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Prevalence and Detection of Multidrug Resistance Bacterial Strains Isolated from the Different Inanimate Surfaces of the Hospital Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nosocomial infections or Hospital-acquired infections (HAI) influence the clinical outcomes in hospital in-patients and indicate a serious global concern in medicine. Fomites of hospital environments carry a deadly pathogen and transmit infectious diseases. The emergence of antimicrobial resistance (AMR) in the hospital environment has increased due to misuse and/or overuse of antibiotics. The present study was aimed to evidence the MDR bacterial pathogens from

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the fomites of the hospital environment. The study was conducted in Acute care hospital, in Cuddalore district, Tamil Nadu, India. Totally 65 samples were collected from the different areas of the hospital including Operation Theatre (OT), Orthopaedics Surgery (OS), Wound and emergency Unit (WEU), Intensive care unit (ICU), Dialysis Unit (DU), Special Ward (SW) from March 2022 to September 2022 and the samples were processed for the isolation of bacteria using Nutrient agar, Macconkey agar and Blood agar. Totally 84 isolates were identified in that 48 and 36 isolates were gram positive and gram-negative respectively. *Bacillus spp, Staphylococcus aureus, Streptococcus spp, Micrococcus spp, E. coli, Salmonella spp* and the *proteus spp.* were the common isolates of this study and most of them showed multidrug resistance. In gram-positive isolates *Bacillus spp.* (22%) was dominant followed by *Staphylococcus aureus* (21%) and in gram-negative bacterial isolates *Salmonella spp.* (17%) were dominant followed by *E.coli (*16%). Among the bacterial isolates, 18% showed multidrug resistance (MDR) followed by 3% and 2% of XDR and PDR respectively. All the studied surfaces of the hospital carried minimum bacterial contamination. So more hygienic practices and effective disinfection practices should be implemented in the studied hospital to prevent the spread of nosocomial infections.

Keywords: Hospital environment; antibiotic resistance; fomites; bacterial contamination; nosocomial infection.

1. INTRODUCTION

Nosocomial infections affect the hospital environment by causing public health problems globally. It increases the loss of social economic status and also induces hospital mortality rates [1]. Nosocomial pathogens often showing multidrug resistance are denoted as ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*.). These six pathogens are highly potential to cause Healthcare-associated infections [2]. Nosocomial pathogens including gram-positive and gram-negative bacteria can survive on fomites of a hospital environment even for a month and also act as a reservoir of infection among the patients and the health care personnel. Environmental factors determine the survival of the bacterial pathogen on the fomites [3].

Nosocomial infections increase the length of hospitalization in addition to the serious complication of chronic diseases like heart diseases, and kidney disorders etc., [4]. Objects such as doorknobs, cutting boards and computer keyboards which transfer nosocomial pathogens are called fomites or inanimate objects [5].

Nosocomial infections were increasing over the decades due to the emergence of new infections, multidrug resistance pathogens and increased hospitalised stays. These infections occurred mainly in developing countries. [6].

Globally healthcare-associated infections are increasing especially in western hospitals. It was reported that 15% of the hospitalised patients were infected by the persistent transmission of the pathogens from hospital surfaces. The emergence of drug-resistant pathogens from the surfaces also increased due to the contamination of 50% of hospital environmental surfaces [7].

The environment of the health care system was seriously contaminated by harmful pathogens, especially ICU. Due to the admission of chronically infected patients in the ICU, there are multiple choices for spreading dreadful pathogens around the areas. In addition, frequent usage of the last line of antibiotics and invasive procedures increases the chances of emerging multidrug-resistant pathogens. So high prevalence of hospital-acquired infections have happened in the ICU by various factors [8].

Worldwide Prevalence of nosocomial infections in developing countries are increasing especially in the ICUs at about 50% and in the regular wards 5% to 15%. The emergence of resistant pathogens and infectious diseases will increase the number of hospitalised patients. This will lead to the high prevalence of nosocomial infections globally [9,6].

