Bacterial flora on hands of nursing staff as a source of health care associated infections at a tertiary care centre

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Abstract
Introduction: Healthcare associated infections are an important cause of morbidity and mortality among hospitalized patients. Bacterial flora on hands of nursing staff act as a potential source for health care associated infections. Study of bacterial flora on hands of HCWs and knowing the importance of hand hygiene will play important role in preventing health care associated infections. Present study was undertaken to isolate aerobic bacterial flora on the hands of nursing staff, to study the antibiogram of isolates and to create awareness among the health care provider regarding hand hygiene.

Materials and Methods: Present study was done over a period of three months. After obtaining informed consent, swabs collected from the hands of nurses working in critical areas of hospital and subjected to bacterial culture. Organisms were identified by standard conventional methods. The antibiotic sensitivity testing of the isolates was done by Kirby Bauer’s Disk Diffusion method according to CLSI guidelines.

Result: Of 100 samples studied, 73(73%) samples showed the growth. Among them 36(49.31%) samples grown pathogenic bacteria, 27(36.98%) samples grown pathogenic bacteria along with probable contaminant non-pathogenic bacteria, 10(13.68%) samples showed only contaminant non-pathogenic bacteria. Among 63 pathogenic bacteria Gram positive bacteria predominate over Gram negative bacteria. Gram positive isolates were Staphylococcus aureus 20(31.7%), CONS 27(42.8%) and Gram negative isolates Klebsiella spp. 6(9.5%) followed by Pseudomonas spp. 4(6.3%) were predominated. Among Staphylococcus aureus 10(50%) isolates were found to be Meticillin resistant.

Conclusion: Awareness should be created among HCWs about significance of hand hygiene to prevent HCAIs.

Keywords: Bacterial flora, Hand hygiene, Healthcare associated infections, Healthcare worker, Pathogenic bacteria.

Introduction
Healthcare-associated infections (HCAIs) are an important cause of morbidity and mortality among hospitalized patients. They are responsible for prolonged hospital stay, increase in the cost of treatment, increase in the transmission of drug-resistant pathogenic organisms and long term disability.1,2

In developed countries, healthcare associated infections have been reported to affect 5%-15% of hospitalized patients while in developing countries, prevalence rates have been estimated to be between 14.8% and 19.1%.3,4

The human skin harbours about 1012 microbes which include both commensal as well as pathogenic organisms. Cultures from the skin have frequently demonstrated bacteria such as Diphtheroids, Staphylococcus, Streptococcus viridans, Streptococcus faecalis, Micrococcus, Corynebacteria, Propionibacteria, Gram positive aerobic spore-bearing bacilli, Gram negative bacilli such as Escherichia coli, Proteus spp. and fungi like Candida albicans.5-7 Pathogenic organisms from infected patients and surrounding environment can be carried from one patient to another by health care workers.5

Proper hand hygiene is the simple, effective and least expensive means of reducing the spread of pathogenic organisms in health care setting.7,8 Adequate hand hygiene among health care worker could prevent an estimated 15% to 30% of the health care associated infections.8

WHO patient safety launched the first global patient safety challenge; Clean care is safer care in 2005. Later, “Save Lives: Clean Your Hands” initiative was declared for global reduction of HCAIs.9,4

Three different methods of hand washing described in literature:
1. The social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil, and different organic substances;
2. The hygienic or antiseptic hand wash, which is the cleaning of hands with antimicrobial or medicated soap and water (“scrub”); most antimicrobial soaps contain a single active agent and are usually available as liquid preparations.
3. The hygienic hand disinfection, which normally consists of the application of an alcohol-based hand rub into dry hands without water.10,5

Bacterial flora on hands of nursing staff act as a potential source for health care associated infections. Study of bacterial flora on hands of HCWs and knowing the importance of hand hygiene amongs health care worker will play important role in preventing health care associated infections.

Aims and Objectives
1. To isolate aerobic bacterial flora on the hands of nursing staff to know the potential source of health care associated infection
2. To study the antibiogram of isolates.
3. To create awareness among the health care provider regarding hand hygiene to prevent Healthcare-associated infection

Materials and Methods

Present study was done in Shimoga Institute of Medical Sciences, Shivamogga. Ethical committee clearance was obtained. Study was done for three month duration from June 2018 to August 2018. Study was carried out on 100 nursing staff. After obtaining informed consent, sterile cotton swabs were pre-moistened in sterile normal saline and sample was taken from the dorsal aspect, ventral aspect and interdigital spaces of hands of the nurses working in critical areas of tertiary care hospital and processed in Department of Microbiology. The swabs were inoculated on Blood agar and MacConkey agar plates. The plates were incubated at 37°C for 24-48hrs aerobically. The bacterial isolates were identified using standard conventional methods.

