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Assessment of Bacterial Contamination on Surgical Instruments commonly used in Dutse General Hospital, Jigawa State, Nigeria

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Abstract
This study investigates the microbial hazards associated with contaminated surgical instruments. In the operating room, medical devices that come into contact with sterile body tissues or fluids are considered critical tools that must be properly sterilized. Failure to maintain sterility can result in microbial contamination, leading to disease transmission and a significant increase in surgical site infections (SSIs). This study aimed to assess the bacterial contamination of surgical instruments at

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General Hospital Dutse and evaluate the antibiotic susceptibility profiles of the isolated bacteria. A total of 20 samples were collected using sterile cotton swabs from surgical instruments both before and after procedures in the Surgery Department of General Hospital Dutse, Jigawa State. The samples were cultured on selective and differential media and incubated at 37°C for 24 hours. The isolates were subsequently identified using standard microbiological techniques, including bacterial count, microscopy, biochemical tests, and culture characterization. The identified microorganisms included Escherichia coli (17%), Staphylococcus aureus (33%), Pseudomonas aeruginosa (33%), Bacillus spp. (17%), and Klebsiella pneumoniae (14%), which were isolated from both autoclaved and non-autoclaved surgical instruments pre- and post-operation. Antibiotic susceptibility testing was performed using the Kirby-Bauer method. E. coli showed the highest sensitivity to meropenem (1.6 mm), while S. aureus was susceptible to gentamicin (5 mm) and novobiocin (10 mm). Bacillus spp. exhibited susceptibility to erythromycin (8 mm), and P. aeruginosa was sensitive to chloramphenicol (4 mm). These findings highlight that surgical instruments can serve as potential sources of nosocomial infections, with bacteria transferred through the hands of operating personnel or redistributed from contaminated surfaces during procedures. To prevent cross-contamination, it is essential to implement rigorous sterilization and decontamination protocols for surgical instruments. Advances in SSI control practices, including improved operating room ventilation, enhanced sterilization methods, optimized surgical techniques, and the use of antimicrobial prophylaxis, should be consistently employed to minimize infection risks.

INTRODUCTION

Sterile processing professionals recognize that surgical instruments are a critical frontline in infection prevention, as they frequently come into contact with body fluids and tissues that may introduce or transmit pathogens to patients (Holm & Dunn, 2022). Surgical site infections (SSIs) are among the most significant complications arising from operative procedures, occurring at the incision site or within tissues, organs, or cavities manipulated during surgery. These infections can develop up to 30 days post-surgery and have profound implications for patient outcomes (Marais, 2021; Mmed *et al*., 2023). The SSIs contribute to approximately 2% of surgical procedures and account for about 20% of healthcare-associated infections, making them a serious concern in modern healthcare (Tan & Sanchez, 2024). Despite advances in infection control measures, including aseptic techniques, environmental controls, and antimicrobial prophylaxis, SSIs remain the third most frequently reported type of healthcare-associated infection globally (Akbarzadeh & Maenhout, 2024). In many hospitals, they are ranked as the first or second most common site of infection, posing significant morbidity and mortality risks to surgical patients (Mbowella, 2024).

Microorganisms, which are pervasive on fomites and biological surfaces, can colonize surgical equipment, thereby serving as reservoirs for infections. Such colonization may include bacteria, prions, yeasts, fungi, protozoans, and viruses (Muteeb *et al*., 2023). Surgical theatres, specialized hospital spaces designed for operative procedures, house various equipment whose sterility is critical due to their contact with internal mucosal surfaces (Vippadapu *et al*., 2022; Herrera *et al*., 2024). Sterilization methods for these instruments include boiling, disinfectants, steam autoclaving, sterilizing gases, and chemical treatments, all designed to eliminate microbial contaminants (Gradisnik *et al*., 2024). Contaminated surgical equipment can result in severe postoperative complications and infections, collectively termed surgical site infections or surgically acquired infections (Alrawi *et al*., 2020; Bali, 2021). These infections have been a persistent challenge in clinical research, with common causative microorganisms including *Staphylococcus aureus* (methicillin-resistant and sensitive strains), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and multidrug-resistant organisms such as *Mycobacterium tuberculosis* (Tilahun, 2022).

