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ISOLATION AND IDENTIFICATION OF BACTERIA FROM MEDICAL DEVICES AND HOSPITAL SURFACES

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Abstract

Medical devices are either directly or indirectly associated with health care and patient treatment. The primary cause of hospital-acquired infections (HAIs) is the colonization of bacteria on medical equipment. On medical equipment, microorganisms such as bacteria and fungus exist in the form of biofilms, which enable their long-term survival. The most common bacteria found in medical devices linked to the most common HAIs, such as catheter-related infections, urinary tract infections, bloodstream surveillance infections, and skin and soft tissue infections, are S. aureus, E. coli, K. pneumoniae, P. aeruginosa, and Enterococcus spp. The risk of bacterial and fungal infections is increased when these microorganisms are present on hospital surfaces and medical equipment. Thus, it is essential that hospital and healthcare personnel are properly informed on the risk factors and preventative strategies for HAIs. In order improve awareness of the risk factors for common bacteria present and the diseases they might cause, the purpose of this study was to identify the bacterial isolates from the local hospital in Havelian based on their biochemical characteristics. Twenty-one samples were collected from hospital surfaces and medical devices, including three from hospital curtains, three from hospital sinks, five from equipment trays, five from indwelling devices such as drips and catheters, and five from patient bed linens. These samples were then taken to the laboratory for additional analysis. Following biochemical analysis, the most common bacterial species were Bacillus (29%), Staphylococcus aureus and Escherichia coli (19%), Pseudomonas, and Neisseria (14%), whereas Enterococcus (5%), which had the lowest count. The significance of this research contributes to an extensive understanding of the sources of infectious microorganisms in medical environments.

INTRODUCTION

Medical devices, which can range in complexity from delicate machinery like MRI scanners to simple items like thermometers, are essential to modern healthcare. These devices are necessary for diagnosis,

monitoring, and treatment of number of different disorders. Catheters and other intravascular devices are crucial for the diagnosis and management of cardiovascular disorders. To monitor, identify, or treat a variety of illnesses, these devices are inserted into blood arteries (Smilowitz et al., 2012). Flexible tubes called catheters are inserted into the body to remove or transmit fluids. They are widely used in medical settings for a variety of purposes, including urine catheterization, body fluid drainage, and intravenous therapy (Saint et al., 2000). To administer medication, collect blood, or perform both, central venous catheters are inserted into a major vein, often in the chest, neck, or groin (Maina et al., 2018).

Hospital bed linens, including blankets, pillows, and sheets, as well as hospital curtains, are crucial for patient comfort and prevention of infection. To maintain the environment healthy and protect people from diseases associated with healthcare (Sehulster et al., 2003). The most diverse devices on which bacteria may establish colonies are implants. The most frequent bacteria that infect urological equipment are Escherichia coli, S. aureus, and E. faecalis. The most common contamination of gastrointestinal implants is A. baumannii. The bacterium A. baumannii may build a biofilm that makes it resistant to a variety of antimicrobials and is resistant to nutrition deficiency and desiccation (Ali et al., 2014). Additionally, the most often isolated bacteria are Staphylococcus species, of which S. aureus is found in practically all implants. It was discovered that fungi, such as Aspergillus species and Fusarium spp., only infected the contact lenses. Actually, the following microbes have been the focus of several important recent research: Escherichia coli, K. pneumoniae, Pseudomonas species, P. aeruginosa, P. fluorescents, Acinetobacter species, A. baumannii, C. acnes, and Pseudomonas species are all members of the Enterobacteriaceae family (Bouhrour et al., 2024).

In patients receiving mechanical ventilation, such as hospitalized COVID-19 patients, it can result in pneumonia (O'Toole et al., 2000). Special attention must be given to Corynebacterium striatum, a strain that is frequently found in human skin and nasal mucosa and can cause nosocomial infections (Akbari et al., 2018). This bacterium can colonize orthopedic implants or catheters due to its exceptional ability to adhere to surfaces and form biofilms. Microbes that share numerous essential traits that increase their resistance to treatment, survival, and proliferation are frequently found contaminating hospital surfaces, implants, and medical equipment. Pathogens create toxins, enzymes, and other substances that enhance their ability to transmit disease (Davies & Davies, 2010). In modern healthcare, microbial infections in embedded medical devices are a major problem. Infections can cause medical equipment to malfunction for a number of reasons, resulting in further procedures or even death. Staphylococcus, particularly Staphylococcus epidermidis and Staphylococcus aureus, Streptococcal species, Gram-negative bacilli, enterococci, and anaerobes like Propionibacterium acnes are frequently the bacteria that cause these infections on the patient's skin (Russotto et al., 2015).