Results

Present study was carried out in the department of microbiology, Shimoga Institute of Medical Sciences, Shivamogga. The observations made from the study are shown in following tables.

Table 1: Sample showing growth of bacteria

| Sample showing growth of bacteria                                      | N  | %    |
|------------------------------------------------------------------------|----|------|
| Only pathogenic bacteria                                               | 36 | 49.31|
| Pathogenic along with probable contaminant non-pathogenic bacteria     | 27 | 36.98|
| Probable contaminant non-pathogenic bacteria                           | 10 | 13.68|
| Total organisms isolated                                              | 73 | 100  |

Of 100 samples studied, 73(73%) samples showed the growth. Among them 36(49.31%) samples grown pathogenic bacteria, 27(36.98%) samples grown pathogenic bacteria along with probable contaminant non-pathogenic bacteria, 10(13.68%) samples showed only contaminant non-pathogenic bacteria like micrococci and aerobic spore bearing bacilli.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing of isolates was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. In the present study the susceptibility testing was carried out using the following antibiotics: Ampicillin (AMP), Ampicillin/sulbactam (A/S), Cefuroxime (CXM), Ceftazidime (CAZ), Co-trimoxazole (COT), Clindamycin (CD), Erythromycin (E), Gentamicin (G), Chloramphenicol (C), Ciprofloxacin (CIP), Doxycycline (DO), Vancomycin (VA), Linezolid (LZ), Imipenem (IMP), Meropenem (MRP), Piperacillin-tazobactam (PT), Amikacin (AK), Cefoxitin (cx), Cefepime (CPM), Aztreonam (AT), Tetracyline (TE).

Table 2: Samples showing growth of pathogenic organisms

| Different working area of hospital        | Number of samples collected | Growth of pathogenic organisms, n(%) |
|-------------------------------------------|----------------------------|-------------------------------------|
| MICU                                      | 12                         | 5 (41.66%)                          |
| Dialysis Unit                             | 12                         | 9(75%)                              |
| NICU                                      | 21                         | 13(61.90%)                          |
| PICU                                      | 13                         | 9(69.23%)                           |
| SICU                                      | 12                         | 7(58.33%)                           |
| Casualty                                  | 12                         | 9(75%)                              |
| Burns ward                                | 6                          | 3(50%)                              |
| OT                                        | 12                         | 8(66.66%)                           |
| Total                                     | 100                        | 63                                  |

MICU – medical intensive care unit, NICU – neonatal intensive care unit, PICU- paediatric intensive care unit, SICU- surgical intensive care unit, OT- operation theatre.

Table 3: Pathogenic organisms isolated from hands of nursing staff.

| Pathogenic organism isolated             | Total n- 63 | %    |
|------------------------------------------|-------------|------|
| CONS                                     | 27          | 42.85|
| Staphylococcus aureus                    | 20          | 31.74|
| Klebsiella spp                           | 6           | 9.5  |
| Pseudomonas aeruginosa                   | 4           | 6.3  |
| Escherichia coli                         | 3           | 4.7  |
| Acinetobacter spp                        | 3           | 4.7  |

Indian Journal of Microbiology Research, January-March, 2019:6(1):30-34
Among 63 pathogenic bacteria Gram positive bacteria predominate over Gram negative bacteria. Gram positive isolates were *Staphylococcus aureus* 20(31.7%), *Coagulase negative staphylococcus* (CONS) 27(42.8%) and Gram negative isolates *Klebsiella spp*. 6(9.5%) fallowed by *Pseudomonas spp.* 4(6.3%) were predominated.

