IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 4 Ser. 4 (April. 2025), PP 18-23
www.iosrjournals.org

An Invitro Comparative Antimicrobial Analysis Of Povidone Iodine, Hydrogen Peroxide And Antibiotics Against Surgical Site Infection Causing Pathogens.

Arunavarsini K, Gayathiri Govindharaju, Mythreyan B, Vikraman Kalidoss And Mahenthiran R

Phd Research Scholar, Department Of Microbiology, Dr. N.G.P Arts And Science College, Coimbatore, India
Clinical Pharmacist, Doctor Of Pharmacy. Sri Ramakrishna College Of Pharmacy, Coimbatore, India.
UG Student, Department Of Microbiology, Dr. N.G.P Arts And Science College, Coimbatore, India
Assistant Professor, Department Of Microbiology, Dr. N.G.P Arts And Science College, Coimbatore, India

Abstract:
Background: *Surgical site infections (SSIs) represent a significant challenge in clinical settings, being among the most prevalent healthcare-associated infections. These infections not only exacerbate patient morbidity but also increase the duration of hospital stays and overall healthcare costs. Understanding the microbial landscape and efficacy of antiseptic agents in clinical settings for improving surgical outcomes.*
Material and methods: *The study isolates and characterizes bacterial strains associated with surgical site infections, specifically,* Escherichia coli, Staphylococcus aureus, *and* Aeromonas sp*. These strains were sourced from Kovai Medical Center and Hospital in Coimbatore. Antimicrobial susceptibility was evaluated using the disc diffusion method, testing hydrogen peroxide, povidone iodine, and antibiotics, with the zone of inhibition measured to assess their effectiveness.*
Results: The study showed that hydrogen peroxide was the most effective at combating the tested pathogens, especially against Aeromonas sp., and also worked well against Staphylococcus aureus and Escherichia coli. *Povidone iodine demonstrated moderate efficacy, particularly against* Staphylococcus aureus, *while antibiotics like vancomycin and ciprofloxacin illustrated variable effectiveness based on bacterial strains.*
Conclusion: *The study highlights the critical role of selecting appropriate antimicrobial agents in preventing SSIs. Hydrogen peroxide emerged as a powerful antiseptic, while povidone iodine remained valuable for its broad-spectrum activity. The results advocate for the implementation of targeted antisepsis protocols in surgical practices to enhance patient safety and minimize infection rates while emphasizing the need for ongoing research to combat antibiotic resistance.*
Keywords: *Surgical site infections, antimicrobial agents, antibiotics, antimicrobial susceptibility and healthcare- associated infections.*

Date of Submission: 13-04-2025 Date of Acceptance: 23-04-2025

I. Introduction

Surgical site infection (SSI) is defined as an infection that occurs at or near the surgical incision site within 30 days of the procedure or within one year if an implant is placed. SSIs are among the most common healthcare-associated infections (HAIs) and can lead to significant morbidity, prolonged hospital stays, increased healthcare costs, and even mortality. The pathophysiology of SSIs involves a complex interplay of factors, including the patient's underlying health status and the nature of the surgical procedure. Microbial contamination can occur after or before surgery[1].

Surgical site infection (SSI) is a significant and common complication that occurs following surgical procedures, characterized by the infection of the surgical wound. Defined by the Centers for Disease Control and Prevention (CDC), surgical site infection can be classified into three categories: 1. superficial, 2. deep, and 3. organ or body cavity infections[2]. Superficial SSIs develop within 30 days post-surgery and involve the skin and subcutaneous tissue, while deep SSIs occur after 30 days or within one year if a foreign body is implanted, affecting the fascia and muscles. Organ or body cavity infections arise in close proximity to the surgical site within the same time frames. The incidence of SSIs is estimated to range from 2% to 11% across various surgical interventions, leading to increased risks in patients hospital stays[3].

The microbiology of surgical site infection is characterized by a diverse array of microorganisms that can be classified as either endogenous or exogenous. Endogenous flora, which originates from the patient's own skin membrane, is responsible for the majority of SSIs. When the skin or mucosal barriers are breached during the surgery, these microorganisms can contaminate the surgical site, leading to infection.