Surgically acquired infections are a subset of nosocomial infections, also known as hospital-acquired infections, which manifest during hospital stays or after discharge (Al Rawas *et al.*, 2023). Microorganisms responsible for these infections can be isolated and identified using microbiological techniques such as spread plate, pour plate, streaking, and most probable number methods. Further identification can be achieved through selective media, biochemical assays, and molecular techniques (Jufri, 2020; Ogodo *et al.*, 2022; Tula *et al.*, 2023). This study aimed to assess the microbial contamination of surgical instruments and their potential role in SSIs, with a focus on identifying pathogens and evaluating their susceptibility to commonly used antibiotics.

MATERIALS AND METHODS

Area of study

The study was conducted at General Hospital Dutse, Jigawa State. The site was selected due to its high patient attendance, being located in the capital city of Jigawa State. It is one of the major referral centers in the state and receives patients from all regions of the state. Surgical operations are performed daily at the hospital. Dutse is situated in the northwest geopolitical zone of Nigeria, at latitude 11°42′04″N and longitude 9°02′31″E. As the capital of Jigawa State, it had an estimated population of 153,000 people in 2009. It is currently the largest city in Jigawa State, followed by Hadejia (111,000), Gumel (43,000), and Birnin Kudu (27,000) (Lawal *et al.*, 2018). The city is served by two general hospitals, several primary health centers, and a few private hospitals. The state's topography is generally characterized by undulating land with expansive sand dunes in some regions. In contrast, the area around Dutse is mainly rocky with some low hills. Like many areas of the state, high temperatures are typically recorded between April and September (Dogara & Ocheje, 2016).

Sample Collection

Samples were collected from surgical instruments in the operating theatre at Dutse General Hospital. A total of 20 samples were obtained using sterile cotton swab sticks. Of these, 10 samples were taken at the beginning of surgical procedures from instruments that had been autoclaved (sterilized). The remaining 10 samples were collected from the same set of instruments after the completion of operations, immediately before any sterilization process, to determine the level of contamination that may have occurred during the procedure. The surgical instruments examined included dissecting forceps, Kocher clamps, Metzenbaum scissors, bone nibblers, Raumplus retractors, Galipots, Deaver retractors, stereotactic systems, curved mosquito artery forceps, Langenberg retractors, Mayo scissors, and shoulder arthroscopes. The surfaces of the instruments and their components were swabbed using sterile swabs moistened with sterile normal saline. The swabs were immediately transported on ice to the Microbiology Laboratory at the Federal University Dutse, Jigawa State, Nigeria, for further analysis.

Sample Processing and Bacterial Culture

The tips of the swab sticks were rinsed in 10 mL of phosphate-buffered saline (PBS) to prepare the primary stock solution. Serial dilutions of the stock were then prepared at a 1:10 dilution factor. From each dilution, 1 mL was inoculated onto nutrient agar (NA) plates using pour plate method as described by Terrones-Fernandez *et al.*, (2023). From dilution factor of 10^{-3}, 1 mL was inoculated onto nutrient agar (NA). The plates were incubated at 37°C for 18–24 hours. After the incubation period, visible colonies were sub-cultured onto selective media, including Eosin Methylene Blue (EMB) agar, MacConkey agar, and Mannitol Salt Agar, to

isolate specific microorganisms. The sub-cultured plates were incubated at 37°C for an additional 18–24 hours to facilitate growth and allow for bacterial identification.

Identification of Bacterial Colonies
The identification of bacterial colonies was carried out through Gram's reaction and a series of biochemical tests, including the catalase test, coagulase test, urease test, and IMViC series.

Isolation of Bacteria
The bacteria were isolated using protocol outlined by Giuliano *et al*. (2019) to classify the isolates based on their gram reaction. Briefly, a bacterial smear was prepared on a clean glass slide and stained with crystal violet solution for 60 seconds. The slide was then rinsed with tap water and drained to prevent dilution of the mordant. It was subsequently flooded with iodine solution (mordant) for 60 seconds and washed again with tap water. Decolorization was performed using acetone, added dropwise until the free color was removed, followed by another rinse with tap water. The slide was then counterstained with neutral red for 30 seconds, washed, and allowed to air-dry. Finally, the stained smear was examined under an oil immersion objective lens to observe the cellular morphology.

