ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 03 | March - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ISOLATION AND IDENTIFICATION OF BACTERIAL NORMAL FLORA ON SKIN OF HEALTHCARE WORKERS AND DETERMINATION OF ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN

Clinical Microbiology

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ABSTRACT

Normal human skin is colonized by huge quantities of microorganisms that live on its surface. When the health care workers (HCWs) do not wash their hands between patients or do not practice standard infection control measures, they can be responsible for transmission of nosocomial infections. The aim of this study was to study the normal flora of healthcare workers' hands during routine patient care and simultaneously to evaluate the state of antibiotic susceptibility of microorganisms isolated from the hands of HCWs. The study also aims to examine whether job title of HCWs can affect the growth of hand flora in order to create awareness on the effective infection control measures. The study group included different groups of healthcare workers like Nurses, Lab technicians, Housekeeping staffs, Pharmacists, CSSD staffs, Substation staffs, Ward boys and Lift operators. Cubital fossa of the hand was wiped with normal saline to remove the contaminants. Using cotton swab, the skin swab was collected. The swab was streaked on the blood agar and MacConkey agar plates. These plates were incubated at 37°C for 24-48 hours. After incubation period, the plates with growth were selected for further identification. A total of 162 bacterial isolates (90.5%) were obtained from 179 samples. Out of 162 isolates, 79 (49%) were *Staphylococcus epidermidis*, 32 (20%) were *Escherichia coli*, 18 (11%) were Micrococcus, 17 (10.5%) were *S. saprophyticus*, 14 (9%) were *Klebsiella* sp. and 2 (1%) were *Pseudomonas* spp. No growth was observed in 17 samples of (9.5%) HCWs. In this study, it showed that *Staphylococcus* species were most resistant to cotrimoxazole and Ciprofloxacin. Among Gram-negative isolates, most of them were resistant to Ampicillin. The role of the hand flora of HCWs in the development of nosocomial infections is significant.

KEYWORDS

Normal flora, Health Care Workers, Nosocomial infections

INTRODUCTION

The skin is a complex human organ functioning to prevent loss of moisture and confine the entry of pathogens. It also provides various microenvironments with varying temperature, pH, moisture, and sebum content for part of the normal flora. Normal human skin is colonized by huge quantities of microbiota that live on its surface.[1, 2] Microbes found on the skin are usually regarded as pathogens or symbiotic organisms.[3] The peripheral layer of the epidermis is constantly in contact with bacteria from the surrounding environment. These organisms are considered as resident flora of skin. The resident organisms live as small microcolonies on the surface of the stratum corneum and within the outermost layers of the epidermis of the skin.[4] There are approximately 1 million resident bacteria per square centimetre of skin.[1]

Microbiome has a major impact on physiological functions including protection against infections, reaction in the immune system, and disposition for inflammation-mediated diseases. The normal flora of the skin, composed primarily of Gram-positive cocci and diphtheroid, may represent a selective barrier against the proliferation of potentially pathogenic organisms. Moreover, small numbers of Gram-negative bacteria or yeasts may also include in the normal human skin flora.[1,2,5]

The number of bacteria on an individual's skin remains relatively constant. Bacterial survival and the extent of colonization depend on the exposure of skin to a particular environment and on the innate and species-specific bactericidal activity in skin. Also, a high degree of specificity is involved in the adherence of bacteria to epithelial surfaces.[6]

Most microorganisms live in the superficial layers of the stratum corneum and in the upper parts of the hair follicles. Some bacteria reside in the deeper areas of the hair follicles and are beyond the reach of ordinary disinfection procedures. These bacteria are a reservoir for recolonization after the surface bacteria are removed. [7]

When the health care workers (HCWs) do not wash their hands between patients or do not practice standard infection control measures, they can be responsible for transmission of nosocomial infections. The aim of this study was to study the normal flora of healthcare workers' hands during routine patient care and to evaluate the state of antibiotic susceptibility of microorganisms isolated from the hands of HCWs. The study also aims to examine whether job title of HCWs can affect the growth of hand flora.

MATERIALS AND METHODS

The study was done at a tertiary care hospital in northern Kerala during the period of 3rd October 2023 to 30th December 2023.

The study group included different groups of HCWs like Nurses, Lab technicians, Housekeeping staffs, Pharmacists, CSSD staffs, Substation staffs, Ward boys and Lift operators.

Cubital fossa of the hand was wiped with normal saline to remove the contaminants. Using cotton swab, the skin swab was collected and streaked on blood agar and MacConkey agar plates. These plates were incubated at 37°C for 24 - 48 hours. After incubation, the plates with growth were selected for further identification.