As the name noted ESKAPE mainly gram-negative bacterial pathogens reported escaping from the wide range of antibiotics and causing deadly nosocomial infections [10,11].

Despite the fact that nosocomial illnesses and deaths in hospitals in developing countries rank fifth globally, the study was not properly documented. According to a comprehensive review, the prevalence of HAIs is 10.1% in low- and middle-income nations and 7.6% in high-income countries [12,13].

Due to Hospital-acquired infections death rate and the financial burden in the hospital sectors are increased. It was estimated that a 20% prevalence rate of nosocomial infection in developing countries [1]. Hospitalized patients are seriously affected by nosocomial infections. It is transmitted mainly through the patients, health care personnel and the fomites from the hospital environment. Harmful pathogens were predominantly transferred from the hospital waste. Nearly 15% of hospitalized patients suffering from nosocomial infections [14].

Most of the nosocomial infections (urogenital, respiratory and intestinal) are mainly caused by the gram-negative rods preferably *E.coli, Proteus spp., Serratia spp., Klebsiella pneumoniae, Pseudomonas aeruginosa. Enterobacter spp., Morganella spp., Providentia spp.* Possibility of the transmission of nosocomial pathogens through the hospital environment chance by the hands of healthcare personals and diseased inpatients. There is a lot of risk of occupying the rooms of diseased persons having MDR pathogens to get same kind of infection to others [8].

XDR and MDR isolates of hospital environment cause Blood stream infections with high mortality. XDR bacteria such as *P.aeruginosa, A.baumanni* and *K. pneumoniae* were showed high resistance to carbapenem antibiotics and mortality associated death than the MDR isolates [15].

According to one prevalence study Multidrug resistant organism (MDRO) causes hospital acquired infections and increased mortality were noted every year in Germany. Out of 1136 infected patients, 215 patients were died due to the illness of the MDRO infections [16].

High mortality of the MDR bacterial nosocomial infections were reported in cancer patients. Amikacin and carbapenem drugs were treated 89.7% of the nosocomial infections caused by the MDR isolates [17].

This study was designed to screen the distribution of gram-positive and gram-negative isolates on the different surfaces of fomites in acute care hospitals. Fomites were analysed due to the frequent usage of health care personnel as well as patients. MDR pathogens were screened among the total isolates, creating awareness of implementing proper procedures of sterilization and disinfection surfaces.

2. MATERIALS AND METHODS

2.1 Study Design

The present study was conducted in an acute care hospital in Cuddalore. It offers services in various specialities including paediatrics, cardiology, neurology, psychiatry and plastic surgery. In addition to inpatient services, it offers 24-hour emergency medical care and a pharmacy and is equipped to provide a wide range of diagnostic testing. The hospital also hosts a blood bank. The procedures of the study were reviewed and accepted by the hospital management. Samples were collected from the six wards of the hospital includes Operation Theatre (OT), Orthopaedics Surgery (OS), Wound and emergency Unit (WEU), Intensive care unit (ICU), Dialysis Unit (DU), Special Ward (SW) from March 2022 to September 2022.

2.2 Sample Collection

The samples were collected from the ten different inanimate surfaces of the Six hospital environmental units. Passive sampling method was used to collect the sample. Swabs were moistened in the 0.9% w/v physiological saline and rubbed over the surface of the objects then labelled and transported to the microbiology laboratory aseptically. Totally, 65 swabs were collected from the ten different inanimate objects including Boyles Machine, Surgical bed, Floor, Surgical lamp, Door, Switches, Monitor, Window, Air Conditioner and Vacuum unit. Sampling process was done in the morning (9.00 to 10.00am) and evening (5.00 to 6.00pm).

2.3 Isolation of Nosocomial Bacterial Isolates

Each swab sample was plated on the three different media such as Nutrient agar, Macconkey agar and Blood agar separately, then incubated under 37 °C for 24 to 48 hours under aerobic condition. Individual Colonies were isolated based on the different morphological characteristics. Pure bacterial cultures were identified by the standard morphological and biochemical characteristics [18]. Gram staining was performed to classify bacteria into Gram-positive and negative, and various Indole, Oxidase, Coagulase, DNase, Triple Sugar Iron (TSI), Catalase, Urease and Citrate were performed for further identification of isolates [19].

