Characterization of Bacterial Pathogens and Their Antimicrobial Susceptibility Profile, from Inanimate Surfaces in Tertiary Care Hospital Peshawar, Pakistan

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ABSTRACT

Aim: To isolate, identify and antimicrobial susceptibility profile of the bacterial pathogens from inanimate surfaces of hospital which contributes to hospital acquired infections.
Methods: A total of 80 samples, including 20 from each unit (surgical ward, Medical ward, ICU and Operation Theater) were aseptically collected from different surfaces. Isolation and identification were made on the basis of different bacteriological media and biochemical tests. API 10 S (Biomerieux France) kits were used for the identification of gram negative bacteria. Antimicrobials susceptibility was performed according to Kirby-Bauer disc diffusion method.
Findings: Over all prevalence of culture positive samples were (75%) from which 9 different bacterial strains were isolated. The prevalence of *Staphylococcus aureus* is (68%), *Staphylococcus epidermidis* (28%), *Streptococcus agalactaie* (1.6%), *Enterococcus faecalis* (1.6%), *Escherichia coli* (3.3%), *Pseudomonas aeruginosa* (1.6%), *Enterobacter aerogenes* (1.6%) *Serratia marcescens* (1.6%). Prevalence rate of MRSA was 21.8%. 50% of Gram negative isolates were resistant to Cefotaxime, 50% to Meropenem, 40% to Amoxicillin, 40% Nitrofurantoin, 50% to Polymyxin B. 100% of the gram negative isolates were sensitive to Fosfomycin and Ciprofloxacin.
Conclusion: The hospital inanimate surfaces are heavily contaminated with resistant pathogenic bacteria which can be a potential source of hospital acquired infections. Attention is required for proper decontamination method to avoid the possible dissemination to the patients and hospital staff.

Keywords: Inanimate surfaces; hospital-acquired infections; bacterial pathogens; antimicrobial susceptibility; MRSA.

1. INTRODUCTION

The hospital environment considerably effects numerous factors in the chain of hospital acquired infections [1]. The environment includes different surfaces like floors, walls, beds, bed sheets, equipment's, water and hands of health care personals [2]. Contamination of these surfaces with pathogenic microorganisms may be responsible for hospital acquired infections. Contact of healthy individuals, patients and health care worker with contaminated surfaces can be the source for transmission of microorganisms [1].

There are two different categories of contaminated surfaces based on their role in the transmission of disease; housekeeping and medical equipment. Housekeeping surfaces such as walls and floors are usually associated with the lowest risk of disease transmission [3]. Medical equipment such as X-ray machines, blood pressure cuffs thermometers, ventilators, nebulizers and other medical machines form a higher risk in terms of the transmission of diseases than housekeeping surfaces [4].

Nosocomial infections are increasing the risk of morbidity and mortality in hospitalized patients.

Basically, infections that occur within 48 h of admission or stay at a healthcare facility and that were not present or incubating at the time of admission, are commonly considered nosocomial infection [5]. These infections affect approximately 1 in 10 patients admitted to hospital [6]. The agents that commonly cause nosocomial infections include *Streptococcus spp., Acinetobacter spp., Enterococci, Pseudomonas aeruginosa,* coagulase-negative *staphylococci, Staphylococcus aureus, Bacillus cereus, Legionella* and *Enterobacteriaceae* family members, namely*, Proteus mirabilis, Klebsiella pneumonia, Escherichia coli, Serratia marcescens.*[7].

This study aimed to characterize the bacterial pathogens and determine their antimicrobial susceptibility profile from inanimate surfaces of the hospital. The hospital surfaces are contaminated with bacterial pathogens and contribute mainly to hospital-acquire infections.

2. METHODS

2.1 Study Design

The current study is undertaken in a tertiary care hospital in Hayatabad Peshawar. Four different tertiary care hospital units (surgical ward, ICU, Medical ward, Operation Theater) were selected. Samples were collected from different high touched inanimate surfaces. A total of 80 samples, 20 from each unit (Surgical ward, ICU, Medical ward and Operation Theater) were collected aseptically from different surfaces. The current study was conducted in three months, from June to August 2017.

2.2 Sample Collection and Transport

Samples were collected using sterile moist swabs. The swabs were rolled over the surfaces covering up to 20 cm^2 area. The swabs were transported aseptically within one hour in rigid cool container to the microbiology laboratory of Sarhad University of Science and Information Technology for further processing.

2.3 Sample Processing

All the samples were first inoculated on nutrient agar and incubated at 37 ̊C for 24hour. After incubation mixed growth was observed and each colony was sub cultured on nutrient agar plates for pure cultures. The pure cultures were then sub cultured on selective and differential media (MacConkey Agar, Eosin Methylene Blue Agar and Sheep Blood Agar) to get selected and differential growth. Mac-Conkey and EMB agar were used for the growth of gram negative bacteria. Sheep blood agar was used to differentiate between hemolytic and non-hemolytic bacteria.