Biochemical Tests
Biochemical characterization of the bacterial isolates was conducted following standard microbiological protocols as outlined by Giuliano *et al*. (2019) to confirm the bacterial isolate.

Coagulase Test
The coagulase test was conducted to detect the presence of coagulase enzyme in bacterial isolates (Parija, 2006). A clean glass slide was divided into two halves with a marking pencil. Two drops of sterile saline were placed on the slide. Using a sterile inoculating loop, a bacterial colony was emulsified in the saline on one half of the slide. A drop of undiluted plasma was added to the suspension and mixed with an applicator stick. The second half of the slide, containing only the bacterial suspension and saline, served as a control. The slide was gently rocked, and clumping of the bacterial suspension within 10–15 seconds was observed as a positive result. Further biochemical tests such as the catalase, urease, and Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) tests were performed to confirm and differentiate bacterial species.

Catalase Test
A single colony of each bacterial isolate was smeared onto a clean glass slide. The slide was then flooded with 1.0 mL of 3% hydrogen peroxide. The reaction was observed and recorded. Effervescence (bubbling) indicated a positive result, demonstrating the presence of the catalase enzyme (Giuliano *et al*., (2019)

Indole Test
The bacterial isolates were cultured in 5 mL of peptone water and incubated at 37°C for 24 hours. Following incubation, 3–8 drops of Kovac's reagent were added to the culture tube, which was gently tilted to mix. A positive reaction was identified by the formation of a red colour in the reagent layer within 10 minutes, while a negative reaction retained its yellow color (Giuliano *et al*., 2019).

Citrate Test
The test was performed by inoculating the bacterial isolates onto Simon's citrate agar slopes. The inoculated slopes were incubated at 37°C. A color change in the medium from green to

blue was considered a positive result, indicating the ability of the bacteria to utilize citrate as a sole carbon source (Giuliano *et al*., 2019).

Methyl-Red Voges-Proskauer (MR-VP) Test

Five milliliters (5 mL) of MR-VP broth were inoculated with the bacterial isolate and incubated at 37°C for 48 hours. After incubation, 1 mL of the broth was transferred into a separate test tube, and 2–3 drops of methyl red indicator were added. A red color indicated a positive test, while a yellow color denoted a negative test. To the remaining broth in the original tube, 5 drops of 40% potassium hydroxide (KOH) and 15 drops of 5% alpha-naphthol in ethanol were added. The tube was shaken, the cap loosened, and the tube placed in a sloping position. Development of a pink color, starting from the liquid-air interface within 1 hour, indicated a positive VP test. Absence of color change denoted a negative result (Giuliano *et al*., 2019).

Urease Test

Urea broth tubes were labeled and inoculated aseptically with the bacterial isolates using a sterilized inoculating loop. The cultures were incubated at 37°C for 24 hours. A positive urease test was indicated by the development of a deep pink color in the medium, showing urease enzyme activity (Giuliano *et al*., 2019).

RESULTS

Table 1 present the bacterial counts Gram reaction and morphology of isolates obtained from the surgical instruments, revealing varying levels of contamination. The highest bacterial load was observed on scissors with mean total viable count of 1.87×10^5 (CFU/mL), while the kidney dish exhibited the lowest bacterial load of 1.7×10^4 (CFU/mL). High number of bacterial load indicates varying degrees of microbial contamination across different instruments. Following stain, Both Gram-positive and Gram-negative bacteria were identified, *Staphylococcus spp* and *Bacillus* spp. representing the dominant Gram-positive cocci and rods, respectively, while *Escherichia coli* and *Pseudomonas spp* are the prevalent Gram-negative bacilli.

