Analysis of microorganisms and antibiotic resistance in medical instruments in the central sterile supply department (CSSD) of hospitals before and after sterilization

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Abstract. Sterilization is essential in preventing the spread of pathogenic bacteria and antibiotic resistance in hospital settings. This study examines the microbial growth and antibiotic resistance of seven medical instruments from the Central Supply Sterile Department, before and after sterilization. The instruments analyzed include arterial clamps, anatomical forceps, tissue scissors, surgical forceps, nal puder, open small tray, and instrument tray. The methodology involved in vitro microbiological testing, including Gram staining, colony counting, and the Vitek 2 system for bacterial identification and antibiotic susceptibility testing (AST). Results showed that before sterilization, nal puder was contaminated with *Staphylococcus hominis* ssp *hominis* (Gram-positive) and *Bacillus sp.* (Gram-positive), while the instrument tray was contaminated with *Pseudomonas stutzeri* (Gram-negative). After sterilization and a 90-day storage period, *Staphylococcus hominis ssp hominis* was detected on the open small tray. AST revealed an increase in bacterial sensitivity to antibiotics, from 12 to 14 types post-sterilization. The minimum inhibitory concentration (MIC) for Benzylpenicillin decreased from 0.25 µg/mL (resistant) to ≤0.03 µg/mL (sensitive). This study highlights the critical role of sterilization in infection control and antibiotic resistance management in healthcare settings.

1 Introduction

Antimicrobial resistance (AMR) is a global health issue that adversely impacts the quality of healthcare services [1]. Nearly 60% of microorganisms isolated from infected surgical wounds show antimicrobial resistance patterns in the surgical site [2]. The incidence of these infections has been reported in 20% to 31% of all hospital-acquired infections (HAIs) among

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inpatient patients indicating antimicrobial resistance [3]. According to a study by Bashaw & Keister (2019), bacteria commonly associated with surgical site infections include *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Enterococcus* spp., and *Escherichia coli*. These pathogens can spread through both direct and indirect contact [3]. *Staphylococcus* species are a major cause of infection on medical instruments due to their lack of self-cleaning capacity, which makes medical instruments vulnerable to contamination during surgery and daily use [4] [5].

Decontamination activities are carried out in the Central Sterile Supply Department (CSSD) [6], which plays a critical role in preventing and controlling infections across healthcare facilities Sterilization is the most common method used for decontaminating medical instruments [7]. The goal of sterilization is to eliminate all potential infectious contaminants on equipment and instruments, ensuring patient safety and optimal healthcare [8][9]. Medical instruments used in surgical procedures are categorized as critical instruments[10], which require sterilization to minimize the risk of infection transmission to patients [11]. However, the decontamination efforts applied in hospitals are often not fully effective in preventing bacterial contamination on medical instruments. According to research by Rubak et al. (2022), once biofilm forms on the surface of instruments, particularly if left to dry, it becomes extremely difficult to remove using standard cleaning methods, as biofilm has a strong adhesive property [4]. Therefore, preventing biofilm formation is more effective when performed at the point of use, i.e., while the instrument is still wet, by applying proper procedures in accordance with operational standards [12]. Meanwhile, controlling invasive pathogens that may contaminate medical instruments, patients, and the hospital environment remains a challenge that needs to be addressed in a more systematic manner [13].

The primary objective of this study is to analyze the growth and resistance patterns of microorganisms on medical instruments that have undergone the sterilization process, with the hypothesis that resistant microbes can survive despite sterilization. This research aims to provide a deeper analysis and understanding of these resistance patterns, which is expected to contribute significantly to infection prevention efforts and the improvement of sterilization procedures in the CSSD. The main focus of this study is on the interaction between microorganisms and the surface of medical instruments, where biofilm formation plays a key role in reducing antibiotic effectiveness [14] and facilitating the development of systemic infections, such as bacteremia [15]. Therefore, a deeper understanding of microorganisms and their resistance patterns on medical instruments is essential to formulate more effective infection prevention strategie.

