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Incidence and Antibiotic Resistance Patterns of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Fomites in Critical Units of General Hospital Katsina

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Abstract

***Nosocomial infections, predominantly caused by* Staphylococcus aureus *and* Pseudomonas aeruginosa*, continue to be a major public health challenge, especially in developing countries, due to their associated morbidity and mortality. This study aimed to isolate and identify bacterial pathogens from fomites in the Accident and Emergency (A&E) unit, male ward, and female ward of General Hospital Katsina and to evaluate their antimicrobial susceptibility patterns. A total of 90 swab samples were collected from frequently touched items such as beddings, door handles, floor surfaces, scissors, and forceps across the three locations. Standard microbiological techniques were utilized for the isolation and identification of* S. aureus *and* P. aeruginosa*, followed by antibacterial susceptibility testing using the disc diffusion method. Bacterial growth was observed in 58 (64.4%) samples, with* S. aureus *accounting for 32 (55.2%) and* P. aeruginosa *for 26 (44.8%) of the isolates. The male ward exhibited the highest contamination levels, with a mean colony count of 725.25 CFU/plate, significantly higher than the female ward (487.67 CFU/plate) and the A&E unit (48 CFU/plate), as confirmed by a one-way ANOVA ($p < 0.05$). Chi-square analysis revealed no statistically significant association between bacterial species and hospital location ($p = 0.823$). The susceptibility testing showed that* S. aureus *isolates were generally susceptible to Gentamycin, Ciprofloxacin, and Pefloxacin but resistant to Amoxicillin. Conversely,* P. aeruginosa *displayed high resistance to Gentamycin and Septrin. Conclusively, these findings revealed the critical role of fomites in the transmission of pathogens and highlighted the need for enhanced infection control measures and targeted antibiotic stewardship programs to mitigate the risks associated with these infections.*

***Keywords:** Nosocomial infections, Staphylococcus aureus, Pseudomonas aeruginosa, Fomites, Antibacterial susceptibility*

INTRODUCTION

Nosocomial infections, called hospital-acquired infections, are those infections acquired due to treatment in a hospital or healthcare service-providing center. Nosocomial infections have been the main problem in healthcare delivery. These frequently result in a prolonged recovery of patients and even death when not treated early. Nosocomial infection often appears 48 hours or more after hospital admission or within 3 days after discharge (Iliyasu *et al.*, 2016). Different microorganisms (bacteria, fungi, and viruses) have been associated with the development of nosocomial infections, possibly leading to prolonged recovery of patients and even death when not treated early (Khan *et al.*, 2015).

Several species of microorganisms have been isolated in different hospitals across the world (Ilic and Marković-Denić, 2017). Even though some of these organisms were not known for causing intractable nosocomial infections, they are opportunistic pathogens.

Pseudomonas aeruginosa and Staphylococcus aureus have both been prominently mentioned as significant nosocomial infections, according to a review of the literature. The most significant finding is that resistant strains of Staphylococcus aureus, like methicillin-resistant S. aureus, have been linked to serious infections of the skin and soft tissues, necrotizing pneumonia, and other complications like

endocarditis, meningitis, and toxic shock syndrome (TSS) (Davoudi *et al.*, 2014).

The environment, medical personnel, other sick patients, contaminated surfaces, and contact with fomites are common sources of nosocomial infection exposure. According to Aminu *et al.* (2017) and Kanamori *et al.* (2017), hospital supplies like IV drip tubes, catheters, and life support equipment, along with personal items like stethoscopes and neckties worn by healthcare professionals, can spread pathogens from patient to patient and act as potential carriers of hospital-acquired infections. Thus, the nosocomial infection caused by nosocomial pathogens has led to a problem of enormous magnitude globally; hospital localities have proven favorable in the transmission of disease due to the existing suitable pathogens–host–environment relationship (Stiller *e. al.*, 2016).

Hospital-acquired infections add to the functional disability and emotional stress of the patient, and in most cases, it may lead to disabling conditions that reduce the quality of life. Since the human hands harbor microorganisms both as residents and transients (Pepper and Gentry 2015), transferring the pathogens between people who access the same area or surface is possible. Amphitrite handwashing can minimize microbes acquired by contact with contaminated surfaces.

One of the main challenges faced in eliminating nosocomial infection is the inadequate human resource base for sanitation. There are no dedicated frontline workers in sub-districts where they are most needed to promote and implement sanitation strategies (Khan *et al.*, 2017).