Every medical equipment is susceptible to the growth of microbial biofilms, which can cause infections. Medical equipment is responsible for 60–70% of hospital infections. Bacteria that create biofilms can adhere to medical equipment as well as human tissues. Bacterial biofilm infections frequently result in tissue loss, device malfunctions, and pathogen transmission, all of which can cause fatal infections (Latif & Wajid, 2024). Numerous bacteria, including methicillin-resistant strains of S. aureus, coagulase-negative staphylococci (including S. epidermidis, S. haemolyticus, S. hominis, and S. warneri), Propionibacterium acnes, P. aeruginosa, Haemophilus influenzae, Providencia, Enterococci, Streptococcus viridans, Escherichia coli, Citrobacter, Lactobacillus, Acinetobacter, Serratia Marcescens, Klebsiella pneumoniae, and Corynebacterium have been found to be responsible for infections linked to prosthetic devices (Veerachamy et al., 2014).

Hospital visitors may breathe in bio-aerosols, which are microscopic biological particles that contain pollutants. Health issues including allergies, harmful impacts, and infectious infections can result from this exposure to pollutants. Overuse of antibiotics can strengthen the infection and make treatment more difficult. In hospitals, bacteria can be found in the air, on surfaces, and on equipment (Oliveira et al., 2021). Carriers can spread pathogens such as Staphylococcus aureus, Staphylococcus pyogenes, and others. Cooling systems emit contaminants from water pipes into the air, which can lead to Legionella infections. Hospital

ventilation systems have the potential to accumulate dust and other pollutants, which can promote the growth of mold. When individuals cough, sneeze, or talk, microscopic droplets can spread certain illnesses, such as the flu and TB. Patients may be exposed to these pollutants through gloves, visiting hands, or dust that comes into contact with surfaces. It may be dangerous for the subsequent patient to be in a room where the prior patient had certain resistant bacteria or yeasts (Bonadonna et al., 2017).

Material and method

Sample collection

A sterile swab stick was used to directly collect samples from several locations throughout the Havelian, Pakistan, local hospital. Due to its non-toxicity to microorganisms, the swabs were initially dipped in Phosphate Buffered Saline (PBS) for sample collection. Three samples were taken from hospital curtains (items 16–18), five samples were taken from equipment trays (items 1–5), five samples were taken from indwelling devices (items 6–10), including drips and catheters, five samples were taken from patient bed linens (items 11–15), and three samples were taken from sinks (items 19–21). The swab samples were then put in Ziploc bags, usually labeled, and transported to the lab in less than two hours.

Media prepration

In order to prepare the nutrient agar medium for bacterial growth, 28g of nutrient agar was added to 1000 ml of solution, and the nutrient agar was measured on a digital balance machine in relation to its standard level. Therefore, in accordance with our sample size, which is 21, seven plates were collected, and we prepared a 200 ml nutrient agar media solution by mixing 5.6 g of nutrient agar with 200 ml of distilled water. We then put 25 ml of media solution into each plate to cover the base. After that, the prepared medium and petri plates were autoclaved at 121°C to remove any remaining contaminants and sterilize them (Azman et al., 2017).

Culturing and Streaking

Following collection, swab samples were inoculated into Nutrient Agar plates and incubated for 24 to 48 hours at 37°C to achieve the proper bacterial growth. Then, using the subsequent procedures, one colony was chosen from each plate and streaked onto a fresh nutrient agar plate: They picked up one colony using a sterile loop. Individual bacterial cells were then isolated by zigzag-stripping the loop across the plate. After that, the plate was incubated at 37°C for 24 to 48 hours to permit the development of bacteria. For 24 to 48 hours, the plates were incubated at 37°C, and the growth of bacteria was monitored. The colonies were distinguished by their size, color, and shape. For purity and further identification, the isolated colonies were subcultured on fresh NA plates.

