetracycline	27	73
Cotrimoxazole	76	24
Ciprofloxacin	49	51
Amoxicillin-clavulanate	19	81

Table-I. Antibacterial susceptibility pattern of palmar isolates

DISCUSSION

When it comes to the study participants' hand-washing habits, the study findings revealed that most of the HCPs practiced hand hygiene, whether they washed their hands with soap or not. However, most of the respondents did not follow the guidelines recommended by WHO for good hand hygiene. Majority of them washed their hands long enough but not with warm water or soap.[15] It was also notable that most people used hand sanitizer with alcohol in it. Also, a lot of HCPs concurred that they performed hand hygiene rarely, one or two times a day, or as needed. These practices were inadequate to prevent or reduce the spread of infection through hand carriage, nor is it enough to ensure adequate hand hygiene.[16,17] The palmar surfaces of the 52 HCPs were cultured on Blood agar plates, and 456 colony-forming units (CFU) were isolated; most of the HCPs had more than 8 CFU on their hands. According to a different study, most physicians, nurses, and non-clinical employees have one or more bacterial strains colonized on their hands.[18]

According to one study, nurses may accumulate 100–1,000 CFU of Klebsiella spp. on their hands while performing "clean activities,"[19] whereas they can acquire 10–600 CFU/ml on their hands after touching patients' groins who have been significantly exposed to Proteus mirabilis.[20] Out of 456 CFU, 91.1% were Gram-positive and only 8.9 % turned out to be Gram-negative. Staphylococcus spp. was observed as the major species isolated from the palmar surfaces of HCPs with S. epidermidis as the predominant one (43.10%). The second common isolate was Micrococcus spp. accounting for 16.8% of all the isolates. Frequency of other members of the gram-positive bacteria including Corynebacterium, Streptococcus and Bacillus was relatively low. These findings supported other studies in the literature that highlighted that S. epidermidis is a prevalent microorganism on the skin.[21] Nearly all the bacteria on the skin of medical professionals and nurses belong to four primary groups: Enterococci, streptococci, micrococci including staphylococci such Staphylococcus epidermidis, and diphtheroid like corynebacteria other than C. diphtheriae.[18]

The transmission of bacteria like S. aureus, MRSA, or S. epidermidis via the hands has been linked to several forms of diseases related to healthcare.[22] The other species that were isolated match the results of earlier research on skin bacteria, albeit in terms of population sizes.[23] This demonstrates that although the skin's bacterial ecology is varied, a few consistent species are recognized as residential bacteria. Although transient bacteria are commonly a reflection of their surroundings, it has been observed that members of the genus Staphylococcus colonize the skin more easily than Corynebacterium spp

and other species. This observation raises the possibility of inaccurate results from surveys based on culture. Sequencing techniques should now be used for detection of members of microbial communities to address this problem.[24] While many of the resident bacteria infrequently cause disease, certain sporadic microorganisms with high pathogenicity can cause illnesses in people with compromised immune systems.[25] According to estimates from the Centers for Disease Control, approximately 33% of people harbor S. aureus, which contributes to its pathogenicity by producing clinically significant enzymes such hemolysins and leucocidins.[26] A research found that 16.9% of MRSA infections were obtained from healthcare workers' hands[27]—a significantly greater percentage than what we found.

In contrast to our findings, another Indian study from Gujarat states that health care professionals' hands are frequently colonized by bacteria, primarily S. aureus.[28] On the other hand, Pseudomonas aeruginosa, a major pathogen associated with morbidity and death in burn patients, is linked to leg ulcers despite being a rare type of gram-negative bacteria on the skin.[29,30] Furthermore, wearing artificial fingernails has been linked to an increased risk of P. aeruginosa colonization[31]; yet, in our investigation, Pseudomonas spp. accounted for just 1.10 percent of isolates.