2 Methods

This study was approved by the Ethics Committee of the Faculty of Medicine, Sebelas Maret University Surakarta, under the number: 207/UN27.06.11/KEP/EC/2023, and protocol ID: 192/02/08/2023. The research was conducted in vitro in a clinical microbiology laboratory, with samples collected from the Central Sterile Supply Department (CSSD), consisting of 7 medical instruments: arterial clamp, anatomical forceps, tissue scissors, surgical forceps, nal puder, open small tray, and instrument tray. These instruments include hemostatic forceps, tissue forceps, tissue scissors, dressing forceps, needle holder, bowl, and container. Sample selection was based on the Spaulding classification criteria for critical instruments, which are those in contact with body tissues. Samples were taken both before and after sterilization in the CSSD, with proper documentation of the instrument's retrieval and return according to procedures. The transportation of instruments to the laboratory was done using a sterilized container to prevent cross-contamination.

The in vitro tests used septic and aseptic techniques, conducted in a biosafety cabinet with biosafety level 2 conditions. The microbiological testing aimed to identify bacterial species present on the medical instrument samples. The tools used in this study included matches, petri dishes, spirit lamps, and sterilized container boxes. The process also involved using sterile swabs, aquabidest, and blood agar (BA) media. The samples were incubated for 24 hours at 37°C to allow bacterial growth. Gram staining was performed using glass slides, staining racks, and reagents such as crystal violet, Lugol's iodine, acetone, and safranin, followed by washing with water. Colony counting was carried out in a biosafety cabinet (BSC) to prevent cross-contamination, using blood agar media incubated for 24 hours.
Bacterial identification was performed using the VITEK 2 system, an automated growth-based technology that provides rapid and accurate information on bacterial sensitivity intermediate resistance, and resistance. The system uses computer equipment, VITEK test tubes, ID cards, AST cards, McFarland turbidity standards, micropipettes, and a DensiChek to measure bacterial density.

The research procedure began by soaking the medical instruments in sterile liquid for 24 hours at 37°C. A swab was then taken from the instrument surfaces and inoculated onto blood agar media. After incubation, colonies were counted. Gram staining was performed by covering the smear with crystal violet for one minute, followed by treatment with Lugol's iodine, acetone, and safranin. The results were observed under a microscope. A single bacterial colony was selected for identification and resistance testing by mixing it with a 0.45% NaCl solution (pH 5.0). Turbidity measurements were taken using a DensiChek.
All data on colony counts, species identification, and antibiotic sensitivity must be recorded and analyzed. The results will be used to evaluate the effectiveness of the sterilization process in the CSSD and to plan better infection prevention strategies.

3 Result

In an in vitro study conducted in a clinical microbiology laboratory, samples were collected from the Central Sterile Supply Department (CSSD) consisting of 7 medical instruments: arterial clamp, anatomical forceps, tissue scissors, surgical forceps, nal puder, open small tray, and instrument tray. The results revealed the presence of *Staphylococcus sp* (Gram-positive cocci arranged in clusters, violet color) on the nal puder instrument, which was identified as *Staphylococcus hominis ssp hominis* (Gram-positive) with 99% probability. Additionally, *Bacillus sp* (Gram-positive bacilli arranged in clusters, violet color) was also detected, but the AST test was unable to identify the species. The instrument tray showed the presence of a Gram-negative bacterium, identified *as Pseudomonas stutzeri* (bacilli, pink color) with 99% probability.

The identification results using the VITEK 2 system showed that *Pseudomonas stutzeri* was identified with 99% probability. This bacterium is Gram-negative, aerobic, and commonly found in water and soil. In immunocompromised individuals, ***Pseudomonas stutzeri*** can enter the body and cause infections in surgical wounds, blood, urine, and the respiratory tract. According to *Table 1*, *Pseudomonas stutzeri* is sensitive to 11 types of antibiotics, indicating that this microbe is highly responsive to certain antibiotics and shows good inhibition effects against these agents.