Gram staining

Using the Gram staining technique, bacteria have been classified into Gram-positive and Gram-negative groups according to the properties of their cell walls. Gram Staining The first step involved spreading a small layer of bacterial suspension (smear) onto sterile glass slides, drying them with air, and then heat-fixing them by passing them over a flame. Second, crystal violet, the principal stain, was applied to each slide's smear and left for one minute. To create a crystal violet-iodine combination, iodine solution (a mordant) was then applied for one minute. The slide was then cleaned with ethanol for 30 seconds in order to distinguish between bacteria that were Gram-positive and those that were Gram-negative. Then, for one minute, a thick coating of safranin was applied. Following staining and decolorizing, each slide was carefully washed with distilled water to get rid of any remaining stains. Finally, all of the slides were examined at 1000x magnification using an oil immersion microscope. Gram-negative bacteria absorbed the safranin and became red or pink, but Gram-positive bacteria kept the crystal violet stain and appeared purple (Coico, 2006).

Figure 1: Preparing smear for gram staining

Microscopy

A light microscope with oil immersion (100x magnification) was used for examining the stained slide. Gram-positive bacteria retained the crystal violet stain and appeared purple, whereas Gram-negative bacteria lost the crystal violet stain and acquired the safranin counterstain, appearing pink (Coico, 2006).

Catalase Test

The Catalase test assist in determining whether or not the bacteria have the catalase enzyme. In order to distinguish between two species of bacteria, specifically Micrococcaceae (catalase-positive) and Streptococceae (catalase-negative), this test is essential. By converting dangerous substances, such as hydrogen peroxide (a reagent), into water and oxygen, the catalase catalyst shields microorganisms from harm (Reiner, 2010).

Slide (Drop) Method

In order to perform the catalase test for each of the samples, microscope slides were first collected, with both ends labeled. In order to make the bacterial suspension, a small amount of organism from an 18–24-hour culture was gathered and put onto the microscope slide using a sterile inoculating loop or wooden toothpick. Next, without mixing, one drop of 3% hydrogen peroxide is applied to the organism on the microscope slide using a dropper or Pasteur pipette. After that, for one to two minutes, the instantaneous bubble generation (O2 + water = bubbles) was observed. Finally, fast bubble development or release indicates favorable reactions. On the other hand, the absence of bubble formation indicates negative reactions (Reiner, 2010).

Coagulase Test

Glass slides, a centrifugation machine to separate plasma from blood, a pipette to add distilled water, an inoculating loop to select and mix the culture, and a permanent marker to identify the slides were all required for the coagulase test. Slides were taken separately for the coagulase test. Each slide was prepared in accordance with the appropriate procedures and guidelines, and the smear was created by combining the distilled water and the culture suspension. Following that, a drop of citrated plasma was added to this sample, and it was well mixed. All of the slides underwent a similar process. The slides were deemed affirmative if they clumped or agglutinated within 5–10 seconds. And those slides that didn't exhibit any clumping were interpreted negatively (Brown et al., 2005).

Oxidase test

The oxidase test determines if an organism contains the enzyme oxidase. The test solution changes color, signifying a positive result, if an organism demonstrates this enzyme. Tetra-methyl-p-phenylenediamine dihydrochloride, also known as Kovacs oxidase reagent, is a colorless fluid used in the

oxidase test that changes color when it comes into contact with the oxidase enzyme. This color shift from colorless to deep blue or purple indicates a favorable outcome (Shields & Cathcart, 2010).

Filter Paper Spot Method

To carry out an oxidase test, circle-shaped pieces of filter paper of the proper size have been cut out for each sample's detection. Next, using a sterile wire loop, a small portion of a freshly cultured bacterial colony that has been well-isolated (18–24 hours old) has been selected. This is accomplished by gently rubbing the colony onto a tiny piece of filter paper. After that, the bacterial culture was given 1-2 drops of a 1% Kovacs oxidase reagent solution (tetra-methyl-p-phenylenediamine di-hydrochloride). Finally, a dark purple hue emerged within 5–10 seconds of the observation, signifying successful outcomes. However, unfavorable outcomes have been demonstrated by either no change or a tiny hue shift that appears bright yellow (Shields & Cathcart, 2010).