Table 4: Pathogenic organisms isolated from hands of nursing staff working in different areas of tertiary care hospital

| Areas                  | CONS | *Staphylococcus aureus* | Klebsiella spp | Pseudomonas aeruginosa | Escherichia coli | Acinetobacter spp |
|------------------------|------|-------------------------|----------------|------------------------|------------------|-------------------|
| MICU                   | 3    | 1                       | 0              | 0                      | 1                | 0                 |
| Dialysis unit          | 4    | 3                       | 1              | 0                      | 0                | 1                 |
| NICU                   | 5    | 5                       | 1              | 1                      | 0                | 1                 |
| PICU                   | 1    | 4                       | 1              | 1                      | 1                | 1                 |
| ICU                    | 2    | 3                       | 0              | 1                      | 1                | 0                 |
| Casualty               | 6    | 1                       | 2              | 0                      | 0                | 0                 |
| Burnsward              | 2    | 0                       | 0              | 1                      | 0                | 0                 |
| OT                     | 4    | 3                       | 1              | 0                      | 0                | 0                 |
| Total pathogenic organisms. n- 63 | 27   | 20                      | 6              | 4                      | 3                | 3                 |

Table 5: Antibiotic sensitivity pattern of Gram positive isolates

| Antibiotics tested | Organisms isolated | MSSA n-10 Number sensitive (%) | MRSA n-10 Number sensitive (%) | MSCONS n-21 Number sensitive (%) | MRCONS n-6 Number sensitive (%) |
|--------------------|--------------------|---------------------------------|---------------------------------|-----------------------------------|----------------------------------|
| AMP                |                    | 3(30%)                          | 0                               | 8(38.09%)                         | 2(33.33%)                        |
| COT                |                    | 6(60%)                          | 3(30%)                          | 13(61.90%)                        | 5(50%)                           |
| CD                 |                    | 8(80%)                          | 4(40%)                          | 15(71.42%)                        | 4(66.66%)                        |
| CIP                |                    | 6(60%)                          | 5(50%)                          | 16(76.19%)                        | 2(33.33%)                        |
| CX                 |                    | 10(100%)                        | 0                               | 21(100%)                          | 0                               |
| C                  |                    | 7(70%)                          | 5(50%)                          | 12(57.14%)                        | 4(66.66%)                        |
| DO                 |                    | 7(70%)                          | 6(60%)                          | 15(71.42%)                        | 4(66.66%)                        |
| E                  |                    | 6(60%)                          | 3(30%)                          | 12(57.14%)                        | 3(50%)                           |
| G                  |                    | 6(60%)                          | 3(30%)                          | 11(52.38%)                        | 2(33.33%)                        |
| LZ                  |                    | 10(100%)                        | 10(100%)                        | 21(100%)                          | 6(100%)                          |
| VA                  |                    | 10(100%)                        | 10(100%)                        | 21(100%)                          | 6(100%)                          |

MSSA - Methicillin sensitive staphylococcus aureus, MRSA – Methicillin resistant staphylococcus aureus. MSCONS - Methicillin sensitive coagulase negative staphylococcus, MRCONS - Methicillin resistant coagulase negative staphylococcus

Among *Staphylococcus aureus* 10(50%) isolates were found to be Methicillin resistant (MRSA) whereas 6 (22.2%) strains of CONS were Methicillin resistant based on the Cefoxitin resistance as per CLSI guidelines.

Table 6: Antibiotic sensitivity pattern of Gram negative isolates

| Antibiotics tested | Organisms isolated | Klebsiella spp n-6 (%) | Pseudomonas aeruginosa, n4 (%) | Escherichia coli n-3 (%) | Acinetobacter spp. n-3 (%) |
|--------------------|--------------------|------------------------|--------------------------------|------------------------|--------------------------|
| AMP                |                    | 0                      | 1(33.33%)                      | 0                      | 0                        |
| GEN                |                    | 3(50%)                 | 0                               | 2(66.66%)              | 2(66.66%)                |
| AK                 |                    | 4(66.66%)              | 2(50%)                          | 3(100%)                | 2(66.66%)                |
| A/S                |                    | 5(83.33%)              | -                               | 3(100%)                | 3(100%)                  |
| CXM                |                    | 4(66.66%)              | -                               | 3(100%)                | 1(33.33%)                |
| CX                 |                    | 4(66.66%)              | -                               | 3(100%)                | 2(66.66%)                |
| CPM                |                    | 6(100%)                | 4(100%)                         | 3(100%)                | 3(100%)                  |
| CIP                |                    | 4(66.66%)              | 3(75%)                          | 2(66.66%)              | 3(100%)                  |
| IPM                |                    | 6(100%)                | 4(100%)                         | 2(66.66%)              | 3(100%)                  |
| MRPA               |                    | 6(100%)                | 4(100%)                         | 2(66.66%)              | 3(100%)                  |
| COT                |                    | 0                      | -                               | 2(66.66%)              | 0                        |
| AT                 |                    | 6(100%)                | 4(100%)                         | 3(100%)                | 3(100%)                  |
| CAZ                |                    | 4(66.66%)              | 1(25%)                          | 3(100%)                | 2(66.66%)                |
| C                  |                    | 3(50%)                 | -                               | 3(100%)                | 1(33.33%)                |
| TE                 |                    | 5(83.33%)              | -                               | 3(100%)                | 0                        |
| PT                 |                    | -                      | 4(100%)                         | -                      | 3(100%)                  |
Discussion