2.4 Antibiogram Category Study of Bacterial Isolates

A total three of bacterial pathogens such as, *Streptococcus pneumoniae* (ATCC - 19615), *Streptococcus pyogenes* (ATCC - 49619), *Pseudomonas aeruginosa* (ATCC - 9027) were purchased from the American Type Culture Collection (ATCC), USA, for the study. The bacterial cultures were sub cultured, maintained on Nutrient agar slants, and stored in a refrigerator at 4 (°C) for further experiments. All the bacterial isolates were tested for their susceptibility to 16 different antibiotics by the kirby- Bauer disk diffusion method in accordance with clinical and Laboratory Standards Institute guidelines (CLSI, 2012) [20]. Bacterial inoculum was prepared by inoculating a loopful of organisms in 5 ml of Nutrient broth and incubated at 37 °C for 12 hours till moderate turbidity was developed. The turbidity was matched with the 0.5 Mc Farland standard and then used to determine bactericidal activity. The suspension was then inoculated onto a Muller-Hinton agar plate. The gram-negative isolates were tested against antibiotics Levofloxacin (5mcg), Amikacin (30mcg), Ceftriaxone (30mcg), Erythromycin (13mcg), Ceftazidime (30mcg), Gentamicin (120mcg), Meropenem(10mcg), Clindamycin (2mcg). Gram positive isolates were tested against the antibiotics, Ciprofloxacin (5mcg), Cefepime (30mcg), Cefazolin (30mcg), Imipenem (10mcg), Amoxiclav (30mcg), Cotrimoxazole (5mcg), Piperacillin (100mcg), Cefuroxime (30mcg).

Then, all plates were incubated at 37°C for 18–24 h, aerobically. After overnight incubation. the susceptibility pattern of the isolates was determined by comparing with the standard chart. Multi-Drug Resistant isolates were screened by showing resistant to three to more antimicrobial categories [21]. Susceptible to one agent in three or more antibiotic classes, atleast one in all or minimum two classes, all antibiotic classes were denoted as MDR, XDR and PDR.

3. RESULTS

In this study 65 samples were collected from the ten different surfaces of the hospital environment. Six different hospital units were screened for the bacterial contamination. Fig. 1 shows the distribution of samples among different hospital sections or units as follows Operation theatre (15%), Orthopaedics surgery (17%), Wound and emergency unit (62%), Intensive care unit (17%), Dialysis unit (17%), special ward (45%). More or less equal proportion of the samples were collected from all the six units of hospital environment. Among the six units of hospitals screened wound and emergency unit showed highest contamination (62%) followed by special ward (45%).

Totally, 84 isolates were identified and belong to eight different genera of both gram positive and gram-negative bacteria such as Bacillus *spp., Staphylococcus aureus, Streptococcus spp., Micrococcus spp., E. coli, Salmonella spp.,* and the *Proteus spp.* Fig. 2 shows that the distribution of gram-positive and gram-negative isolates obtained from the different hospital inanimate surfaces. A maximum number of gram-positive isolates and gram-negative bacterial isolates were obtained from the surface of the door (23%) and surgical bed (22%) respectively (belongs to which ward or hospital unit). All the other fomites are moderately contaminated with bacterial isolates. Fig. 3 shows the number of isolates on the different surfaces of the hospital environment. Among the six wards or units screened wound and emergency unit was contaminated more followed by the special ward.