The risk of infection increases significantly when the surgical site is contaminated with a high bacterial load, typically exceeding 10ˆ5 microorganisms per gram of tissue, or even lower inoculum levels when foreign materials such as implants are present[4].

Studies indicate that approximately 70-95% of SSIs are caused by patients own microbiome, with common pathogens including *Staphylococcus aureus*, coagulase-negative staphylococci, and *Escherichia coli*[5].

Staphylococcus aureus is a major contributor to the incidence of surgical site infections. It possesses various virulence factors that contribute to its ability to cause infections. These include the production of toxins that can damage tissues and evade the host's immune responses. Its ability to form biofilms can also complicate treatment, particularly on implanted devices during surgeries. During surgical procedures, Staphylococcus aureus can be transmitted from the skin or nasal passages of carriers to the surgical site. This transmission can occur via direct contact by healthcare personnel or through contaminated instruments[6].

Escherichia coli is commonly recognized as both a commensal inhabitant of the human gastrointestinal tract and a significant pathogen associated with various infections, including surgical site infections. *E. coli* has been identified as one of the predominant pathogens responsible for SSIs, particularly in cases involving prolonged hospitalization or invasive procedures. Studies indicate that *E. coli* accounts for a notable percentage of SSIs, with its presence being linked to factors such as patient immunocompromisation, surgical techniques, and antibiotic usage patterns[7].

In the study, *E. coli* was identified as the most common organism isolated from surgical wounds, accounting for approximately 30.23% of infections in elective surgeries. *E. coli* is often linked to certain risk factors that contribute to the development of SSIs. These include the nature of surgical procedures (those involving entry into the gastrointestinal tract are particularly at risk), the presence of contaminated wounds that can impair healing and immune response[8].

Aeromonas species are increasingly recognized as pathogens associated with surgical site infections, particularly following procedures involving exposure to water or contaminated environments. These bacteria are commonly found in aquatic habitats and can enter the body through penetrating wounds, especially in healthy individuals who may not have underlying health conditions. The mechanism of action of *Aeromonas* ., in surgical site infections primarily involves their ability to adhere to host tissues, evade the immune response, and produce a range of virulence factors. Upon entering the body through contaminated wounds, Aeromonas can adhere to epithelial cells using specific surface proteins, facilitating colonization on the open wounds[9].

Antibiotics have long been regarded as critical agents in the treatment and management of bacterial infections, including those arising from wounds. Antibiotics function by targeting specific processes within bacterial cells, such as cell wall synthesis, protein synthesis, DNA replication, or metabolic pathways. This mechanism of action effectively inhibits bacterial growth or leads to bacterial cell death, aiding in the control of infections that may arise from contaminated wounds. SSI can lead to severe complications or systemic infections such as sepsis. Antibiotics can significantly reduce the risk of these complications by controlling bacterial proliferation, thereby aiding in faster healing of wounds and enhancing patient recovery[10].

One of the well-recognized functions of hydrogen peroxide in wound management is its antimicrobial capacity. Hydrogen peroxide generates reactive oxygen species (ROS) that effectively kill a wide range of pathogens, thereby reducing the risk of infection in wounds. Hydrogen peroxide also acts as a second messenger in various signaling pathways involved in cellular responses to injury. Upon a cutaneous injury, the concentration of hydrogen peroxide increases in surrounding tissues, which modulates signaling pathways related to inflammation and tissue repair[11].

Inflammation is a critical phase of wound healing, and hydrogen peroxide plays a dual role in this process. While it can promote oxidative stress, excessive levels of hydrogen peroxide are associated with chronic inflammation, leading to impaired healing[12].

Povidone iodine has been extensively studied as an antiseptic agent in the prevention of surgical site infections across various surgical settings. The accumulation of evidence highlights its efficacy and safety at multiple stages of surgical interventions, including preoperative skin preparation, intraoperative irrigation, and postoperative wound management[13].

According to WHO, povidone iodine is widely recognized for its role in preoperative antisepsis. It is one of the most commonly used antiseptics for surgical site preparation and has been associated with lower rates of SSIs compared to other agents[14].

Povidone-iodine, used during surgery, reduces surgical site infection incidence. Its broad-spectrum antimicrobial activity lowers infection risk and enhances wound healing in postoperative care, playing a dual role in prevention and recovery[15].