The microorganisms transmitted in the health care settings are infective to both patients and health-care workers and many of them will be multidrug resistant pathogens. Contaminated hands of health care worker plays a major role in transmission of infection in the hospital and act as a potential source for health care associated infection. Maintaining hand hygiene plays an important role in preventing health care associated infections.

Hand hygiene importance was first realized in a Vienna hospital in the 19th century. Maternity patients were dying at a high rate. Dr. Ignaz Semmelweis started ordering his staff members to wash their hands before treating the patients. As a result death rate was reduced drastically. In our study, out of 100 samples studied, 73 (73%) samples showed the bacterial growth. Among them 36 (49.31%) samples grown pathogenic bacteria, 27 (36.98%) samples grown pathogenic bacteria along with probable contaminant non-pathogenic bacteria, 10 (13.68%) samples showed only contaminant non-pathogenic bacteria like micrococi and aerobic spore bearing bacilli. Study done in Lucknow in the month of August 2015 showed among 60 samples analysed, 48 (80%) yielded bacterial growth, while 12 (20%) showed no bacterial growth. Study done at Bhopal in 2013 showed out of 100 swabs collected from the dominant hands of nurses working in critical areas, 83 showed the growth. All the samples showed the presence of 2 or more type of organisms, major contaminant nonpathogenic bacterium isolated were Diphtheroids and Aerobic Spore Bearing (ASB) bacilli. Study done at Uttar Pradesh in 2016 reported among 200 samples collected from HCWs, 106 (53%) samples showed no growth while 95 (47.5%) samples showed growth of microorganisms. Another study done in Salem for a period of four months from September-December 2017 observed bacterial growth in all the HCWs (100%) and fungus was isolated from eight HCWs (6.2%).

In our study, Among 63 bacterial pathogens, Gram positive bacteria predominate over Gram negative bacteria. Gram positive isolates were Staphylococcus aureus 20 (31.7%), Coagulase negative staphylococcus 27 (42.8%), and Gram negative isolates were Klebsiella spp. 6 (9.5%), followed by Pseudomonas spp. 4 (6.3%), Escherichia coli 3 (4.7%), Acinetobacter spp 3 (4.7%). Concordant results were found in study done at bhopal in 2013. Study done at lucknow on the bacterial isolates from hand swab samples of health care workers in 2015 reported 22 (45.8%) Escherichia coli, 4 (8.3%) Pseudomonas aeruginosa, 10 (20.8%) Klebsiella pneumoniae and 12 isolates of Staphylococcus spp, of which 8 (16.6%) were Staphylococcus aureus and 4 (8.3%) were Coagulase Negative Staphylococcus (CONS).

In our study, Among Staphylococcus aureus 10 (50%) isolates were found to be Methicillin resistant. MRSA were 100% sensitive to Vancomycin, Linezolid, 80% sensitive to Clindamycin, 70% sensitive to Chloramphenicol, Doxycycline, 60% sensitive to Co-trimoxazole, Erythromycin, Gentamicin and 30% to Ampicillin. In our study most of the MRSA were resistant to many drugs compare to MSSA.

Another study done in Salem for a period of four months from September-December 2017 reported 17% of Staphylococcus isolates were resistant to Cefoxitin which indicates both MRSA and MR-CONS are colonising the skin of HCWs. In our study among 27 CONS, 6 (22.2%) were Methicillin resistant. MRCONS were 100% sensitive to Vancomycin and Linezolid, 66.66% sensitive to Doxycycline, Chloramphenicol, Clindamycin, 50% sensitive to Erythromycin, Co-trimoxazole, 33.33% sensitive to Ampicillin, Ciprofloxacin, Gentamicin. MSCONS were 100% sensitive to Vancomycin, Linezolid, 76.19% sensitive to Cefoxitin, 71.42% sensitive to Clindamycin, Doxycycline, 61.90% sensitive to Co-trimoxazole, 57.14% sensitive to Erythromycin, Chloramphenicol, 52.38% sensitive to Gentamicin, 38.09% to Ampicillin.

