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BACTERIOLOGICAL PROFILING OF WOUND INFECTIONS AND ANTIBACTERIAL POTENTIAL OF SELECTED ANTISEPTICS AGAINST ISOLATED PATHOGENS

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Abstract

A wound is a type of injury that occurs due to any cut, puncturing or tearing of skin that may lead to damage to the first line of defense resulting in a variety of microbial infections. The present study aimed to assess the bacteriological profile of wound infections in Hyderabad, Sindh. Wound samples including (pus, aspirates, and tissue) were collected aseptically using sterile swabs/syringes. A total of 150 samples were collected from acute and chronic wounds. Isolation and identification of bacteria were carried out as per standard methods. All the samples were cultured on nutrient agar, MacConkey's agar and blood agar plates simultaneously. The antibacterial potential of the selected antiseptic was assessed by the agar well diffusion method. The data showed that 54.67% (n=82) were from males and 45.33% (n=68) from females. Among the samples, 92.67% (n=139) yielded the growth of a single bacterial isolate while 7.33% (n=11) demonstrated the growth of two bacterial isolates. Gram-positive 54% (n=81) were more prevalent as compared to Gram-negative 38.67% (n=58). The majority of bacteria belonged to Methicillin Resistant Staphylococcus aureus (MRSA) 31.33% (n=47) followed by Pseudomonas aeruginosa 18% (n=27), Methicillin sensitive S. aureus (MSSA) 15.33% (n=13). Other isolates of wounds included Escherichia coli 8% (n=12), Enterobacter spp. 4.67% (n=07), β-hemolytic Group A Streptococci (4.67% (n=07), Klebsiella pneumoniae 2.67% (n=04), Proteus mirabilis 1.33% (n=2), Proteus vulgaris 2% (n=03), Acinetobacter spp. 0.67% (n=1) and mixed cultures 7.33% (n=11). Acetic acid and Benzalkonium chloride showed high activity against both Gram-negative as well as Gram positive bacteria. Boric acid, however, showed activity against E. coli but not against other bacteria tested in this study. In summary, MRSA was the most frequent followed by P. aeruginosa bacteria causing wound infections at Hyderabad.

Keywords: *Acetic acid, Antibacterial activity, Antiseptic, Boric acid, Wound infections*

INTRODUCTION

Human skin contains many bacteria (normal flora), which are normally harmless if the skin is intact. The main function of intact skin is to protect underlying tissue from invasion by potential pathogens (1). Any skin abrasion whether trauma, accident, surgical procedures or burns will lead to damage to the first line of defense and result in a variety of microbial infections (2, 3). Exposed subcutaneous tissue (i.e., wound) provides a favorable environment for a wide variety of microorganisms to contaminate, colonize and proliferate within the body (4). A wound is a type of injury that occurs due to any cut, puncturing or tearing of skin and this type of wound is generally called an open wound. Any wound that occurs due to blunt force trauma and results in a contusion is called a closed wound. Simply, a wound refers to a sharp injury which damages the dermis of the skin. Wound infection is indicated by the presence of clinical signs of infection such as tenderness, edema, erythema, abscess pus, and pain. Wound infections depend on virulence, bacterial inoculum, and pathogenicity and when the body's immune defenses are overwhelmed or cannot cope with normal bacterial growth (5, 6). Infection in the wound results in further tissue damage and may prolong wound healing and chronicity which will result in active disease (7). Infection in wounds by microorganisms and their tolerance to common antibiotics is a major problem in healthcare settings. The hospital-acquired infections are a serious health hazard and constitute more than 80% of the mortality (8). Wound infections have been perceived as the most common crucial problem, especially in the presence of foreign agents that enhance the risk of serious infections even with moderately small bacterial infections (9). *S. aureus* (including

MRSA and MSSA), P. aeruginosa and the members of the family Enterobacteriaceae, such as *E. coli*, *P. vulgaris*, *Klebsiella spp.*, are most commonly isolated from acute and chronic wound infections (10). The current study aimed to assess the etiology of wound infections and the antibacterial potential of selected antiseptics for proper management and control of wound infections caused by MDR bacteria.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING

A total of 150 wound specimens were collected from in-patients and out-patients from various medical centres and hospitals of Hyderabad for six months period. All wound specimens (pus, aspirates, tissue) were collected by convenient collection methods either with the help of a sterile swab or injection in the case of pus samples. Duplicate isolates were excluded. The samples were processed for isolation of pure culture of potential wound pathogens and their subsequent identification at the species level. The tissue samples were inoculated in broth media at the first instance. The pus swabs and aspirate specimens were directly inoculated on the surface of Nutrient agar, MacConkey's agar, and Blood agar.

ISOLATION AND IDENTIFICATION OF BACTERIA

Isolation and identification were carried out as described previously (11). Isolates were identified based on Gram staining reaction, morphology (shape, size and arrangement), and cultural characteristics biochemical characterization of isolates (Fig. 1) was performed based on Urease test, Simmons' citrate agar test, Triple sugar iron (TSI) media, Sulphide, Indole, Motility (SIM) media, Oxidase test, Catalase test, Coagulase test, and DNase Test.