Antibiotics are pivotal in preventing surgical site infections, which can significantly hinder recovery and increase healthcare costs. The use of topical antibodies directly on surgical wounds healing by primary intention has been shown to reduce infection rates compared to no treatment or antiseptics, with studies indicating a reduction in surgical site infection rates. Systemic antibiotics are also crucial, especially for high-risk patients when administered before surgery. However, judicious use is essential to combat antibiotic resistance. Overall, antibiotics are key in enhancing patient outcomes and reducing the incidence of surgical site infections[16].

In the preliminary study, the clinical pathogens causing surgical site infection are collected from the hospital. Later, the in vitro antibacterial study was performed, and comparatively, the findings have shown the potential of antibiotics, hydrogen peroxide, and povidone-iodine against surgical site infections.

II. Material And Methods

Bacterial strains and maintenance: The surgical site infection-causing bacteria, strain 1 Escherichia coli, strain 2 Staphylococcus aureus, and strain 3 Aeromonas sp., were obtained from Kovai Medical Centre and Hospital (KMCH), Coimbatore. The strains were grown and maintained on nutrient broth and nutrient agar plates for further use.

Test sample collection: Commercially available Povidone iodine was purchased from Apollo Pharmacy, Coimbatore. Nice hydrogen peroxide solution and Himedia antibiotic discs were used in the current study.

Chemicals used: Himedia Nutrient Agar, Himedia Nutrient Broth, and Himedia Muller Hinton Agar .

Phenotypic characterization: The clinically obtained bacterial isolates undergone Gram's staining for Gram reaction by microscopic observation at 100x and also for culture characteristics. For further confirmation of bacterial isolates, IMViC tests and catalase and oxidase tests were performed for biochemical characterization according to Bergey's Manual[17].

Antimicrobial Activity (Disc Diffusion Method): The antibacterial activity was evaluated using the agar disc diffusion method. Muller Hinton agar was prepared and poured into a sterile plate. A 24-hour-old bacterial culture was taken and moistened using a sterile swab and applied to the sterile MHA plates. Sterile discs with a 6 mm diameter were loaded with 50 µl of povidone iodine and hydrogen peroxide along with the standard antibiotic disc and incubated at 37℃ for 24 hours, and the zone of inhibition was measured[18].

III. Results

Phenotypic characterization: All three clinically obtained strains were reconfirmed by performing phenotypic characterization, i.e., microscopic observation, IMViC, catalase, and oxidase for biochemical characterization **(Table no: 1)**. Microscopic observation was performed for strain 1. It is observed as gram-negative rods at a magnification of 100x. The biochemical characterization revealed that the strain tested positive for indole and methyl red and negative for Voges-Proskauer and citrate. Furthermore, strain 1 is found to be catalase positive and oxidase negative. Hence, strain 1 is confirmed as *Escherichia coli.*

Strain 2 was identified microscopically as gram-positive cocci appearing in clusters, which indicates its staphylococcal arrangement. In biochemical characterization, the strain tests negative for indole and positive for methyl red, the Voges-Proskauer test, and citrate. Also tested positive for oxidase and negative for catalase. By the following test, strain 2 is identified as *Staphylococcus aureus.*

Microscopic observation tested positive for indole and methyl red, negative for the Voges-Proskauer, and exhibited positive results for citrate, catalase, and oxidase. So the strain-3 is confirmed to be *Aeromonas sp*.

S. No	Types of tests performed	List of clinical isolates used		
		Escherichia coli	*Staphylococcus aureus*	*Aeromonas sp.,*
1	Microscopic observation	Rods	Cocci	Rods
2	Grams stain	Gram negative	Gram positive	Gram negative
3	Indole	+ve	-ve	+ve
4	Methyl Red	+ve	+ve	+ve
5	Voges proskauer	-ve	+ve	-ve
6	Citrate	-ve	+ve	+ve
7	Catalase	+ve	+ve	+ve
8	Oxidase	-ve	-ve	+ve

Table no: 1 Morphological and Biochemical characterization of Strain 1, Strain 2 and Strain 3.