Motility Test

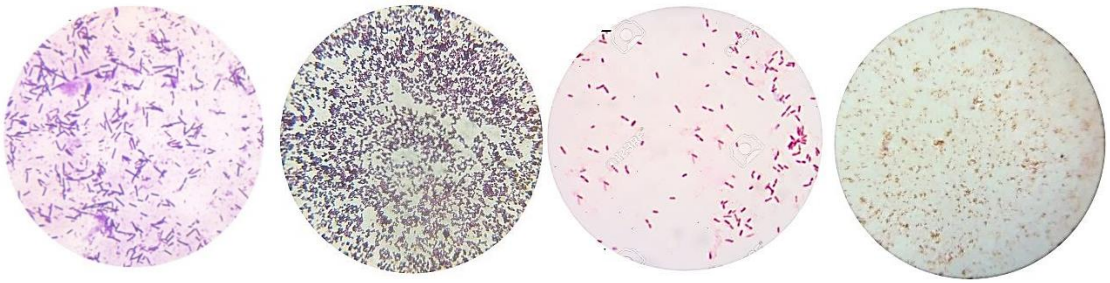
A flask containing 300 milliliters of distilled water and six grams (6 g) of motility medium was autoclaved to sterilize it. After that, sterile test tubes were obtained, filled with sterile motility media, and sealed with paper or a cap to prevent contamination. The medium was then allowed to solidify for 15 to 30 minutes in the refrigerator. The culture was then infected by putting the heated inoculation needle straight down into the middle of the test tubes to a depth of approximately ⅔ of medium, and then pulling the needle back along the same straight line, known as the stab line. All of the samples underwent the same process, and each test tube was put in an incubator set to a temperature of around 37 C, which is ideal for bacterial species. Following a 24-48-hour incubation period, motility was noted. The growth that radiated or disseminated from the stab line was identified as motile bacteria in each test tube. The tubes that showed a straight line of bacterial growth were considered negative (Mishra & Makharia, 2012).

Results

Gram Staining

Gram staining was done to determine whether the isolated bacteria belonged to the Gram positive or Gram negative group after the bacteria that were found on samples of medical equipment and hospital surfaces were cultured. Gram staining results showed that slides 1, 5, 12, 14, 15, and 19 showed purple-colored Positive rods, slides 2, 8, 13, and 17 showed orange-pink-colored Negative cocci, and slides 3, 10, and 16 showed purple-colored Positive cocci. Slides 4, 6, 7, and 9 showed pink-colored Negative bacilli, slides 11, 20, and 21 showed pink-colored Negative rods, and slide 18 showed purple-colored Positive staphylococcus.

Microscopy



Sample 1: Gram positive bacilli
Sample 12: Gram positive bacilli
Sample 15: Gram positive bacilli

Figure 2: Bacterial isolates appeared as Gram positive

Table 1. Identification of bacterial isolates on the basis of Gram staining

Slide number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Gram staining	+	-	+	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-

Catalase Test

After Gram Staining test, we performed another biochemical i-e, catalase test in order to differentiate bacteria based on their ability to produce enzyme i-e catalase enzyme. The results were observed as; Sample 1, 3, 9, 19 is indicated as catalase negative while sample 2, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21 is indicated as Catalase Positive.

Figure 3: Catalase test results of bacterial isolates

Table 2: Identification of bacterial isolates on the basis of Catalase Test

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Catalase	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+

Coagulase Test

Then, in order to distinguish between staphylococcus species and bacteria that were isolated from samples, we conducted another biochemical test, namely, coagulase. Samples 1, 5, 6, 9, 10, 13, 14, 15, 17, 18, 19, and 21 were found to be Coagulase Positive, whereas samples 2, 3, 4, 7, 8, 11, 12, 16, and 20 were found to be Coagulase Negative.

Figure 4: Coagulase test results of bacterial isolates

Table 3: Identification of bacterial isolates on the basis of Coagulase Test

Slide number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Coagulase	+	-	-	-	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-	+

Oxidase Test

In order to distinguish between various bacterial groups according to their capacity to manufacture the oxidase enzyme, oxidase test was carried out. Oxidase negative results are shown in samples 1, 2, 3, 4, 6, 10, 13, 14, 15, 17, 18, and 20. Oxidase positive results are shown in samples 5, 7, 8, 9, 11, 12, 16, 19, and 21.