The identification results using the VITEK 2 system showed that *Staphylococcus hominis ssp hominis* was identified with 99% probability. The VITEK 2 test indicated an intermediate (I) interpretation for erythromycin, and resistance to benzylpenicillin, clindamycin, and tetracycline. In cases of central line-associated bloodstream infections (CLABSI), 20% of Gram-positive bacterial infections showed multi-drug resistance, with a 75% increase in growth patterns and antibiotic resistance.

The antibiotic resistance testing (AST) on medical instruments after sterilization, using the VITEK 2 system, showed that *Staphylococcus hominis ssp hominis* was identified on the open small tray with 97% probability. The VITEK 2 test results for *Staphylococcus hominis ssp hominis* on medical instruments before and after sterilization indicated an intermediate interpretation, where there was a shift from sensitive to resistant, but not fully resistant. Resistance is defined as the condition where *Staphylococcus hominis ssp hominis* is not killed by the antibiotic. The development of antimicrobial resistance occurs due to selection pressure from the use of antibiotics, which is linked to the spread of resistant microbes. Bacteria within the *Staphylococcus* genus, which cause hospital infections, show that biofilm formation is a virulence factor in coagulase-negative staphylococci.

Table 1. Microscopic Characteristics of Bacteria on Medical Instruments Before Sterilization

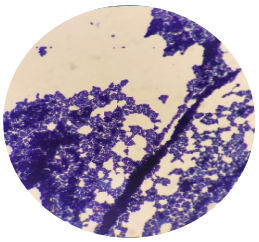
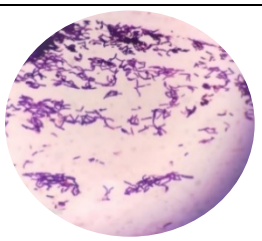
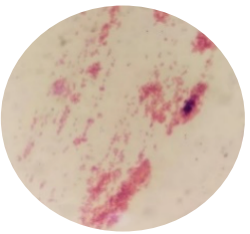
No	Medical Instruments	Bacteria	Image
1	Nal puder	*Staphylococcus sp.*: Gram-positive, cocci arranged in clusters, violet color *Identified as Staphylococcus hominis ssp hominis* (Gram-positive) with 99% probability	
2	Nal puder	*Bacillus sp*: Gram-positive, bacilli arranged in clusters, violet color AST test not performed or Unidentified	
3	Instrument trays	Gram-negative bacteria**:** Bacilli, pink color *Identified as Pseudomonas stutzeri* (Gram-negative) with 99% probability	

Table 2. Microscopic Characteristics of Bacteria on Medical Instruments After Sterilization (Stored for 90 Days in the CSSD)

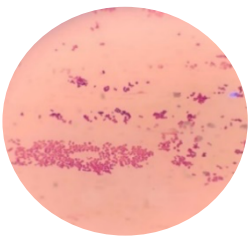
No	Medical Instruments	Bacteria	Image
1	Open Small Tray	*Staphylococcus sp* Gram-positive, cocci arranged in clusters, violet color *Identified as Staphylococcus hominis ssp hominis* with 97% probability	

Table 3. AST Results Using the VITEK 2 System on Medical Instruments *Pseudomonas stutzery*

No	Antimicrobial	MIC	Interpretation
1	Piperacillin	<= 4 µg/mL	Sensitive
2	Ceftazidime	<=1 µg/mL	Sensitive
3	Cefriaxone	<=1 µg/mL	Sensitive
4	Cefepime	<=1 µg/mL	Sensitive
5	Aztrenonam	<=1 µg/mL	Sensitive
6	Meropenem	<=0,25 µg/mL	Sensitive
7	Amicacin	4 µg/mL	Sensitive
8	Gentamycin	<=1 µg/mL	Sensitive
9	Ciprofloxacin	<=0,25 µg/mL	Sensitive
10	Tigecycline	<=0,5 µg/mL	Sensitive
11	Trimethoprim//Sulfamethoxole	<=20 µg/mL	Sensitive