Antimicrobial susceptibility of bacterial agents was performed by disc diffusion method according to Clinical and Laboratory Standards Institute's (CLSI) recommendations. The following antibiotics were used: Meropenem (MRP), Ciprofloxacilin (CIP), CoTrimoxazole (COT), Ampicillin (AMP), Cefepime (CPM), Cefaperazone / Sulbactum (CFS) and Novobiocin (NV).

RESULT

HCWs were classified according to their job titles. There were 106 (59%) Nurses, 25 (14%) Housekeeping staff, 14 (8%) Lab technicians, 14 (8%) Ward boys, 12 (7%) Pharmacists, 4 Substation staff, and 2 (1%) each lift operators and CSSD staffs. A total of 179 skin sample were collected.

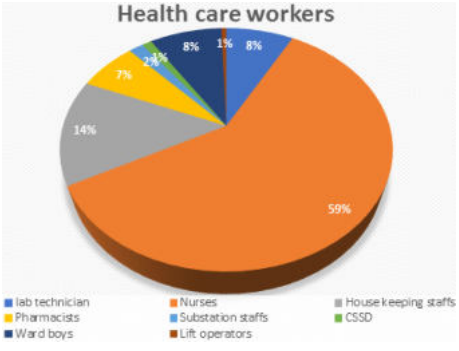

Figure 1. Distribution of HCWs (n=179)

A total of 162 bacterial isolates (90.5%) were obtained from 179 samples. The organisms isolated were *Staphylococcus epidermidis, Staphylococcus saprophyticus*, Micrococcus, *Escherichia coli, Klebsiella* species, and *Pseudomonas* species.

Image 1: Bacterial growth on MacConkey agar

Image 2: Bacterial growth on blood agar

Out of 162 isolates, 79 (49%) were *Staphylococcus epidermidis*, 32 (20%) were *Escherichia coli*, 18 (11%) were Micrococcus, 17 (10.5%) were *S. saprophyticus*, 14 (9%) were *Klebsiella* sp. and 2 (1%) were *Pseudomonas* spp. No growth was observed in 17 samples of (9.5%) HCWs.

Table No.1: Number And Percentage Of Organisms Isolated

Organisms isolated	Number of individuals	Percentage
Staphylococcus epidermidis	79	49%
Staphylococcus saprophyticus	17	10%
Micrococcus	18	11%
Escherichia coli	32	20%
Klebsiella species	14	9%
Pseudomonas species	2	1%

Antibiotic resistance among isolated Gram-positive and Gram-negative bacteria are shown in Tables 2 – 7.

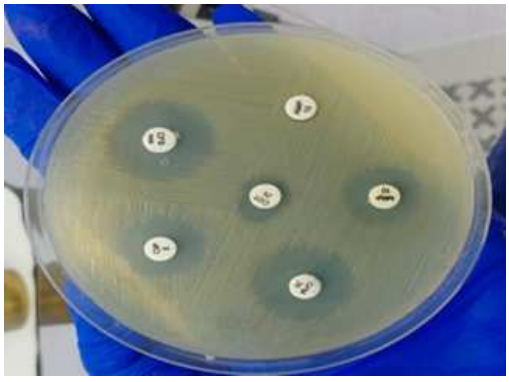

Image 3: Antibiotic Sensitivity Test

Table No. 2: Sensitivity Pattern Of *Staphylococcus Epidermidis*

S. epidermidis (N=79)	Number	Sensitivity (%)
Ampicillin	62	78%
Cefepime	79	100%
Cotrimoxazole	32	40%
Ciprofloxacin	51	64%
Meropenem	79	100%
Novobiocin	79	100%
Cefaperazone/sulbactum	79	100%

Table No.3: Sensitivity Pattern Of *Staphylococcus Saprophyticus*

S. saprophyticus (N=17)	Number	Sensitivity (%)
Ampicillin	11	64%
Cefepime	17	100%
Cotrimoxazole	9	52%
Ciprofloxacin	11	64%
Meropenem	17	100%
Novobiocin	0	0%
Cefaperazone/sulbactum	17	100%

Table No.4: Sensitivity Pattern Of Micrococcus

Micrococcus (N=18)	Number	Sensitivity (%)
Ampicillin	17	94%
Cefepime	18	100%
Cotrimoxazole	16	88%
Ciprofloxacin	17	94%
Meropenem	18	100%
Cefaperazone/sulbactum	18	100%

Table No.5: Sensitivity Pattern Of *Escherichia Coli*

Escherichia coli (N=32)	Number	Sensitivity (%)
Ampicillin	0	0%
Cefepime	32	100%
Cotrimoxazole	31	96%
Ciprofloxacin	18	56%
Meropenem	32	100%
Cefaperazone/sulbactum	32	100%