Table 1: Bacterial count, Gram Reaction and Morphology of Isolates

Samples	Total Viable Count (CFU/mL)	Gram Reaction	Microscopy	Shapes
S1	2.0×10^4	Gram positive	*Staphylococcus*	Cocci
S2	3.5×10^4	Gram positive	*Bacilli*	Rod
S3	2.3×10^4	Gram positive	*Bacilli*	Rod
S4	2.8×10^4	Gram positive	*Staphylococcus*	Cocci
S5	3.9×10^4	Gram negative	*Bacilli*	Rod
S6	1.7×10^4	Gram negative	*Bacilli*	Rod
S7	1.87×10^5	Gram positive	*Streptococcus*	Chain
S8	1.8×10^4	Gram positive	*Streptococcus*	Chain
S9	4.0×10^4	Gram negative	*Bacilli*	Rod
S10	7.2×10^4	Gram negative	*Bacilli*	Rod
S11	3.0×10^4	Gram positive	*Staphylococcus*	Cocci
S12	4.5×10^4	Gram positive	*Streptococcus*	Chain
S13	6.1×10^4	Gram positive	*Staphylococcus*	Cocci
S14	7.5×10^4	Gram negative	*Bacilli*	Rod
S15	3.8×10^4	Gram negative	*Bacilli*	Rod
S16	2.7×10^4	Gram positive	*Staphylococcus*	Cocci
S17	1.62×10^5	Gram positive	*Staphylococcus*	Cocci
S18	6.0×10^4	Gram positive	*Streptococcus*	Chain
S19	5.2×10^4	Gram negative	*Bacilli*	Rod
S20	3.7×10^4	Gram positive	*Staphylococcus*	Cocci

The results in Tables 2 highlight the bacterial isolates from autoclaved and non-autoclaved surgical instruments based on their Gram stain characteristics. Among autoclaved instruments, *Staphylococcus spp* and *Pseudomonas spp* had the highest prevalence (33%), while *Escherichia coli* and *Bacillus* spp. accounted for 17% each. For non-autoclaved instruments, *E. coli* and *Pseudomonas spp* showed the highest prevalence (29%), while *Staphylococcus spp*, *Klebsiella spp*, and Bacillus spp. accounted for 14% respectively.

Table 2: Gram Staining Characteristics of Bacterial Isolates

Source of Equipment	Biochemical Test							Suspected Organism	Occurrence	Prevalence (%)
	CG	CT	ID	MR	CR	VP	UR			
Autoclaved	-	+	+	+	-	-	-	*Escherichia coli*	1	17
	+	+	-	+	+	+	+	*Staphylococcus spp*	2	33
	-	+	-	-	+	-	-	*Pseudomonas spp*	2	33
	+	+	-	-	+	+	-	*Bacillus spp*	1	17
Non-Autoclaved	-	+	+	+	-	-	-	*Escherichia coli*	2	29
	+	+	-	+	+	+	+	*Staphylococcus spp*	1	14
		+	-	-	+	+	+	*Klebsiella spp*	1	14
	-	+	-	-	+	-	-	*Pseudomonas spp*	2	29
	+	+	-	-	+	+	-	*Bacillus spp*	1	14

Keys: CG – Coagulase, CT- Catalase, ID- Indole, MR- Methyle Red, CR= Citrate, VP- Voges proskaeur, UR- Urease, +/-: Positive/Negative Gram reaction

Table 3 summarizes the antibiotic susceptibility profiles of the bacterial isolates from surgical instruments. The results highlight that E. *coli* is susceptible to meropenem and chloramphenicol and resistant to nalidixic acid and sulfamethoxazole, indicating potential overuse or selective pressure by these antibiotics in the hospital. *Staphylococcus spp* exhibited susceptibility to vancomycin, gentamycin, novobiocin, and chloramphenicol while resistant to nalidixic acid and sulfamethoxazole, consistent with widespread antibiotic resistance patterns seen in nosocomial pathogens. *Pseudomonas spp* is susceptible to meropenem and chloramphenicol and resistant to gentamycin, demonstrating its well-known multidrug-resistant nature. *Bacillus* spp. are susceptible only to erythromycin, highlighting its distinct antibiotic sensitivity compared to other isolates. *Klebsiella spp* isolates were susceptible to meropenem and chloramphenicol and resistant to nalidixic acid and sulfamethoxazole, mirroring resistance patterns observed in *E. coli*.