MIC: Minimum Inhibitory Concentration

Table 4. AST Results Using the VITEK 2 System on Instruments Before Sterilization Nal Puder: Identified Bacteria Coccus Gram (+) *Staphylococcus hominis ssp hominis*

No	Antimicrobial	MIC	Interpretation
1	Benzylpenicilin	0,25 µg/mL	*Resistant*
2	Oxacillin	<=0,25 µg/mL	Sensitive
3	Gentamicin	<=0,5 µg/mL	Sensitive
4	Ciprofloxacin	<=0,5 µg/mL	Sensitive
5	Levofloxacin	*<=0,12* µg/mL	Sensitive
6	Moxifloxacin	<=0,25 µg/mL	Sensitive
7	Erytrhromycin	<=0,25* µg/mL	*Intermediate*
8	Clindamycin	>=8 µg/mL	Sensitive
9	Quinupristin/Dalfopristin	1 µg/mL	Sensitive
10	Linezolid	2 µg/mL	Sensitive
11	Vancomycin	<=0,5 µg/mL	Sensitive
12	Tetracyclin	>=16 µg/mL	*Resisten*
13	Tigecycline	<=0,12 µg/mL	Sensitive
14	Nitrofurantoin	<=16 µg/mL	Sensitive
15	Rifampicin	<=0,5 µg/mL	Sensitive
16	Trimethoprim/Sulfamethozole	<=10 µg/mL	Sensitive

MIC: Minimum Inhibitory Concentration

Table 5. AST Results Using the VITEK 2 System on Instruments After Sterilization Open Small Tray: Bacterial Species Identified Coccus Gram (+) Staphylococcus hominis ssp hominis

No	Antimicrobial	MIC	Interpretation
1	Benzylpenicillin	< 0,03 µg/mL	Sensitive
2	Oxacillin	<=0,25 µg/mL	Sensitive
3	Gentamycin	<=0,5 µg/mL	Sensitive
4	Ciprofloxacin	<=0,5 µg/mL	Sensitive
5	Levofloxacin	<=12 µg/mL	Sensitive
6	Moxifloxacin	<0,25 µg/mL	Sensitive
7	*Erytromycin*	*>=8* µg/mL	*Resistant*
8	*Clindamycin*	*>=8* µg/mL	*Resistant*
9	Quinupristin/Dalfopristin	<=0,25 µg/mL	Sensitive
10	Vancomycin	<=0,5 µg/mL	Sensitive
11	Tetracycline	<=1 µg/mL	Sensitive
12	Tigecycline	<=0,12 µg/mL	Sensitive
13	Nitrofurantoin	<=16 µg/mL	Sensitive
14	Rifampicin	<=0,5 µg/mL	Sensitive
15	Trimethoprim/Sulfamtetazole	<=10 µg/mL	Sensitive

MIC: Minimum Inhibitory Concentration

4 Discussion

This study aims to identify and analyze antibiotic resistance patterns in microorganisms found on medical instruments after sterilization. Two types of bacteria identified in this study were *Pseudomonas stutzeri* [16] and *Staphylococcus hominis* subsp. *hominis*. The identification results using the Vitek 2 system [17] showed a high probability of identification (99%) for both bacterial species. These findings provide a clear picture of the types of microorganisms that could potentially cause infections in hospital environments [18], especially in patients with immunocompromised status [15][19]. The accuracy of microorganism identification performed using the Vitek 2 system [20] is noteworthy, as it can provide highly probable identification (99%) for both bacteria tested. This system not only enables rapid and precise identification but also provides useful data regarding the antibiotic sensitivity of each microorganism [21][22].

For *Pseudomonas stutzeri*, antibiotic sensitivity testing indicated that this bacterium was sensitive to 11 different antibiotics, suggesting that it can be effectively controlled with several antibiotic treatment options [23]. This is significant because it provides multiple therapeutic choices for managing infections caused by *Pseudomonas stutzeri*.