2.4 Identification of Isolated Bacterial Strains

Identification of different bacterial strains was done based on phenotypic colonial characteristics, gram staining and biochemical. Biochemical tests oxidase, catalase, motility, indole, coagulase, methyl red, DNase, CAMP and API 10 S (Biomerieux France) were used for the identification of bacteria. ATCC control organisms were used for identification.

2.5 Determination of Antimicrobial Susceptibility Profile

All the isolates were subjected to in vitro testing for the determination of their antimicrobial susceptibility profile to various antimicrobials using the Kirby-Bauer disc diffusion method. In the current study a total of 20 antimicrobials were used, zone of inhibition was interpreted according to CLSI 2016.

3. RESULTS

In the present study, overall prevalence of culture positive sample was (75%), while culture negative was (25%) as shown in Fig. 1.

The overall prevalence observed for gram-positive bacteria was (91%) while Gram-negative was (9%) shown in Table 1.

Among the grampositive bacteria, high prevalence (53%) was observed for *Staphylococcus aureus* followed by *Staphy lococcus epidermidis* (35%) *Streptococcus agalactaie* (1.6%) and *Enterococcus faecalis* (1.6%) as shown in Table 2.

Table 1. Overall prevalence of isolated strains

Strains	Number and percentage %
Gram-positive	55 (91%)
Gram-Negative	5 (9%)
Total	60 (75%)

Table 2. Overall prevalence of gram positive bacteria

Name	Number and percentage
Staphylococcus aureus	32 (53%)
Staphylococcus epidermidis	21 (35%)
Streptococcus agalactaie	1 (1.6%)
Enterococcus faecalis	1 (1.6%)

Among Gram negative bacteria high prevalence (3.3%) was observed for *Escherichia coli,* followed by *Pseudomonas aeruginosa* (1.6%), *Enterobacter aerogenes* (1.6%) and *Serratia marcescens* (1.6%) as shown in Table 3.

Table 3. Over all prevalence of gram negative bacteria

Name	Number and percentage
Escherichia coli	2 (3.3%)
Pseudomonas aeruginosa	1 (1.6%)
Enterobacter aerogenes	1 (1.6%)
Serratia marcescens	1 (1.6%)