Fig. 4. shows the resistant pattern of gram-positive isolates.. Most of the gram-positive isolates were resistant to the majority of the antibiotics used. *Bacillus spp.,* showed resistance against a greater number of antibiotics followed by *Staphylococcus aureus*, *Streptococcus spp.,* and *Micrococcus spp*. The maximum number of gram-positive isolates were resistant to the antibiotic Imipenem (100%) followed by Cefepime (90%) and Cefazolin (73%) as presented in All the isolates were sensitive to Ciprofloxacin, Amoxyclav, Piperacillin and Cefuroxime. Fig. 5. Shows the resistance percentile of gram-negative isolates. Maximum resistance has shown against the antibiotics Meropenem, Erythromycin, and Clidamycin. *Proteus spp*. showed maximum resistance against Meropenem (100%), Erythromycin (40%) and Clindamycin (40%) followed by *Salmonella spp*. and Levofloxacin, Certazidime, Gentamicin and Amikacin showed sensitive to all gram negative isolates used in the study.

Table 1 shows the frequency of the bacterial isolates from the surface of the different fomites. Among the seven genera identified *Bacillus spp.* Shows the maximum percentage followed the *Staphylococcus aureus.* Table 2. Shows the number of isolates of the different resistant categories. Totally 18% of the isolates were identified as MDR followed by 3% XDR and 2% PDR.

Table 1. Frequency of Bacterial species among the different fomites of hospital units

Organism	Frequency of bacterial isolates on fomites (%) N= 84									
	BM	**F**	**D**	**SB**	**L**	**M**	**VU**	**W**	**AC**	**S**
Bacillus sp	2	2	2	2	2	1	1	2	2	3
Staphylococcus aureus	2	2	3	2	-	2	2	2	1	2
Streptococcus sp	-	2	2	-	-	-	1	-	1	-
Micrococcus sp	-	-	2	2	1	-	-	-	-	
Proteus sp	-	1	1	2	-	-	2	-	1	-
E. coli		2	1	2	2	-	2	1	1	1
Salmonella sp	2	-	3	3	2	2	1	2	-	-
Total	8	9	14	13	7	5	9	7	6	6

BM- Boyles machine, F- Floor, D- Door, SB- Surgical Bed, L- Lamp, M-Monitor , VU- Vaccum unit, W- Window, AC- Air Conditioner, S- Switch

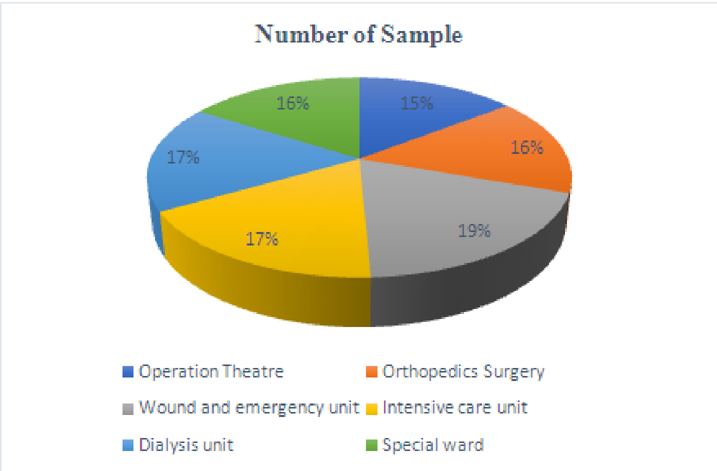

Fig. 1. Distribution of sample among hospital units used in the study

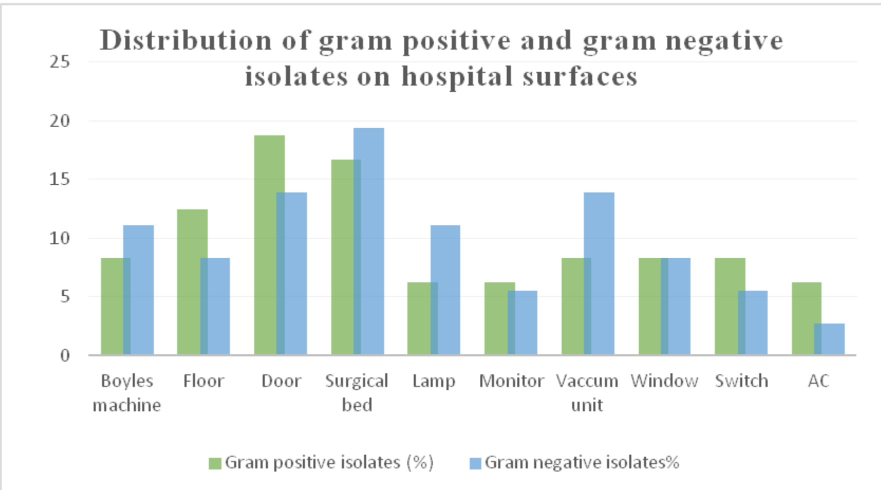

Fig. 2. Distribution of gram positive and gram-negative isolates on hospital surfaces