In recent years, Staphylococcus epidermidis is considered as an agent of hospital and community acquired infections. Study at Lucknow the month of August 2015 found CONS were (100%) sensitive to Cefoxitin, Amikacin, Levofoxacin; (75%) to Amoxycillin/ clavulanic acid, Clindamycin; (50%) to Erythromycin, Ciprofloxacin and least sensitive to Ceftriaxone (25%). All of the CONS were Methicillin sensitive.

Study in Turkey during a three-month period from January to March 2008 reported Among 143 CONS, 43 were (30.1%) MRCONS, 100 were (69.9%) MCONS. All CONS were sensitive to Vancomycin and Linezolid and 96% of MCONS strains were resistant to Penicillin. It was found out that 41 (95.3%) of MRCONS strains were erythromycin-resistant, and 16 (37.2%) of them showed constitutive Clindamycin resistance. It was found out that 68% of MCONS strains were erythromycin-resistant. Constitutive Clindamycin resistance was seen in 13% of MCONS strains.

In our study among Gram negative bacteria, Klebsiella spp. Showed 100% sensitive to Cefepime, Meropenem, Imipenem, Aztreonam. 83.33% sensitive to Tetracycline and Ampicillin/subbacantam. 66.66% sensitive to Cefazidime, Ciprofloxacin, Cefoxitin, Cefuroxime, Amikacin, 50% sensitive to Gentamycin, Chloramphenicol and all the isolates were resistant to Ampicillin, Co-trimoxazole. Pseudomonas spp. - showed 100% sensitive to Imipenem, Ciprofloxacin, Gentamicin and 30% to Ampicillin.
Meropenem, Aztreonam, Piperacillin–Tazobactam, Cefepime. 75% to Ciprofloxacin, 50% to Amikacin, 25% to Ceftazidime. E. coli - showed 100% sensitive to Amikacine Ampicillin/sulbactam, Cefuroxime, Cefoxitin, Cefepime, Aztreonam, Ceftazidime, Chloramphenicol, Tetracycline. 66.66% sensitive to Gentamicin, Ciprofloxacin, Imipenem, Co-trimoxazole, and 33.33% to Ampicillin. Acinetobacter showed 100% sensitive to Ampicillin/sulbactam, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Aztreonam, Piperacillin – Tazobactem, 66.66% to Gentamicin, Amikacin. Cefoxitin, Ceftazidime and 33.33% Cefuroxime, Chloramphenicol.

In Study at Lucknow during August 2015, Escherichia coli showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin; but were resistant to Amoxycillin/clavulanic acid (81.8%), Ceftriaxone (72.7%) and Co-Trimoxazole (63.6%). Pseudomonas aeruginosa showed 100% sensitivity to Ciprofloxacin, Levofloxacin, Piperacillin/Tazobactam, Imipenem; but were resistant to Ceftazidime (75%), Cefepime (50%), Gentamycin (25%), Cefotaxime (75%). Klebsiella Pneumoniae showed 100% sensitivity to Ciprofloxacin, Doxycycline, Amikacin, Gentamycin, Levofloxacin; but were resistant to Amoxycillin/ clavulanic acid (60%), Ceftriaxone (60%) and Co-Trimoxazole (50%).

Study in Vinayaka Mission’s Kirupananda Varayar Medical College, Salem for a period of four months from September-December 2017 reported among Gram-negative isolates, most of them were resistant to Ampicillin and Cotrimoxazole. Resistance was also observed to third generation Cephalosporins and Fluoroquinolones though at a lower rates. All the isolates were sensitive to Carbapenems, fourth generation Cephalosporins as well as to β-lactamase inhibitor combination.

Conclusion
Bacterial flora on hands of health care worker act as a potential source for development of healthcare associated infections due to their pathogenicity and drug resistance. Proper infection control measures like regular hand washing and creating awareness among the health care workers are responsible for prevention of these infections. Use of personal protective equipment also plays an important role in reducing healthcare associated infections. There is need for regular training and continuing medical education programs on hand washing and hand disinfection practices among of health care workers for prevention of healthcare associated infections.

Conflict of Interest: None.

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How to cite this article: Vedavati BI, Halesh LH. Bacterial flora on hands of nursing staff as a source of health care associated infections at a tertiary care centre. Indian J Microbiol Res 2019;6(1):30-34.