Gram-negative bacteria can colonize a hand with as many as 13,000,000 cells 32, and they can do so for a considerable amount of time.[33] Bacillus spp. is another bacterium that has been identified. 6% of patients had that, which is less than in research where 15% of healthcare personnel' hands were found to be culture positive.[34] For better understanding the phenomenon of resistance/susceptibility pattern, organisms showing an intermediate reaction to antibiotics were categorized as resistant during the data interpretation process. In this investigation, the isolates' resistance to chloramphenicol was just 7–17%. Despite the well-documented nature of resistance to chloramphenicol, improved susceptibility to this antibiotic could possibly be attributed to the fact that chloramphenicol is not frequently used because of its side effects.[35]

Coagulase-negative staphylococci i-e S. epidermidis, are gram-positive cocci that were isolated from HCPs in considerable proportions. These pathogens have been reported to cause clinically significant ocular symptoms in recent years.[30] Because CoNS, like S. epidermidis, share an ecological niche with pathogenic S. aureus and may act as repositories of genes that allow MRSA infection following horizontal transfer, antibiotic resistance in CoNS is extremely important.[36]

The results show that CoNS are more vulnerable to the effects of antibiotics. Nonetheless, both species of the Staphylococci genus have poor susceptibilities to fumidic acid, erythromycin, and ciprofloxacin, but very high susceptibilities to tetracycline and clindamycin. Similarly, less than half of the isolates in a research conducted in Osun, Nigeria, shown resistance to sulphonamide, novobiocin, and tetracycline.[37] Unlike other research, like that of Petrillo et al.[30], which found strong resistance to tetracycline but high vulnerability to trimethoprim and sulfonamide. The variance in antibiotic resistance trends may be caused by variations in the practices of prescribers and over-the-counter use. In comparison to our study (9%), similar investigations conducted in Nigeria[38] reported an intermediate resistance to chloramphenicol (45%). S. saprophyticus was assumed to be the staphylococci that showed little or no zone of inhibition to novobiocin. The fact that rare human isolates that are not S. saprophyticus may potentially be resistant to novobiocin, despite the test's simplicity, poses a restriction. Consequently, it is advised that additional biochemical, molecular, or immunological testing techniques be used to confirm identification.[39]

CONCLUSION

This study highlights the importance of proper hand washing and awareness of its importance and monitoring among hospital staff. Hand hygiene practices, profiling of bacterial isolates from the palmar surfaces of HCPs and subsequent antibiotic susceptibility testing point towards inadequate hand hygiene behaviors, which

often contribute to the hand carriage of bacteria. Staphylococcus spp. was the commonest genus among the isolates from the respondents' hands, with S. epidermidis being the predominant bacteria. Antibiotics including amoxicillin-clavulanate, fusidic acid, and ciprofloxacin demonstrated high resistance. The isolates responded best to rifampicin, cotrimoxazole, and chloramphenicol. To address the rising incidence of antimicrobial resistance, local antibiograms need to be investigated and antibiotic stewardship initiatives implemented.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This study was funded by the researchers.

REFERENCES

1. Habboush Y, Guzman N. **Antibiotic resistance.** [Updated 2023 Jun 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513277/

2. World Health Organization**. Antibiotic RESISTANCE.** 2020. [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Accessed 2021 Feb 04.

3. Davies J. **Where Have All the Antibiotics Gone?** Can J Infect Dis Med Microbiol. 2006; 17(5):287-90. doi:10.1155/2006/707296.

4. Ventola CL. **The antibiotic resistance crisis: part 1: causes and threats.** P T. 2015; 40(4):277-83.

5. World Health Organization. **WHO guidelines on hand hygiene in health care: First Global Patient Safety Challenge Clean Care Is Safer Care.** Geneva; 2009. [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK144004/.

6. Duong MC, Nguyen HT, Duong BT, Vu MT. **Assessment of hand hygiene practices of university students in vietnam amid the COVID-19 Pandemic: A brief report.** Disaster Med Public Health Prep. 2022 Oct; 16(5):1844-47. doi: 10.1017/dmp.2021.256.

7. Almoslem MM, Alshehri TA, Althumairi AA, Aljassim MT, Hassan ME, Berekaa MM. **Handwashing knowledge, attitudes, and practices among students in eastern province schools, Saudi Arabia.** J Environ Public Health. 2021; 2021:6638443. doi:10.1155/2021/6638443.