Disinfection of surfaces is necessary to prevent infection from transient microbes, especially surfaces that come in frequent contact with the hand. Nosocomial infections, particularly those caused by drug-resistant pathogens, present a significant challenge in hospital settings. This study investigates the role of fomites in the transmission of S. aureus and P. aeruginosa in General Hospital Katsina, focusing on their antimicrobial resistance patterns to inform better infection control practices.

METHODOLOGY

Study location

This study was conducted at General Hospital Katsina, located along Muhammad Dikko Road Katsina. The laboratory analysis was performed at the Microbiology Research Laboratory of Umaru Musa Yar'adua University (UMYU), Katsina, Katsina State.

Sample Collection and Preparation

A stratified random sampling method was employed to ensure comprehensive coverage of various fomites within the Accident and Emergency (A&E) unit, female ward, and male ward at General Hospital Katsina. Cochran's formula was used to calculate the appropriate sample size, resulting in the collection of 90 duplicate swab samples. These samples were aseptically obtained from beddings, door handles, floor surfaces, forceps, and scissors within the three wards. Each swab stick was moistened with sterile normal saline to enhance microbial recovery and was promptly transported to the laboratory for analysis (Park *et al.*, 2017).

Upon arrival at the laboratory, the swabs were inoculated into nutrient broth and incubated at 37°C for 24 hours. Following incubation, the samples were plated onto cetrimide agar and mannitol salt agar and further incubated in an inverted position at 37°C for another 24 hours. To facilitate bacterial enumeration, 1 mL of the incubated nutrient broth was serially diluted into test tubes containing 9 mL of distilled water, producing dilutions ranging from 10^{-1} to 10^{-6}. From each final dilution, 0.5 mL was transferred onto Petri plates and incubated at 37°C for 24 hours. Plates containing 30 to 300 colonies were selected for counting using the Quebec colony counter, with results expressed as colony-forming units (CFU) per milliliter (Ben-David & Davidson, 2014).

Identification of Bacterial Isolates

Routine laboratory techniques were carried out, including Gram staining and biochemical identification tests.

Gram Staining

The Gram-staining was carried out in order to differentiate the bacteria and their Gram reaction. A smear was made on a glass side and fixed using a Bunsen flame. The fixed smear was covered with crystal violet for 60 seconds, followed by iodine for 60 seconds. It was then decolorized with acetone and immediately washed with water. The smear was then covered with safranin stain for two minutes and washed with water. The samples were examined under an oil immersion objective (Chesbrough, 2018).

Catalase Test

A few drops of hydrogen peroxide solution were poured into a glass slide using a sterile wooden stick; several colonies of the test organism were removed and immersed in the hydrogen peroxide solution and observed for bubbles (Chesbrough, 2018).

Coagulase Test

A drop of distilled water was placed on a slide. A colony of the test organism was emulsified on the drop to make a thick suspension. A loop-full of plasma was added, gently mixed observed for champing within 10 seconds (Chesbrough, 2018).

Motility Test

A sterile needle was used to pick the colony of bacteria motility media was stabbed, within 1cm of the tube's bottom, with an organism under investigation. If the bacterium is motile, there will be growth going out away from the stab or inoculation line, indicating a positive test. If the test is negative, there will only be growth along the stab line (Chesbrough, 2018).

Oxidase Test

Three drops of Kovac's oxidase reagent were added to a filter paper. A colony of the test organism was picked, smeared on the filter paper, and obscured to develop a blue-purple color within 10 seconds (Cheesbrough, 2018).

Indole Test Using Tryptone Water

The test organism was incubated in a test tube containing 4 mL of sterile tryptone water at 37°C for 72 hours. After incubation, 15 drops of Kovac's indole reagent were added, the tube was gently shaken, and the reaction was examined within 10 minutes for the presence of a red color in the surface layer (Cheesbrough, 2018).

Citrate Test (Using Simmons Citrate)

Slants of the medium were prepared in test tubes. Using a sterile wire loop, the slants were streaked with the suspension of the test organism and incubated at 35°C for 48 hours. The medium was then examined for the appearance of a bright blue color (Cheesbrough, 2018).

Antibacterial susceptibility test

The antibiotic screening was carried out by the disc diffusion method. The isolates were aseptically sub-cultured by streaking onto prepared Mueller-Hinton Agar plates. Standardization of the inoculum was achieved by adjusting the turbidity of the bacterial suspension to match a 0.5 McFarland standard.