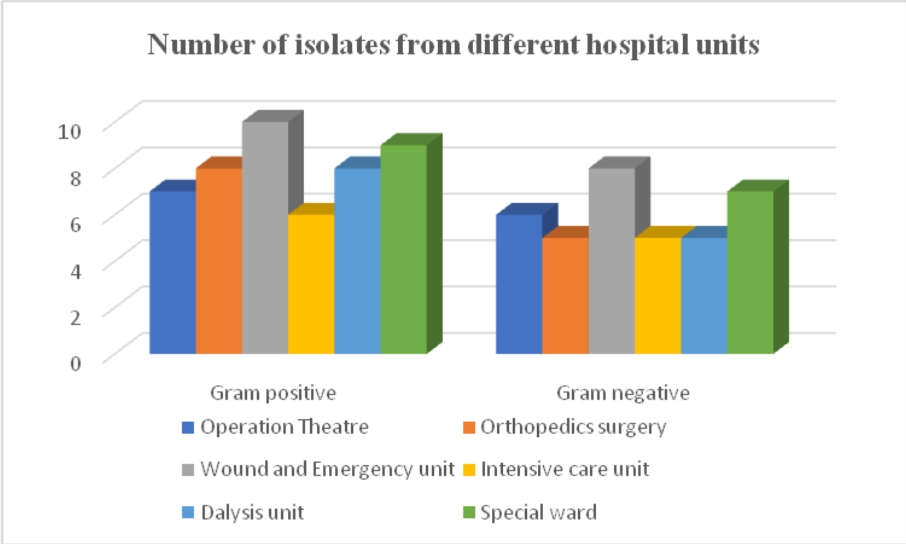

Fig. 3.Number of isolates from the different units of Hospital

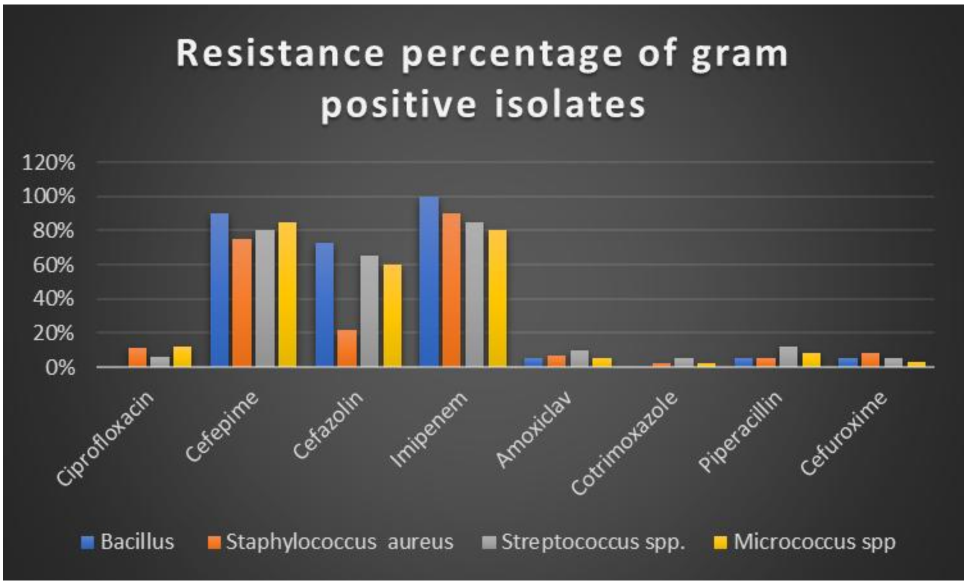

Fig. 4. Resistant pattern of gram-positive bacterial isolates in percentage

Table 2. The distribution of number and species of isolates according to their resistance pattern