8. Sultana M, Mahumud RA, Sarker AR, Hossain SM. **Hand hygiene knowledge and practice among university students: Evidence from Private Universities of Bangladesh.** Risk Manag Healthc Policy. 2016; 9:13-20. doi:10.2147/RMHP.S98311.

9. Ogai K, Nagase S, Mukai K, Iuchi T, Mori Y, Matsue M, et al. **A comparison of techniques for collecting skin microbiome samples: Swabbing versus tape stripping.** Front Microbiol. 2018; 9:2362. doi:10.3389/fmicb.2018.02362.

10. Pitt TL, Barer MR. **Classification, identification and typing of microorganisms.** In: Greenwood D, Barer M, Slack R, Irving W, editors. Medical Microbiology (18th edition). 2012; 24-38.

11. Shoaib M, Muzammil I, Hammad M, Bhutta ZA, Yaseen I. **A mini-review on commonly used biochemical tests for identification of bacteria.** Int J Res Pub. 2020; 54. doi:10.47119/IJRP100541620201224.

12. Hudzicki J. **Kirby-Bauer disk diffusion susceptibility test protocol.** American Society for Microbiology (ASM); 2009.

13. Bayot ML, Bragg BN. **Antimicrobial susceptibility testing.** Encyclopedia of Microbiology. 2021; 166-75.

14. CLSI. **Performance Standards for Antimicrobial Susceptibility Testing,** 33rd Edition.

15. Lee H, Choi S. **Hand washing awareness among students in seoul and antibiotic resistance of staphylococcus aureus isolated on their hands.** J Environ Health Sci. 2009; 35:278-86.

16. Xun Y, Shi Q, Yang N, Yang N, Li Y, Si W, et al. **Associations of hand washing frequency with the incidence of illness: A systematic review and meta-analysis.** Ann Transl Med. 2021 Mar; 9(5):395. doi: 10.21037/atm-20-6005. PMID: 33842616; PMCID: PMC8033386.

17. **Centers for Disease Control and Prevention.** When and How to Wash Your Hands. 2023. [Internet]. Available from: https://www.cdc.gov/handwashing/when-how-handwashing.html. Accessed 2024 Feb 05

18. Sarfraz A, Bhattacharyya S, Ansari MA, Jaiswal NK, Roy H, Kumar R, et al. **Study of bacterial flora of hands of health care givers in a tertiary care hospital in Eastern India.** J Evol Med Dent Sci. 2015 Apr 2; 4:4644-8.

19. Casewell M, Phillips I. **Hands as route of transmission for Klebsiella species.** Br. Med J. 1977; 2:1315-17.

20. Ehrenkranz NJ, Alfonso BC. **Failure of bland soap handwash to prevent hand transfer of patient bacteria to urethral catheters.** Infect. Control Hosp. Epidemiol. 1991; 12:654-62.

21. Landemaine L, Costa GD, Fissier E, Francis C, Morand S, Verbeke J, et al. **Staphylococcus epidermidis isolates from atopic or healthy skin have opposite effect on skin cells: Potential implication of the AHR pathway modulation.** Front Immunol. 2023 May 26; 14:1098160. doi: 10.3389/fimmu.2023.1098160. PMID: 37304256; PMCID: PMC10250813

22. Reboli AC, John JF, Levkoff AH. **Epidemic methicillin-gentamicin-resistant Staphylococcus aureus in a neonatal intensive care unit.** Am. J. Dis. Child. 1989; 143:34-39.

23. Davis CP. **Normal Flora.** In: Baron S, editor. Medical Microbiology (4th edition). 1996. University of Texas Medical Branch at Galveston.

24. Byrd AL, Belkaid Y, Segre JA. **The human skin microbiome.** Nat Rev Microbiol. 2018; 16(3):143-55. doi:10.1038/nrmicro.2017.157

25. Grice EA, Segre JA. **The skin microbiome.** Nat Rev Microbiol. 2011; 9(4):244-53. doi:10.1038/nrmicro2537.

26. Vandenesch F, Lina G, Henry T. **Staphylococcus aureus hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors?** Front Cell Infect Microbiol. 2012; 2:12.