Commercially prepared antibiotic discs were placed onto the inoculated agar plates using sterile forceps. The plates were then incubated at 37°C for 24 hours and observed for zones of inhibition. The sensitivity of the isolates was determined by measuring the diameter of each zone of inhibition around the discs. These values were compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Cheesbrough, 2018).

RESULTS

The colony count data (Table 1) indicate that the male ward had the highest average number of colonies, with a mean of 725.25 CFU/plate. Within the male ward, forceps exhibited the highest colony count at 211 CFU/plate, followed by beddings at 147 CFU/plate. Door handles had the lowest colony count, with 86 CFU/plate. The female ward recorded the second-highest average colony count at 487.67 CFU/plate. In this ward, forceps again had the highest colony count, at 164 CFU/plate, followed by floor surfaces with 98.5 CFU/plate and beddings with the lowest count at 80 CFU/plate. In contrast, the Accident and Emergency (A&E) unit had the lowest colony count, with colonies detected only on floor surfaces, averaging 48 CFU/plate. No colonies were observed on door handles, beddings, forceps, or scissors in the A&E unit.

A one-way ANOVA revealed a statistically significant difference in the mean colony counts across the three wards (A&E, Female Ward, and Male Ward) ($p < 0.05$), leading to the rejection of the null hypothesis. This indicates that at least one ward had a significantly different mean colony count.

The colonies of bacterial isolates on mannitol salt agar appeared round with cocci arranged in clusters and exhibited yellow pigmentation, which is characteristic of *Staphylococcus aureus*. In contrast, bacterial colonies on cetrimide agar were glossy, circular, grape-like, and displayed blue-green pigmentation, which is characteristic of *Pseudomonas aeruginosa* (Table 2).

Table 3 shows that the Male ward had the highest number of positive bacterial plates, 34 (58.61%), followed by the female ward with 18 (31.03%) positive plates, and A & E with the least

number of 6 (10.35%) positive plates. Out of the 32 *S. aureus* isolates the Male ward had the highest number of 18 (31.03%) isolates, followed by the Female ward with 10 (14.24%) isolates and A & E with 4 (6.90%). Similarly, the table shows that out of the 26 *P. aeruginosa* isolates, the Male ward had the highest number of 16 (27.58%), followed by the Female ward and A & E ward, respectively.

The Chi-square test for independence shows there is no statistically significant association (p = 0.823) between the bacterial species (*S. aureus* vs. *P. aeruginosa*) and the hospital location. This suggests that the distribution of bacterial isolates across the wards is not significantly different across the wards is not significantly different.

The *S. aureus* isolates produced clumping in coagulase plasma and tested positive for catalase, citrate, and coagulase while testing negative for indole, motility, and oxidase. Conversely, the *P. aeruginosa* isolates tested positive for catalase, oxidase, and citrate utilization for growth, as indicated in Table 4.

Figure 1 shows the number of positive and negative bacterial plates obtained from the three locations in the hospital. The male ward had the highest number of positive plates (18), followed by the female ward with 10 plates, and the Accident and Emergency (A & E) unit with the fewest positive plates (4).

Table 5 showed that *P. aeruginosa* was intrinsically resistant to Amoxicillin in all wards, while *S. aureus* showed resistance in the A&E and female wards but was sensitive in the male ward. Gentamycin and Septrin were ineffective against both pathogens, displaying high levels of resistance. Augmentin and Sparfloxacin exhibited moderate intermediate resistance, indicating limited effectiveness. In contrast, Pefloxacin, Tarivid, Streptomycin, Chloramphenicol, and Ciprofloxacin demonstrated high efficacy against both pathogens, with large zones of inhibition indicating sensitivity.

Moreover, the study identified Pefloxacin, Tarivid, Streptomycin, Chloramphenicol, and Ciprofloxacin as the most effective antibiotics for treating infections caused by *S. aureus* and *P. aeruginosa* in this healthcare setting. Amoxicillin, Gentamycin, and Septrin were found to be poor treatment options due to resistance. These findings highlighted the importance of antimicrobial susceptibility testing in guiding the appropriate use of antibiotics and minimizing the risk of resistance in hospital environments (Weinstein and Lewis, 2020).