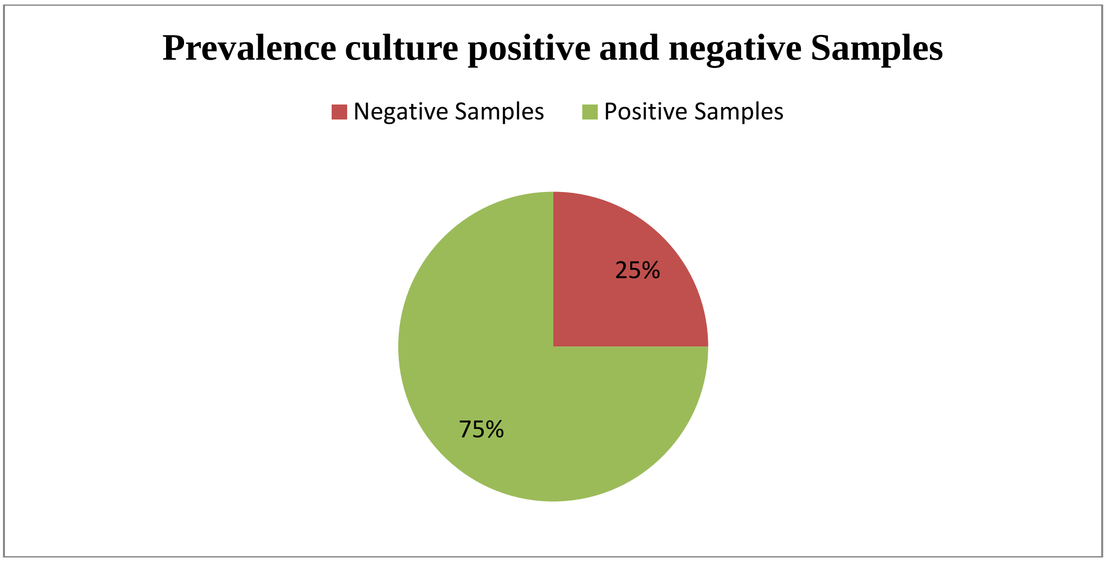

Fig. 1. Percentage of culture-positive and culture-negative samples

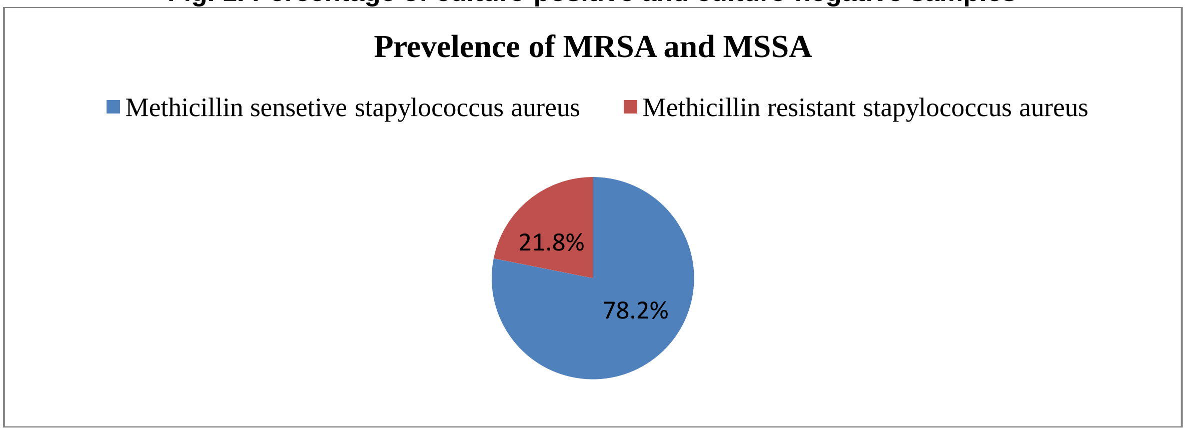

Fig. 2. Prevalence of MRSA and MSSA

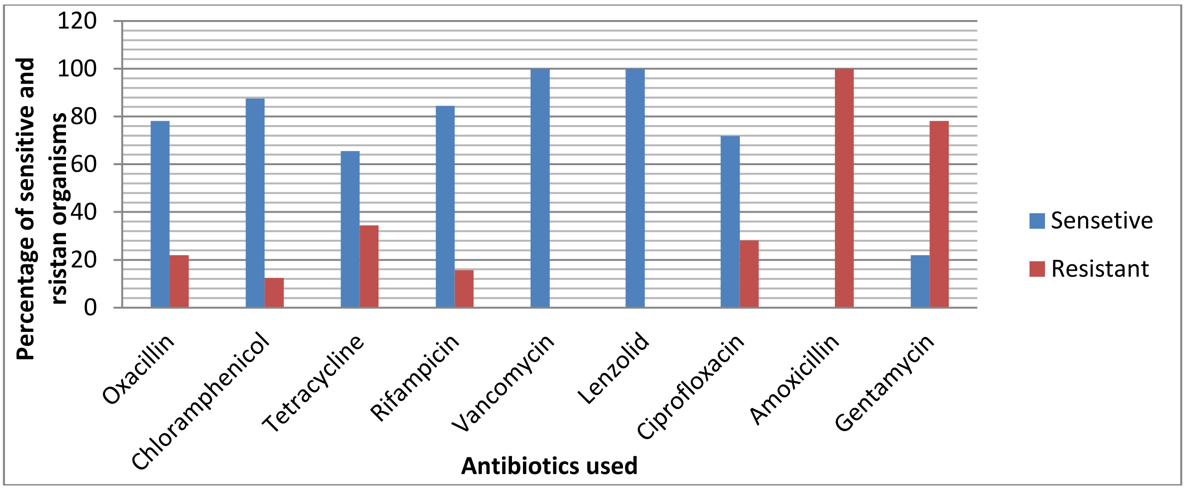

Fig. 3. Percentage of antimicrobials resistance to the antimicrobials used against gram-positive bacteria