Bacteria	MDR	XDR	PDR
Staphylococcus spp	*5*	*3*	*2*
Bacillus spp.	*3*	*0*	*0*
Micrococcus spp.	*2*	*0*	*0*
Proteus spp	*3*	*0*	*0*
Salmonella spp.	*2*	*0*	*0*

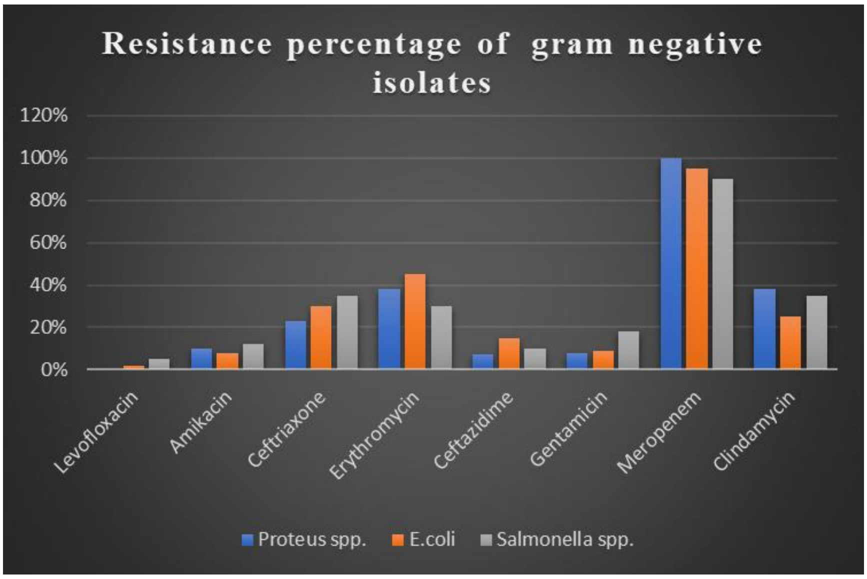

Fig. 5. Resistant pattern of gram-negative bacterial isolates in percentage

4. DISCUSSION

Totally six different environments of acute care hospitals were analysed in this study, among these Wound and emergency units (62%) showed maximum contamination followed by Special ward (45%). Related work was done by Birru et al. [22] who studied the bacterial contamination on the surface of different patient care equipment and they revealed the maximum contamination was in the surgical ward 76.4%, followed by the paediatric ward 66.6% and neonatal intensive care unit 71.8%. Predominant Contaminated bacterial isolates were coagulase-negative *Staphylococci*, *Staphylococcus aureus, Acinetobacter spp and klebsiella spp.* Similar studies conducted to screen the pathogens among the hospital surfaces in Morocco revealed that 51.5% of gram-positive and 48.5% of gram-negative isolates were contaminated the hospital environment [23]. Most of the study were concentrated on the one or two of the hospital environment but in the present study was done in six different environments of the acute care hospital.

In this study, both gram positive and gram-negative isolates were identified from the fomites of an acute care hospital, Cuddalore. Predominant isolates were as Bacillus *spp, Staphylococcus aureus, Streptococcus spp, Micrococcus spp, E. coli, Salmonella spp,* and the *Proteus spp.* Similar works conducted in the health care department in Morocco revealed the bacterial contamination of different objects. But the major contaminants were *Enterobacteria, Staphylococcus aureus, Pseudomonas aeruginosa* and *Acinetobacter*. Gram-negative isolates were more 51.5% than the gram-positive isolates 48.5%. Antibiotic resistant profile of the isolates showed the emergence of MDR pathogens. 31.7 % of gram-negative and 44.7% of gram positive [23]. One of the studies reported that *Acinetobacter spp.* were the predominant contaminant of hospital equipment [24].