Table 1: Average Colony Count of the Swapped Samples and one-way ANOVA showing the differences between the

Fomites	Mean colony count (CFU/plates)			One-way ANOVA	
	A & E n (%)	Female ward n (%)	Male ward n (%)	*F*-test	*p*-value
Beddings	0 (0)	80 (16.40)	147(20.27)	17.09	0.0003
Door handles	0 (0)	87.67 (17.98)	86 (11.86)		
Floor surface	48 (100)	98.5 (20.20)	137.25 (18.92)		
Forceps	0 (0)	164 (33.63)	211 (29.09)		
Scissors	0 (0)	57.5 (11.79)	136 (18.75)		
Total	48 (100)	487.67 (100.00)	725.25 (100.00)		

Key: CFU: Colony Forming Unit, A&E = Accident and Emergency

Table 2: Phenotypic appearance of the isolated bacteria on different culture media

Media	Colony	Shape	Pigmentation	Suspected organism
Mannitol salt agar	Round	Coccus in clusters	Yellowish pigment	*S. aureus*
Cetrimide agar	Glossy in circular	Grape-like appearance	Blue-green pigment	*P. aeruginosa*
Nutrient agar	White, smooth, and round	Circular with convex elevation	Creamy (milk) pigment	*S. aureus*
Nutrient agar	Thin and spherical	Bacillus	Green with glossy pigment	*P. aeruginosa*

Table 3: Distribution of Bacterial Isolates among the three Hospital Locations and Chi-square test indicating the differences between bacterial species.

Organisms	Hospital locations			Chi-square Test	
	A & E no (%)	Female ward no (%)	Male ward no (%)	X^2	P-value
S. aureus	4(6.90)	10(17.24)	18(31.03)	0.39	0.823
P. aeruginosa	2(3.45)	8(13.79)	16(27.58)		
Total	6(10.35)	18(31.03)	34(58.61		

Keys: A & E = Accident and Emergency

Table 4 shows the results of the biochemical screening for the isolated bacteria.

Biochemical test	*S. aureus*	*P. aeruginosa*
Catalase	+	+
Citrate	+	+
Coagulase	+	-
Indole	-	-
Motility	Non-motile	Motile (unipolar)
Oxidase	-	+
Gram reactions	+	-