Antimicrobial activity: The zone of inhibition for all three isolates was performed using the disc diffusion method and measured **(Table no: 2*)***. *Escherichia coli* shows a diameter of 18mm for hydrogen peroxide, 11mm for povidone iodine, and 16mm for the penicillin antibiotic, which shows **(Figure no: 1)** lower sensitivity to povidone iodine when the hydrogen peroxide shows the strongest inhibitory effect.

S.No	Sample	Zone of inhibition (mm)
1	Hydrogen peroxide	18
2	Povidone iodine	11
3	Penicillin (Antibiotic)	16

Table no: 2 Determination of zone of inhibition against *Escherichia coli*

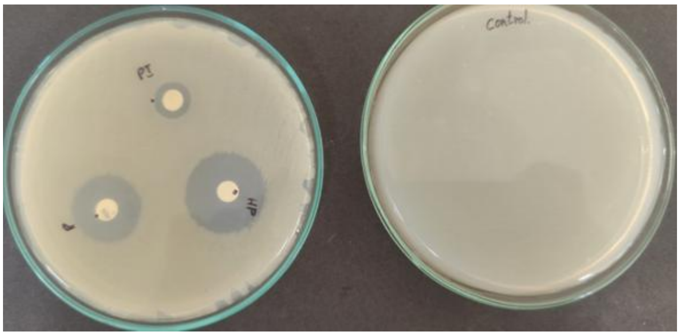

Figure no: 1 Zone of inhibition against *Escherichia coli*

In *Staphylococcus aureus*, the zone of inhibition **(Figure no: 2)** was measured as 23mm for hydrogen peroxide and 13 mm for 21ovidone iodine, and the strongest inhibition effect was measured in the vancomycin antibiotic, which had a 26 mm zone of inhibition **(Table no: 3)**.

S.No	Sample	Zone of inhibition (mm)
1	Hydrogen peroxide	23
2	Povidone iodine	13
3	Vancomycin (Antibiotic)	26

Table no: 3 Determination of zone of inhibition against *Staphylococcus aureus*

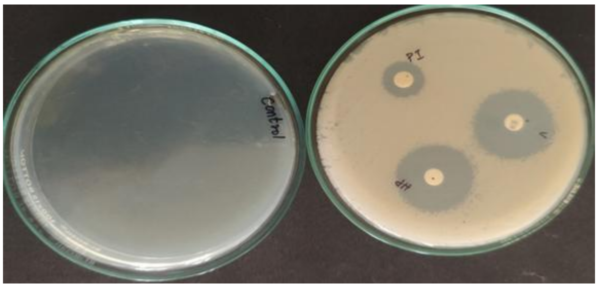

Figure no: 2 Zone of inhibition against *Staphylococcus aureus*

The antimicrobial susceptibility of *Aeromonas sp.,* was measured **(Figure no: 3)**. This strain demonstrated a substantial **(Table no: 4)** zone of inhibition of 32mm to hydrogen peroxide, which is the strongest one compared to 21ovidone iodine and antibiotics. Meanwhile, a diameter of 14mm was observed for 21ovidone iodine and 28mm for the ciprofloxacin antibiotic disc.

S.No	Sample	Zone of inhibition (mm)
1	Hydrogen peroxide	32
2	Povidone iodine	14
3	Ciprofloxacin (Antibiotic)	28

Table no: 4 Determination of zone of inhibition against *Aeromonas sp.,*

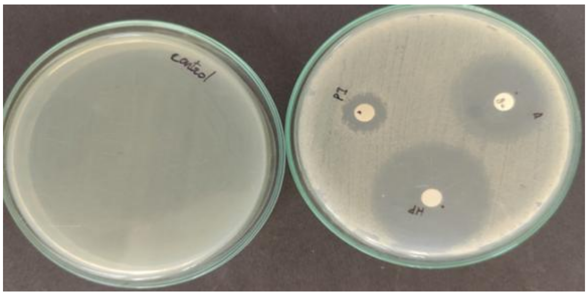

Figure no: 3 Zone of inhibition against *Aeromonas sp.,*

IV. Discussion

The study investigates the microbiological profiles of surgical site infections (SSIs) and evaluates the antimicrobial efficacy of selected antimicrobial agents, including hydrogen peroxide and povidone iodine, in combating the surgical site infections. By identifying and characterizing the bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Aeromonas sp*., the study aims to conduct in vitro assessments of the antimicrobial efficacy of selected treatments through povidone iodine, hydrogen peroxide, and antibiotics.