Density of bacterial load on the hospital environment vary depends upon the health status of inpatients: Heavy load of pathogens presents in the areas of patients with wound and urinary tract infection than other areas [25]. In this present study also identified the various group of bacterial isolates from the different environments of acute care hospital. Specifically door and surgical bed surface carries pathogens in large number when compare with other units of hospital.

Our findings concluded that 20 % of Door handles were contaminated with *Staphylococcus aureus*, *Bacillus spp* and the *salmonella spp*. These results were more or less similar to the study of Oie et al. [26] who revealed 27% of the door handle contaminated with *Staphylococcus aureus*. According to the work conducted by Sserwadda et al. [27] *Staphylococcus aureus* (75.4%) and *Klebsiella pneumonia* (11.5%) were the dominant contaminants of the surface objects of the hospital environment. Infusion stands and Beds showed maximum contamination with bacteria. The antibiotic profile of the isolates revealed that the multi-drug resistant

Staphylococcus aureus showed more percentage than the gram-negative isolates.

Bacterial contamination of nineteen frequently touched objects in the hospital environment was studied and the most prevalent bacteria were *E. coli* followed by *Klebsiella spp*. All the isolates were resistant to Erythromycin, Gentamicin, and Amikacin [28]. Similar to this result, in this study gram-negative isolates showed resistance against Ceftriaxone, Amikacin and Erythromycin. A survey study of 5 years conducted in nine hospitals stated that *Bacillus cereus* was the serious cause of intra and inter-hospital cross-contamination and was also revealed for the first time [29]. Similarly, in this current study, Bacillus cereus (39%) was the dominant isolate and also showed maximum resistance to antibiotics.

One of the studies revealed that the resistant pattern of nosocomial isolates as follows, *P. aeruginosa* was resistant to ciprofloxacin and sensitive to colistin and the staphylococcus aureus was resistant to Augmantine, Ceftriaxone, Ciprofloxacin, Ceftazidime [30]. But in the present study, all the isolates showed maximum sensitivity to Ciprofloxacin, Amoxiclav, Piperacillin and Cefuroxime and showed resistance to beta-lactam antibiotics, Cefepime and Cefazolin especially Carbapenem drugs such as Imipenem and Meropenem. Comparative study of the MDR pathogens from the hospital and non-hospital environment resulted in more differences such as 81.5% from the hospital and 54.2% in the non-hospital environment [31]. This showed the increasing chances of arising MDR pathogens from the hospital environment. Similarly in our study, 23% of resistant pathogens were identified among that 18% MDR, 3% XDR and 2% PDR were noted. From comparison of the different works, our study discovered the carbapenem resistant MDR bacterial isolates from the six different environment of the acute care hospital. Among that few species were showed XDR and PDR.

The data we got from our study insists that the risk of bacterial contamination through the fomites and there will be a reservoir to cause serious infections. The majority of the bacterial isolates showed multidrug resistance leading to the threat of immunocompromised, bedridden patients and healthcare workers. The maximum risk of cross-contamination to the patients of the surgical ward followed by the paediatric ward was noted [32]. This is occurred mainly due to the poor hand hygiene practices followed by the healthcare professionals after contact with the fomites around the patients leading to serious concern in the epidemy of healthcare-related infections [33,34].

5. CONCLUSION

Different hospital environments such as operation theatre, intensive care unit, and dialysis unit were the rich source of bacterial pathogens. Even though surfaces are sequentially sterilized some bacterial pathogens resist on the surface areas such as the floor, door, surgical lamp etc. The antimicrobial susceptibility pattern of the study showed the seriousness of the occurrence of fomites-associated transmission of nosocomial infection. So, precautionary measures should be taken to prevent the spread of infection. From the current study, we concluded that the emergence of MDR pathogens in the hospital environment occurs by contaminated fomites. Regular sterilization of the equipment, as well as the environment of the hospital with effective disinfectant, will reduce this problem. However, identification of the multidrug-resistant pathogen from the fomite surfaces of the hospital environment insists on the importance of hand hygiene of health care personnel.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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