Keys: +: Positive reaction -: Negative reaction

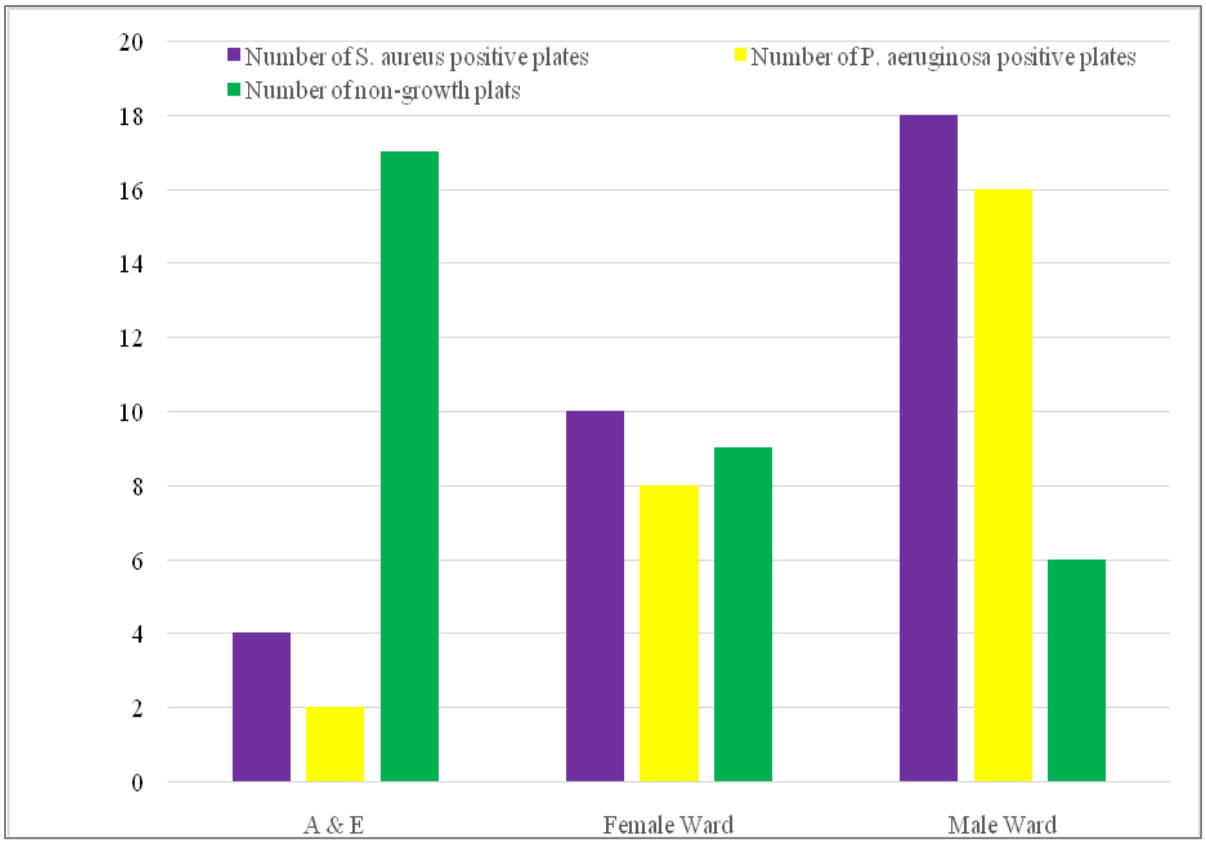

Figure 1: Number of positive bacterial plates from the three locations in the hospitals

Table 5: Antimicrobial Susceptibility Test for *S. Aureus* and *Pseudomonas aeruginosa* Isolated from Hospital Fomites.

ANT	*Staphylococcus aureus*						*Pseudomonas aeruginosa*					
	A & E unit (mm)		Female ward (mm)		Male ward (mm)		A & E unit (mm)		Female ward (mm)		Male ward (mm)	
	Dia	S, R, or I	Dia	S, R, or I	Dia	S, R, or I	Dia	S, R, or I	Dia	S, R, or I	Dia	S, R, or I
AMO	-	R	-	R	≥20	S	-	R	-	R	-	R
AUG	14-19	I	14-19	I	14-19	I	14-19	I	14-19	I	14-19	I
GEN	≤12	R	≤12	R	≤12	R	≤12	R	≤12	R	≤12	R
PEF	≥20	S	≥20	S	≥20	S	≥20	S	≥20	S	≥20	S
TAR	≥18	S	≥18	S	≥18	S	≥18	S	≥18	S	≥18	S
STR	≥15	S	≥15	S	≥15	S	≥15	S	≥15	S	≥15	S
SEP	≤10	R	≤10	R	≤10	R	≤10	R	≤10	R	≤10	R
CHL	≥18	S	≥18	S	≥18	S	≥18	S	≥18	S	≥18	S
SPA	14-17	I	14-17	I	14-17	I	14-17	I	14-17	I	14-17	I
CIP	≥21	S	≥21	S	≥21	S	≥21	S	≥21	S	≥21	S

Key: S = Sensitive; I = Intermediate; R = Resistance; Dia = diameter zone of inhibition; ANT = Antibiotics; AMO = Amoxicillin; AUG = Augmentin; GEN = Gentamycin; PEF = Pefloxacin; TAR = Tarivid; STR = Streptomycin; SEP = Septrin; CHL= Chloramphenicol; SPA = Sparfloxacin; CIP = Ciprofloxacin

DISCUSSION

This study identified *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the predominant bacterial pathogens isolated from fomites in the male and female wards, as well as the Accident & Emergency (A&E) unit at General Hospital Katsina. The presence of these pathogens on high-touch surfaces like forceps, beddings, and door handles suggests a significant risk of nosocomial infections, particularly under poor hygienic conditions.

The colony count analysis revealed that the male ward had the highest average colony count at 725.25 CFU/plate, with forceps being the most contaminated. This finding is consistent with other studies, such as Otter et al. (2016), which highlight that medical equipment and frequently touched surfaces often harbor high levels of contamination, particularly in wards with high patient turnover. The female ward followed with an average colony count of 487.67 CFU/plate, while the A&E unit had the lowest contamination levels, with colonies detected only on floor surfaces. The one-way ANOVA confirmed a statistically significant difference in colony counts across the wards ($p < 0.05$), reinforcing the need for ward-specific infection control strategies.

The identification of *S. aureus* and *P. aeruginosa* from these fomites aligns with findings from other studies conducted in similar healthcare settings. For example, Omololu-Aso *et al.* (2017) reported the widespread presence of *S. aureus* in hospital environments, highlighting its role as a major nosocomial pathogen. The phenotypic characteristics observed in this study, such as the yellow pigmentation and cocci arrangement for *S. aureus* and the blue-green pigmentation of *P. aeruginosa*, are consistent with established microbiological profiles of these bacteria.