The microbiological landscape of surgical site infections consists of both endogenous flora and exogenous contaminants. The predominance of endogenous bacteria, often stemming from the skin and mucosal surfaces, indicates a need for effective preoperative screening and decolonization strategies to mitigate infection risk. The presence of foreign materials such as surgical implants further complicates the situation, as the biofilm-forming bacteria elevate the potential for serious infection.

Povidone iodine demonstrated a moderate level of efficacy against *Escherichia coli*, producing a discernible zone of inhibition. Its mechanism is thought to involve the disruption of cellular membranes and the denaturation of proteins, suggesting that it can effectively curtail the growth of these pathogens, which are a common contributor to postsurgical infections.

Staphylococcus aureus exhibited an even greater sensitivity to povidone iodine, with a larger zone of inhibition compared to E. coli. This enhanced effect can be attributed to the ability of povidone iodine to penetrate staphylococcal biofilms and the organism’s susceptibility to oxidative damage. Consequently, povidone iodine serves as a potent antiseptic in clinical environments where *Staphylococcus aureus* is prevalent.

Hydrogen peroxide showed marked antimicrobial activity across all tested strains, with particularly strong inhibitory effects observed against *Aeromonas sp*., where it produced the largest zone of inhibition. The reactive oxygen species generated by hydrogen peroxide likely play a critical role in its bactericidal action, leading to significant oxidative damage to bacterial cells. However, its effectiveness varied, indicating that while it can be highly effective against certain pathogens, its requisite concentrations must be carefully managed to avoid tissue damage during wound management.

Antibiotics, including penicillin, ciprofloxacin, and vancomycin, were also tested, with results indicating effective inhibition against *E. coli* and *Staphylococcus aureus*. However, their performance against *Aeromonas sp*., was less consistent, highlighting potential intrinsic resistance mechanisms within this species. The reduced effectiveness underscores the necessity for judicious antibiotic use and the importance of understanding bacterial susceptibilities, especially in clinical settings where treatment strategies must be tailored to the specific pathogens involved.

V. Conclusion

The study highlights the significant antimicrobial efficacy of povidone-iodine, hydrogen peroxide, and antibiotics against *Escherichia coli*, *Staphylococcus aureus*, and *Aeromonas sp*., which was properly evaluated. The results demonstrated that hydrogen peroxide exhibited the strongest inhibitory action across all tested pathogens, particularly with substantial zones of inhibition recorded, highlighting its potent antimicrobial properties. While povidone iodine also showed significant efficacy, its performance was comparatively lower than that of hydrogen peroxide, yet it remains a valuable antiseptic for surgical applications due to its broad-spectrum activity and safety profile.

Antibiotics demonstrated varying effectiveness, with specific agents like vancomycin, penicillin, and ciprofloxacin displaying strong inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Aeromonas sp.*, respectively. This study reinforces the importance of selecting appropriate antiseptics and antibiotics to combat surgical site infections caused by these prevalent pathogens. Ultimately, integrating these antimicrobial agents into effective infection control protocols can enhance patient outcomes and reduce the incidence of postoperative complications. Further research should focus on optimizing the use of these agents in clinical settings to combat the rising challenge of antibiotic resistance and improve overall infection management strategies.

References

[1]. Ching, P. R. (2024). Care Bundles In Surgical Site Infection Prevention: A Narrative Review. Current Infectious Disease Reports, 26(6), 163-172.

[2]. Kubo, A., Fujita, K., Tanaka, S., Kimura, Y., & Wada, N. (2025). The Impact Of Perioperative Oral Care On Postoperative Infection In Patients With Esophageal Cancer: A Retrospective Cohort Study Before And During COVID-19. Surgery Today, 1-9.

[3]. Kolasiński, W. (2019). Surgical Site Infections–Review Of Current Knowledge, Methods Of Prevention. Polish Journal Of Surgery, 91(4), 41-47.