27. Mody L, McNeil SA, Sun R, Bradley SE, Kauffman CA. **Introduction of a waterless alcohol-based hand rub in a long-term-care facility.** Infect. Control Hosp. Epidemiol. 2003; 24:165-71.

28. Kapil R, Bhasvsar HK, Madan M. **Hand hygiene in reducing transient flora on the hands of healthcare workers: An educational intervention.** Indian J Med Microbiol. 2015; 33:125-8

29. Buivydas A, Pasanen A, Senčilo A, Daugelavičius R, Vaara M, Bamford DH. **Clinical isolates of Pseudomonas aeruginosa from superficial skin infections have different physiological patterns.** FEMS Microbiol Lett. 2013; 343(2):183-89.

30. Petrillo F, Folliero V, Santella B, Franci G, Foglia F, Trotta MC, et al. **Prevalence and antibiotic resistance patterns of ocular bacterial strains isolated from pediatric patients in University Hospital of Campania "Luigi Vanvitelli," Naples, Italy.** Int J Microbiol. 2020; 1-6.

31. Foca M, Jakob K, Whittier S, Della Latta P, Factor S, Rubenstein D, et al. **Endemic Pseudomonas aeruginosa infection in a neonatal intensive care unit.** N. Engl. J. Med. 2000; 343:695-700.

32. Ayliffe GA, Babb JJR, Davies JG, Lilly HA. **Hand disinfection: A comparison of various agents in laboratory and ward studies.** J. Hosp. Infect. 1988; 11:226-43.

33. Larson EL. **Persistent carriage of gram-negative bacteria on hands.** Am. J. Infect. Control. 1981; 9:112-19.

34. Birch BR, Perera BS, Hyde WA, Ruehorn V, Ganguli LA, Kramer JM, et al. **Bacillus cereus cross-infection in a maternity unit.** J. Hosp. Infect. 1981; 2:349-54.

35. Abdollahi M, Mostafalou S. **Chloramphenicol.** In: Wexler P, editor. Encyclopedia of Toxicology (Third Edition). Academic Press. 2014; 837-40. doi:10.1016/B978-0-12-386454- 333 3.00709-0.

36. Otto M. **Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: Staphylococcal commensal species such as Staphylococcus epidermidis are being recognized as important sources of genes promoting MRSA colonization and virulence.** Bioessays. 2013; 35(1):4-11.

37. Akinwumi F, Okorafor CI, Ukatu L, Akinkunmi E. **Hand Washing Practices and Antibiotic Susceptibility of Palmar Bacterial Flora in a Tertiary Education Institution in Nigeria.** EMJ Microbiol Infect Dis. 2024; 5[1]:77-88.

38. Ezeh CK, Eze CN, Dibua MEU, Emencheta SC. **A meta-analysis on the prevalence of resistance of Staphylococcus aureus to different antibiotics in Nigeria.** Antimicrob Resist Infect Control. 2023; 12:40. doi:10.1186/s13756-023-01243-x.

39. McTaggart LA, Elliott TSJ. **Is resistance to novobiocin a reliable test for confirmation of the identification of Staphylococcus saprophyticus?** J Med Microbiol. 1989; 30:253-66.

AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Maria Khan:** Study conceptualization, study design, development of study protocol, data collection, data analysis, writing, review and editing.
2	**Saba Khan:** Study conceptualization, data collection, performance of experiments, data analysis, results, editing.
3	**Shifa Basharat:** Study conceptualization, data collection, data entry, data analysis, results, editing.
4	**Syed Shahkar Ahmed Shah:** Study conceptualization, data collection, data analysis, manuscript review.
5	**Asfandyar Shah Roghani:** Study conceptualization, data entry, data analysis, manuscript review.
6	**Amina Gul:** study conceptualization, study protocols, data entry, interpretation, manuscript review.