The male ward not only had the highest colony count but also the greatest number of positive bacterial plates, with *S. aureus* and *P. aeruginosa* being the most prevalent. This distribution mirrors the findings of Omololu-Aso *et al.* (2017) and Nwankwo *et al.* (2014), who also observed *S. aureus* as a leading healthcare-associated pathogen, particularly in settings where hygiene practices might be compromised. In contrast, the A&E unit had the fewest positive bacterial plates, likely due to more stringent cleaning protocols and shorter patient stays, a factor also noted by Hodi *et al.* (2014).

The Chi-square test indicated no significant association between the type of bacteria and the hospital location ($p = 0.823$), suggesting a relatively uniform distribution of these pathogens across the wards. This finding, however, differs from the study by Odigie *et al.* (2017) in Abuja, which found higher contamination levels in female toilets compared to other areas. The current study's results suggest that bacterial contamination in the male ward of General Hospital Katsina may be more widespread, possibly due to differing hygiene practices or patient demographics.

The antimicrobial susceptibility testing revealed that *S. aureus* isolates were generally susceptible to antibiotics like Gentamycin, Zinacef, Ciprofloxacin, Streptomycin, Septrin, Erythromycin, and Pefloxacin but resistant to Amoxicillin and Rocephin. This pattern is

consistent with findings from Adegoke *et al.* (2015) and Nwankwo *et al.* (2014), who also reported high resistance to Amoxicillin among *S. aureus* isolates in Nigeria. The resistance to Amoxicillin, a commonly prescribed antibiotic, underscores the need for ongoing surveillance and proper antibiotic stewardship to prevent the emergence of more resistant strains.

In comparison, *P. aeruginosa* isolates showed significant resistance to Gentamycin and Septrin, a finding that resonates with studies by Odumosu *et al.* (2016) and Khan *et al.* (2014), who reported high levels of multidrug resistance in *P. aeruginosa* isolates from Nigerian hospitals. However, the sensitivity of *P. aeruginosa* to Pefloxacin, Tarivid, Streptomycin, Chloramphenicol, and Ciprofloxacin observed in this study is somewhat more optimistic than reports from Igbinosa *et al.* (2017), who noted increasing resistance to these drugs. This discrepancy could be attributed to local variations in antibiotic use, the specific strains of *P. aeruginosa* present, or differences in healthcare infrastructure.

The findings of this study align with much of the existing literature on nosocomial pathogens in hospital settings in Nigeria and other developing countries. For example, the high prevalence of *S. aureus* and *P. aeruginosa* is a common theme, reflecting the significant role these pathogens play in hospital-acquired infections (HAIs). Studies like those by Omololu-Aso *et al.* (2017) and Adegoke *et al.* (2015) provide further evidence of the pervasive nature of these bacteria in hospital environments.

However, there are notable differences in the resistance patterns observed in this study compared to others. While some studies, such as Ekwealor *et al.* (2016), report widespread resistance of *P. aeruginosa* to many antibiotics, the current study found that this pathogen remains susceptible to several key antibiotics, including Pefloxacin and Ciprofloxacin. This suggests that while antibiotic resistance is a growing problem, there may still be viable treatment options available in specific settings. These variations underscore the importance of local surveillance data in informing treatment guidelines and infection control policies, as highlighted by (Falagas *et al.*, 2014).

The findings from this study emphasize the need for targeted infection control measures and robust antibiotic stewardship programs in hospitals. The identification of high-touch surfaces like forceps and beddings as major sources of contamination highlights the need for stringent cleaning protocols and frequent monitoring.

However, the study has limitations. It was restricted to 90 swab samples from specific wards in a single hospital, which may not provide a comprehensive overview of the prevalence and resistance patterns of the pathogens across other settings. The sampling period, limited to July to October 2017, does not account for potential seasonal variations in bacterial contamination or resistance patterns. Furthermore, the reliance on phenotypic identification methods rather than molecular techniques, may have limited the precision in identifying bacterial strains and resistance mechanisms.

CONCLUSION

In conclusion, this study shows valuable insight into the patterns of bacterial contamination and antibiotic resistance in a healthcare setting, with specific focus on *S. aureus* and *P. aeruginosa*. The findings support the ongoing need for effective infection control and antibiotic stewardship, particularly in resource-limited settings like Nigeria. Future research should aim to include larger sample sizes, broader geographic coverage, and advanced molecular techniques to better understand the dynamics of hospital-acquired infections and resistance patterns. This will help to develop more effective strategies for mitigating the impact of these pathogens on patient health.

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