[4]. Krizek, T. J., & Robson, M. C. (1976). Evolution Of Quantitative Bacteriology In Wound Management. Journal Of Trauma And Acute Care Surgery, 16(4), 327.

[5]. Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., Jarvis, W. R., & Hospital Infection Control Practices Advisory Committee. (1999). Guideline For Prevention Of Surgical Site Infection, 1999. Infection Control & Hospital Epidemiology, 20(4), 247-280.

[6]. Humphreys, H., Becker, K., Dohmen, P. M., Petrosillo, N., Spencer, M., Van Rijen, M., ... & Garau, J. (2016). Staphylococcus Aureus And Surgical Site Infections: Benefits Of Screening And Decolonization Before Surgery. Journal Of Hospital Infection, 94(3), 295-304.

[7]. Pu, L., Li, G., Qi, B., Li, C., Bu, P., Li, Y., ... & Xu, Y. (2023). Prevalence And Drug Resistance Of Escherichia Coli Among Patients With Orthopaedic Surgical Site Infections In China: A Systematic Review And Meta-Analysis. Preventive Medicine Reports, 36, 102452.

[8]. Saravanakumar, R., & Devi, B. P. (2019). Surgical Site Infection In A Tertiary Care Centre-An Overview-A Cross Sectional Study. International Journal Of Surgery Open, 21, 12-16.

[9]. Rutteman, B., Borremans, K., Beckers, J., Devleeschouwer, E., Lampmann, S., Corthouts, I., & Verlinde, P. (2017). Aeromonas Wound Infection In A Healthy Boy, And Wound Healing With Polarized Light. JMM Case Reports, 4(10), E005118.

[10]. Owusu, E., Ahorlu, M. M., Afutu, E., Akumwena, A., & Asare, G. A. (2021). Antimicrobial Activity Of Selected Medicinal Plants From A Sub-Saharan African Country Against Bacterial Pathogens From Post-Operative Wound Infections. Medical Sciences, 9(2), 23.

[11]. Zhu, G., Wang, Q., Lu, S., & Niu, Y. (2017). Hydrogen Peroxide: A Potential Wound Therapeutic Target. Medical Principles And Practice, 26(4), 301-308.

[12]. Van Der Vliet, A., & Janssen-Heininger, Y. M. (2014). Hydrogen Peroxide As A Damage Signal In Tissue Injury And Inflammation: Murderer, Mediator, Or Messenger?. Journal Of Cellular Biochemistry, 115(3), 427-435.

[13]. Monstrey, S. J., Govaers, K., Lejuste, P., Lepelletier, D., & De Oliveira, P. R. (2023). Evaluation Of The Role Of Povidone-Iodine In The Prevention Of Surgical Site Infections. Surgery Open Science, 13, 9-17.

[14]. Maiwald, M., & Widmer, A. F. (2017). WHO's Recommendation For Surgical Skin Antisepsis Is Premature. The Lancet Infectious Diseases, 17(10), 1023-1024.

[15]. Bigliardi, P. L., Alsagoff, S. A. L., El-Kafrawi, H. Y., Pyon, J. K., Wa, C. T. C., & Villa, M. A. (2017). Povidone Iodine In Wound Healing: A Review Of Current Concepts And Practices. International Journal Of Surgery, 44, 260-268.

[16]. Heal, C. F., Banks, J. L., Lepper, P. D., Kontopantelis, E., & Van Driel, M. L. (2016). Topical Antibiotics For Preventing Surgical Site Infection In Wounds Healing By Primary Intention. Cochrane Database Of Systematic Reviews, (11).

[17]. Hemalata, V. B., & Virupakshaiah, D. B. M. (2016). Isolation And Identification Of Food Borne Pathogens From Spoiled Food Samples. International Journal Of Current Microbiology And Applied Sciences, 5(6), 1017-1025.

[18]. Nurkhaliza, F., Risana, M. Z., Pubasari, A., Priatmoko, S., Prastya, M. E., & Andreani, A. S. (2024). Comparative Study Of Well Diffusion And Disc Diffusion Method To Investigate The Antibacterial Properties Of Silver Nanoparticles Synthesized From Curcuma Longa Extracts. In E3S Web Of Conferences (Vol. 503, P. 09003). EDP Sciences.