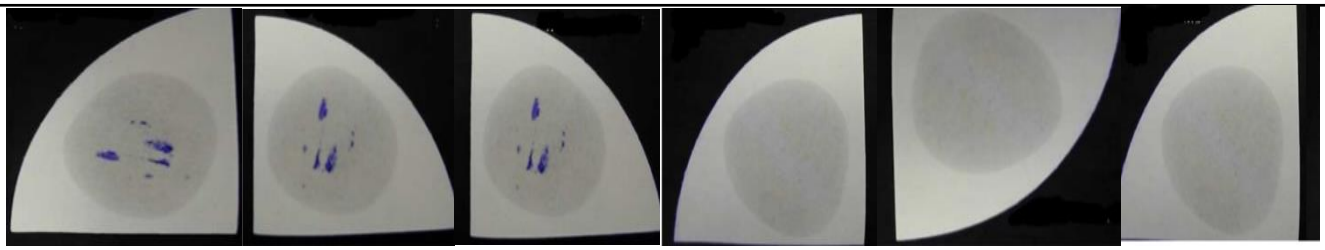


Figure 5: Oxidase test results of bacterial isolates

Table 4: Identification of bacterial isolates on the basis of Oxidase Test

Slide no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Oxidase	-	-	-	-	+	-	+	+	+	-	+	+	-	-	-	+	-	-	+	-	+

Motility test results

Then we performed another biochemical test i-e; Motility test in order to determine the motility of bacteria that either which bacteria has ability to move or not the result of this biochemical test are; in sample 1, 4, 7, 8, 13, 15, 19 were indicated as motile while in sample 2, 3, 5, 6, 9, 10, 11, 12, 14, 16, 17, 18, 20, 21 were indicated as non-motile.

Figure 6: Motility test results of bacterial isolates

Table 5: Identification of bacterial isolates on the basis of Motility

Slide no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Motility	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	-	-	+	-	-

After the biochemical characterization, 21 bacterial isolates were obtained with several characters. The results of biochemical tests shown 10 Gram positive and 11 Gram negative from gram staining, 17 Catalase positive and 4 catalase negative, 12 coagulases positive and 9 coagulase negative, 9 oxidase positive and 12 oxidase negative, 7 motile and 14 non-motile bacteria.

Table 6: List of source equipment and identified bacteria

Sample No.	Source	Results
1	Equipment tray	Bacillus
2	Equipment tray	Neisseria
3	Equipment tray	Enterococcus
4	Equipment tray	Escherichia coli
5	Equipment tray	Bacillus
6	Drips and Catheters	Escherichia coli
7	Drips and Catheters	Pseudomonas aeruginosa
8	Drips and Catheters	Neisseria

9	Drips and Catheters	Pseudomonas aeruginosa
10	Drips and Catheters	Staphylococcus aureus
11	Bedlinens	Pseudomonas aeruginosa
12	Bedlinens	Bacillus
13	Bedlinens	Neisseria
14	Bedlinens	Bacillus
15	Bedlinens	Bacillus
16	Curtains	Staphylococcus aureus
17	Curtains	Staphylococcus aureus
18	Curtains	Staphylococcus aureus
19	Sinks	Bacillus
20	Sinks	Escherichia coli
21	Sinks	Escherichia coli