Table 7: Antibiotic Susceptibility of Bacterial Isolates

Bacterial Isolate	Meropenem	Vancomycin	Gentamycin	Nalidixic Acid	Sulfamethoxazole	Novobiocin	Chloramphenicol	Erythromycin
Escherichia coli	S	NA	NA	Resistant	Resistant	NA	Susceptible	NA
Staphylococcus spp	NA	S	Susceptible	Resistant	Resistant	Susceptible	Susceptible	NA
Pseudomonas spp	S	NA	Resistant	NA	NA	NA	Susceptible	NA
Bacillus spp.	NA	NA	NA	NA	NA	NA	NA	Susceptible
Klebsiella spp	S	NA	NA	Resistant	Resistant	NA	Susceptible	NA

Keys: NA= Not applicable, S= Susceptible

Discussion

The study reported that surgical instruments in the operating theatre at Dutse General Hospital harbor significant bacterial contamination, some of which are known to cause nosocomial infections. This is in line with previous study that reported the presence of bacterial pathogens on surgical instruments and other inanimate objects in healthcare settings (Owusu *et al.,* 2022). The bacterial load observed ranged from 1.87 x 10^5 (CFU/mL) to 1.7 x 10^4

(CFU/mL) with scissors having the highest level of contamination and the kidney dish with the lowest contamination which is consistent with the findings of Oluwagbemiga *et al*. 2017). These findings indicated the critical role that contaminated surgical instruments play in the transmission of hospital-acquired infections (HAIs) which correspond to the findings reported by Aljamali, & Al Najim (2020). A diverse microbial population dominated by both Gram-positive and Gram-negative bacteria was reported. Among the Gram-positive isolates, *Staphylococcus spp* and *Bacillus* spp. were most prevalent, whereas *Escherichia coli*, *Pseudomonas spp*, and *Klebsiella spp* represented the major Gram-negative pathogens. This bacterial diversity is consistent with earlier research by Sabre *et al*. 2020) which highlights the pervasive nature of these microorganisms in clinical environments.

Autoclaved instruments were predominantly contaminated by *Staphylococcus spp* and *Pseudomonas spp*, while non-autoclaved instruments exhibited higher contamination by *E. coli* and *Pseudomonas spp* which is consistent with the findings of Saleem and Aslam (2017). These findings indicated the critical importance of proper sterilization in preventing cross-contamination and supports previous studies that highlight autoclaving as an essential infection control measure. The higher contamination by *Pseudomonas spp* and *Staphylococcus spp* highlight their prevalence and pathogenic potential in nosocomial infections as suggested by Sabat *et al.* (2021). The presence of *Pseudomonas spp* is particularly concerning due to its resistance to commonly used antibiotics and disinfectants. This adaptability makes it a significant threat in hospital environments, as noted in prior studies. Similarly, *E. coli* was frequently isolated and is a known contributor to HAIs, particularly in surgical settings (Oyedum *et al*., 2023). *E. coli* isolates were found to be susceptible to meropenem, while *Staphylococcus spp* displayed sensitivity to vancomycin, gentamycin, and novobiocin which is in line with the reports of Amsalu *et al.* (2024). The resistance of certain isolates to common antibiotics such as nalidixic acid and sulfamethoxazole highlights the growing concern of antibiotic resistance due to the overuse of these agents in hospital settings. These findings is consistent with previous reports that emphasize the increasing resistance of hospital pathogens to commonly used antibiotics (Muteeb *et al*., 2024).

Conclusion

This study highlights the high prevalence of bacterial contamination on surgical instruments at Dutse General Hospital, with *P. aeruginosa* and *S. aureus* being the most frequently isolated pathogens. The findings suggest the urgent need for improved sterilization practices and infection control measures to reduce the risk of postoperative infections. Continued vigilance and adherence to established protocols are essential in maintaining patient safety and preventing nosocomial infections. Antibiotics such as meropenem, gentamicin, and novobiocin were effective against specific isolates, indicating the potential for targeted therapies.

Based on this study, the following are recommended to mitigate the risk of nosocomial infections by implementing stringent infection control policies, including the use of aseptic techniques and immediate instrument sterilization after surgery. Enhance cleaning and sterilization protocol should be introduced through automated cleaning systems and providing training for healthcare personnel on sterilization procedures to minimize human error. Patient safety can be improved by regularly inspecting instruments before packaging to detect residual contaminants, to prevent cross-infection.

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