In contrast, *Staphylococcus hominis ssp hominis* exhibited resistance to several antibiotics, including benzylpenicillin, clindamycin, and tetracycline. However, sensitivity testing revealed an intermediate interpretation for erythromycin. This suggests that, despite resistance, treatment with certain antibiotics may still be possible [24], although with special considerations. This finding underscores the importance of performing antibiotic sensitivity testing before selecting therapy, particularly in cases of healthcare-associated infections in hospitals.

The study also found a shift in antibiotic sensitivity patterns in *Staphylococcus hominis ssp hominis* on medical instruments after sterilization. Based on testing on medical instruments, there was a change in interpretation from sensitive to intermediate or even resistant. This indicates that, although the instruments underwent sterilization and were stored for 90 days, resistant microorganisms can still be found, highlighting the importance of strict adherence to sterilization procedures in hospitals [25][26].

This study also has several limitations that should be considered. One limitation is the scope of the antibiotic testing. While *Pseudomonas stutzeri* [27] was found to be sensitive to 11 antibiotics, this study did not include all antibiotics that might be relevant for clinical therapy [28]. Therefore, further research including a broader range of antibiotics would provide a more comprehensive understanding of the resistance patterns of this microorganism. Additionally, the study did not specifically identify the molecular or genetic mechanisms underlying antibiotic resistance in both bacteria. While there is an indication that resistance in *Staphylococcus hominis* may be due to selective pressure from improper antibiotic use [29], the exact genetic or enzymatic factors contributing to resistance have not been elucidated. Future research should investigate the molecular mechanisms or related genes involved in resistance.

Another limitation that should be noted is the influence of biofilm formation [30], which was not fully explored in this study. Since biofilm is an important virulence factor for *Staphylococcus hominis* [31] and serves to protect microorganisms from the effects of antibiotics [32], further studies are needed to evaluate the role of biofilm on medical instrument surfaces. Biofilm formation could explain why this bacterium persists even after instruments have been sterilized, opening up opportunities for developing strategies to prevent biofilm formation on medical instruments.

The findings of this study have important implications for infection control practices in hospitals. The resistance of *Staphylococcus hominis* and *Pseudomonas stutzeri* to several antibiotics highlights the importance of implementing prudent antibiotic use policies (antibiotic stewardship) [1] in hospitals. Proper and sensitivity-based antibiotic use can prevent the further development of microbial resistance [33]. The study also demonstrates that, despite sterilization of medical instruments, contamination with resistant microorganisms can still occur. Therefore, hospitals need to assess their current sterilization procedures. It may be necessary to enhance sterilization protocols or use antimicrobial materials that can prevent biofilm formation on the surfaces of medical instruments. Additionally, medical instruments should be stored in ways that prevent recontamination after sterilization.

5 Conclusion

This study provides important insights into the antibiotic resistance patterns of *Pseudomonas stutzeri* and *Staphylococcus hominis ssp hominis* on medical instruments after sterilization. Although both bacteria showed sensitivity to several antibiotics, resistance to others, as well as shifts in sensitivity on medical instruments, highlight the urgent need for enhanced infection control measures in hospitals. These findings emphasize the critical importance of continuous monitoring of antibiotic resistance and the effectiveness of sterilization procedures to prevent the spread of resistant pathogens. As resistance to certain antibiotics in these bacteria could lead to increased rates of hospital-acquired infections, it is vital to address these concerns to safeguard patient safety and reduce healthcare-associated risks. Future research should further investigate the mechanisms underlying antibiotic resistance in these strains, particularly with regard to biofilm formation on medical instruments, and explore more effective sterilization techniques or combinations of antibiotics. Collaborative efforts between clinical microbiologists and infection control specialists will be essential in developing strategies to combat emerging resistance patterns and improve patient outcomes.

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