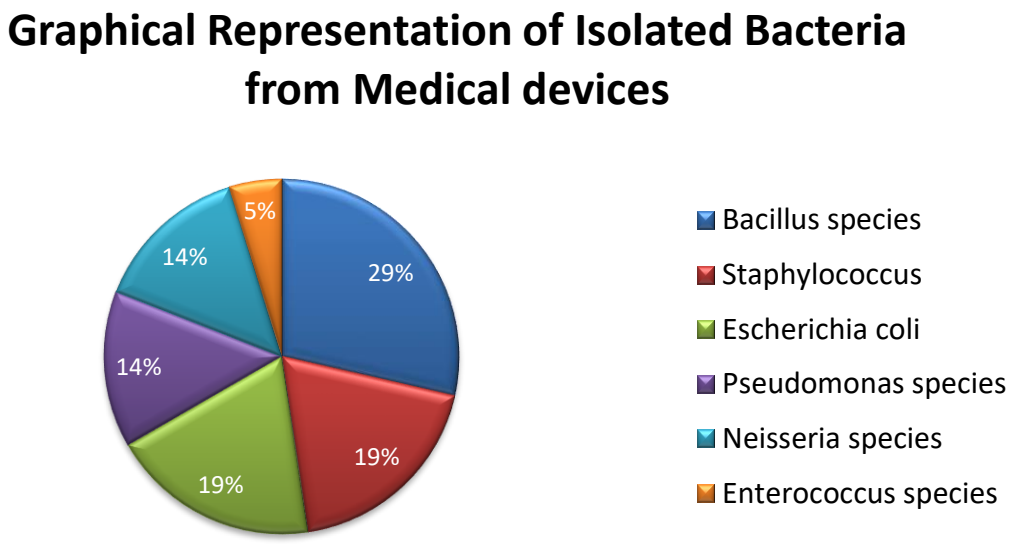


Figure 7: Pie chart of Identified Isolated Bacteria

Discussion

Hospital surfaces and medical equipment may serve as harbors for bacterial colonization, which can result in the transmission of illnesses. Medical equipment and hospital surfaces are home to a diverse array of bacterial species, including coagulase-negative These bacteria, which are frequently associated with nosocomial infections, can colonize indwelling medical devices and create biofilms. They include Staphylococcus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas species, Proteus mirabilis, Enterococcus, and Klebsiella species. The production of biofilms by these bacteria, which are a primary source of device-related illnesses, can make treatment more difficult and raise the risk of infection transmission (Shaheen & Baqai, 2016). The serious issue of infections linked to healthcare is often caused by inanimate items, especially catheters. Although a variety of microorganisms can operate as pathogens and cause nosocomial infections, coagulase-negative staphylococci are likely to be the primary source of infections linked to foreign bodies (Von Eiff et al., 2005). Furthermore, certain strains of Acinetobacter calcoaceticus, Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis exhibit virulence traits like biofilm formation that have been strongly linked to a variety

of catheter-related HAIs, including those involving urinary catheters, arterial catheters, central venous catheters, and other medical devices (Götz & Peters, 2000). Bacterial persistence raises the danger of transmission in hospital environments. Bacteria that are resistant to antibiotics and have the capacity to create robust biofilms can multiply and spread to various healthcare settings. According to (Jabłońska-Trypuć et al., 2022) the most common strains of bacteria that can cause the diseases that are difficult to treat can include: Klebsiella pneumoniae, Clostridium difficile, Escherichia coli, Acinetobacter species, Enterobacter sakazakii, Enterobacter cloacae, Pseudomonas
aeruginosa and Staphylococcus aureus.

The aim of our research was to examine the diversity and existence of bacteria on hospital surfaces and medical equipment, with an emphasis on their isolation and identification. The study's findings somewhat coincide with those of other investigations that have found comparable bacterial species in hospital settings, including Neisseria, Bacillus, Pseudomonas, Enterococcus, Escherichia coli, and Staphylococcus aureus. Medical trays and hospital drapes were discovered to be heavily polluted with bacteria. Because bacteria like these can cause deadly HAIs, this is concerning. In order to strengthen infection control measures, provide improved patient security, and prevent illnesses linked to healthcare, our study's findings emphasize the importance of high awareness and well-established methods.

Conclusion

As a result, our study revealed that Bacillus, Staphylococcus, and Escherichia coli species are commonly detected on hospital surfaces and medical equipment. In order to lower the danger of diseases spreading in hospitals, we must enhance our infection control procedures and cleanliness standards. This is a major concern for patient safety. Our study seeks to further our understanding of this crucial subject and highlight the necessity of healthcare environments being more cautious and efficient in order to stop the transmission of illnesses. This implies that hospitals and other healthcare institutions must strengthen infection control procedures, increase cleaning and disinfection techniques, make sure that staff members are properly trained, and use efficient techniques to lessen the growth of bacteria on surfaces.

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