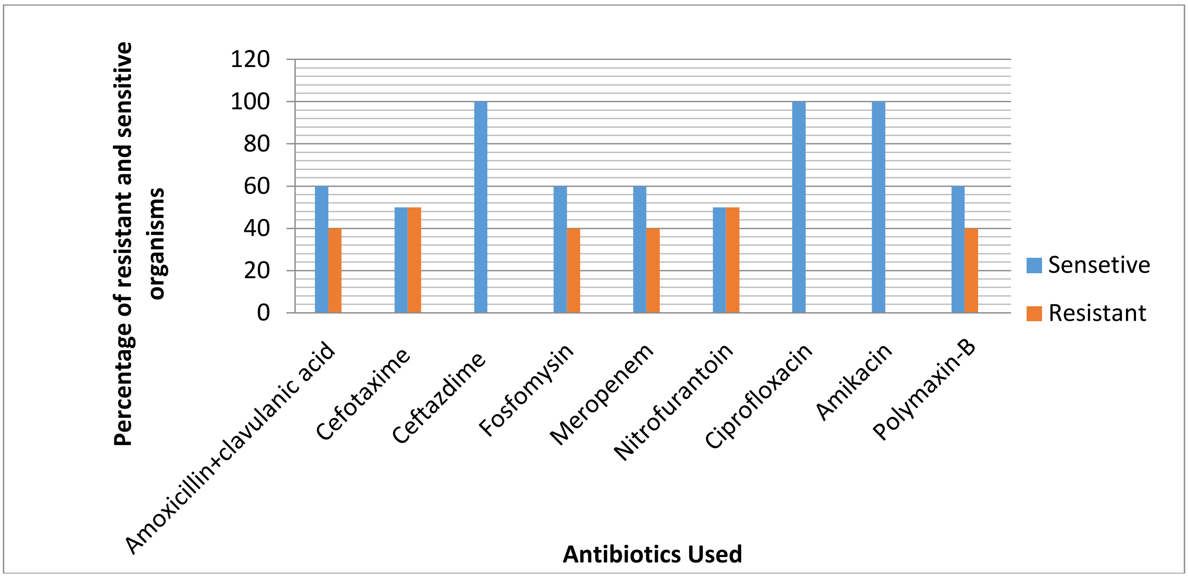

Fig. 4. Percentage of antimicrobials resistance to the antimicrobials used against gram Negative bacteria

4. DISCUSSION AND CONCLUSION

The hospital environment and inanimate surfaces serve as a hot spot for the growth of pathogenic microorganisms. These contaminated surfaces can lead to the spread of infection in hospital settings like the spread of nosocomial infections. There are number of evidences that support the role of contaminated inanimate surfaces in transmission of hospital-acquired infections (HAI) [8]. In this study we analyzed different inanimate surfaces for bacterial contamination.

Different gram negative bacteria like *Pseudomonas aeruginosa, Acinetobacter baumannii,* members of *Enterobacteriaceae* have been reported extensively from the inanimate surfaces and objects by [9]. In the current study, we found four different strains of gram-negative bacteria, *Pseudomonas aeruginosa, Escherichia coli, Serratia marcescens* and *Enterobacter arogene*. Although the ratio of our gram negative isolates were smaller but significant and may be a potential risk to the health care system.

In our study, all the samples collected from bedsheets in the ward were found contaminated. The most common organisms isolated was *Staphylococcus aureus and Staphylococcus epidermidis*. These two organisms are the dominant normal flora of the skin and are disseminated from skin to other surfaces. *Staphylococcus aureus* is considered as the infectious flora and found in most infections [10]. Samples collected from tables and chairs were also found contaminated. These findings are similar to the report of [11]. Use of single nebulizer over time for different patients is a common practice in hospitals. In the current study *Staphylococcus aureus* was isolated from nebulizers, a similar to the findings to that of [12].

Samples collected from the surfaces in ICU were also found contaminated similar to the study of [13], who isolated same type of bacteria from both patients and surfaces using PCR technique.

Staphylococcus aureus, *Pseudomonas aeruginosa* and members of *Enterobacteriaece* were frequently reported from surfaces in operation theaters. Emmanuel (2012) [14] reported 30% prevalence rate while [15] reported 58% of culture positive samples collected from different surfaces and objects used for surgery in operation theater. In the current study, some of the samples collected from surfaces in operation theater were found contaminated.
In the current study, Vancomycin (100%), Linezolid (100%) Chloramphenicol (96%), Rifampicin (92%), Ciprofloxacin (84%) and Tetracycline (76%) were found to be sensitive and drug of choice for methicillin sensitive *Staphylococcus aureus.* High resistance was observed to amoxacillin (100%) and gentamycin (68%). While antibiotic susceptibility profile determined for methicillin resistant *Staphylococcus aureus,* showed that Vancomycin (100%), Lenzolid (100%) and Rifampicin (71.4%) were found to be sensitive.

Antimicrobials susceptibility profile performed for gram negative bacteria in which high sensitivity pattern was noted for Ciprofloxacin (100%), Amikacin (100%) and Ceftazidime (100%). While a combination of Amoxacillin+cluvanevic acid, Fosfomycin, and Polymyxin B showed (60%) sensitivity. In the present study high resistance was noted for Cefotaxime (50%) and Nitrofurantoin (60%) in gram negative bacteria.

In conclusion, that inanimate surfaces in tertiary care hospital are heavily contaminated with pathogenic bacteria. Prevalence of isolated bacterial strains were higher in wards as compared to operation theater. All the surfaces were found highly contaminated as compared to the instruments and objects. Antibiotics susceptibility pattern of gram positive bacteria were higher than gram negative bacteria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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