Table No 6: Sensitivity Pattern Of *Klebsiella* Species

Klebsiella species (N=14)	Number	Sensitivity (%)
Ampicillin	0	0%
Cefepime	12	85%
Cotrimoxazole	10	71%
Ciprofloxacin	10	71%
Meropenem	11	78%
Cefaperazone/sulbactum	12	85%

Table No. 7: Sensitivity Pattern Of *Pseudomonas* Species

Pseudomonas species (N=2)	Number	Sensitivity (%)
Ampicillin	0	0%
Cefepime	1	50%
Cotrimoxazole	2	100%
Ciprofloxacin	2	100%
Meropenem	2	100%
Cefaperazone/sulbactum	2	100%

Staphylococcus epidermidis was obtained from all the categories of HCWs. *S. saprophyticus* was obtained from Nurses, laboratory technicians, housekeeping staff as well as pharmacists. Micrococcus was obtained from nurses, laboratory technicians and ward boys. *E. coli* and *Klebsiella* sp. were obtained only from the hands of nurses whereas *Pseudomonas* sp. was obtained from laboratory technicians ie. Microbiologists only.

Table 8. Distribution Of Bacterial Growth Among HCWs

HCWs Organism	Nurses	Lab technicians	Pharma cists	Ward boys	Lift operators	CSSD staff	House keeping staff	Sub station staff	Total
S. epidermidis	37	05	09	07	02	01	17	01	79
S. saprophyticus	14	01	01	-	-	-	01	-	17
Micrococcus sp.	09	04	-	05	-	-	-	-	18
E. coli	32	-	-	-	-	-	-	-	32
Klebsiella sp.	14	-	-	-	-	-	-	-	14
Pseudomonas	-	02	-	-	-	-	-	-	02
No growth	-	02	02	02	-	01	07	03	17
Total	106	14	12	14	02	02	25	04	179

DISCUSSION

Out of total 179 samples collected, 162 (90.5%) bacterial growth of both Gram-positive (114 – 70%) and Gram-negative (48 – 30%) organisms were identified. *Staphylococcus epidermidis* (49%) was the predominant organism isolated from Gram-positive organisms followed by Micrococcus (11%) and *Staphylococcus saprophyticus* (10%). The Gram-negative bacteria isolated were *Escherichia coli* (20%), *Klebsiella* species (9%) and *Pseudomonas* species (1%) which was in concordance with the findings of similar studies.[1,8,9] In contrast, a study showed, *Micrococcus* species as the common isolated organism.[10]

In this study, *Staphylococcus* species were most resistant to Cotrimoxazole and Ciprofloxacin. Among Gram-negative isolates, most of them were resistant to Ampicillin. This is similar to study where all the isolates were sensitive to Carbapenems, fourth generation Cephalosporins as well as to β-lactamase inhibitor combination. [1]

Colonization rates of Gram-negative bacteria on the hands of HCWs ranged from 21 - 86.1%.[8] In the current study it was estimated as 14 – 43%.

SUMMARY AND CONCLUSION

The study was done at a tertiary care hospital in northern Kerala. Out of 179 samples collected, a total of 162 samples showed bacterial growth. Among them 114 (70%) were Gram-positive and 48 (30%) Gram-negative bacteria were isolated. The most common Gram-positive organism isolated was *Staphylococcus epidermidis* (49%) followed by *Micrococcus* species (11%) and *Staphylococcus saprophyticus* (10%) and the Gram-negative organisms isolated were *Escherichia coli* (20%), *Klebsiella* species (9%) and *Pseudomonas* species (1%). Both Gram-positive and Gram-negative organisms showed resistance to antibiotics ampicillin, ciprofloxacin and cotrimoxazole and were mostly sensitive to cefepime, meropenem and cefaperazone / sulbactam combination.

Staphylococcus epidermidis was obtained from all the categories of HCWs. *Staphylococcus saprophyticus* was obtained from Nurses, Laboratory technicians, Housekeeping staff and Pharmacists. *Micrococcus* was obtained from Nurses, Laboratory technicians and Ward boys. *E. coli* and *Klebsiella* sp. was obtained only from the hands of Nurses whereas *Pseudomonas* sp. was obtained from Laboratory technicians ie. Microbiologists only.

In this study, *Staphylococcus epidermidis* was observed as the main bacterial normal flora. Though commensals were predominantly isolated, few pathogenic organisms like *E. coli*, *Klebsiella* species and *Pseudomonas* species were also isolated from the HCWs. These organisms also exhibited high level of resistance to antibiotics like Ampicillin, Ciprofloxacin and Cotrimoxazole.

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