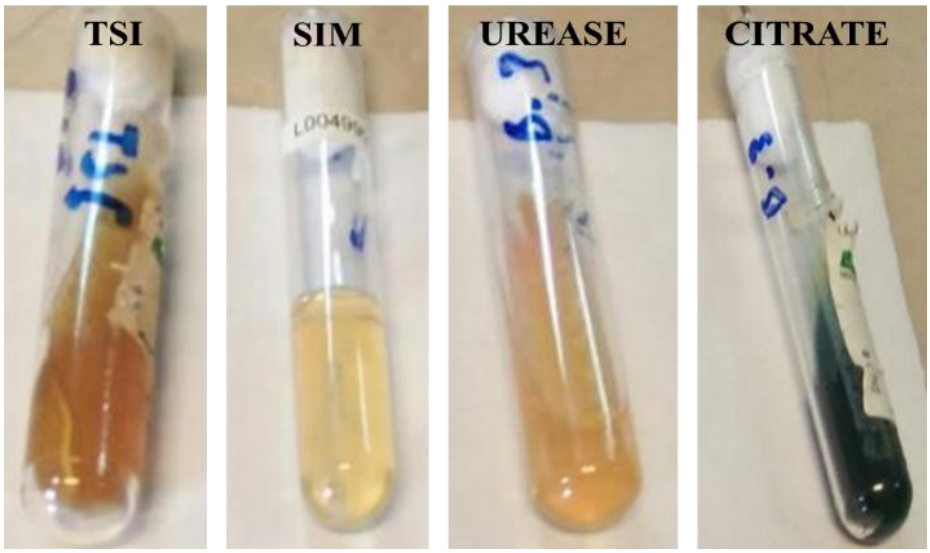

Fig. 1. Representative picture showing biochemical characterization of *E. coli* isolated from wound samples

EVALUATION OF ANTIBACTERIAL POTENTIAL OF SELECTED ANTISEPTICS ON THE WOUND PATHOGENS

Three different antiseptics including Acetic acid, Boric acid and Benzalkonium chloride were used to investigate their effectiveness as antibacterial substances against wound isolates of this study using agar well diffusion method. Multidrug resistant bacteria were used as representative test isolates to determine the efficacy of antiseptics used in the study (12).

RESULTS

GENDER-WISE DISTRIBUTION OF THE PATIENTS OF THIS STUDY

Infection in wounds by microorganisms and their resistance to common antibiotics is a major problem in healthcare settings. The data showed that 54.67% (n=82) of total samples were from males and 45.33% (n=68) from females (Fig. 2).

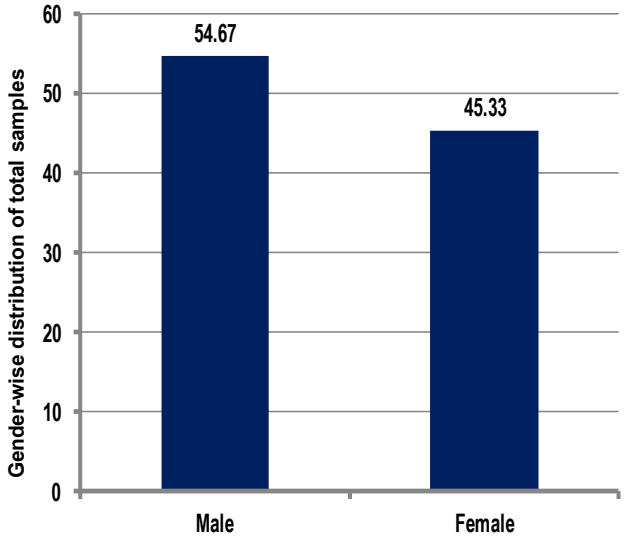

Fig. 2. Gender wise distribution of samples of wounds

Among all samples, 92.67% (*n*=139) yielded a single bacterial isolate, while 7.33% (*n*=11) samples contained mixed growth of two bacterial isolates. Data from Gram staining showed both groups of bacteria. They were either present as mono or dual/mixed organisms (Fig. 3). Overall, Gram-positive 54% (*n*=81) were more prevalent as compared to Gram negative bacteria 38.67% (*n*=58) among the wound infections at Hyderabad (Fig. 4).

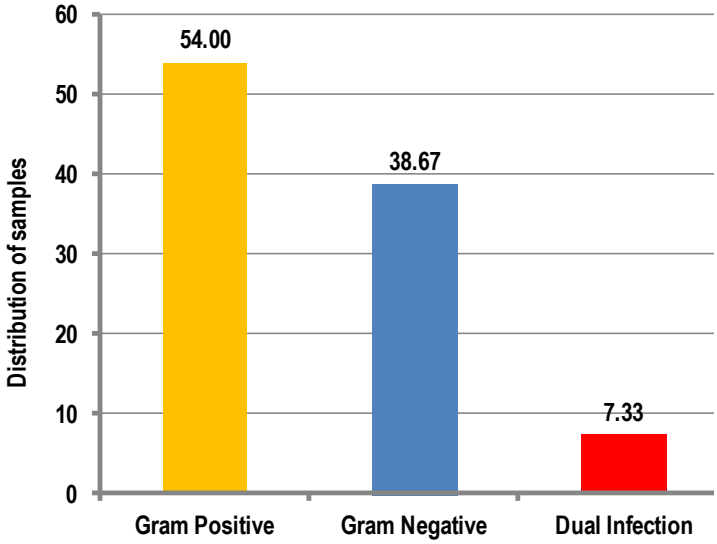

Fig. 3. Distribution of pathogens based on Gram's reaction

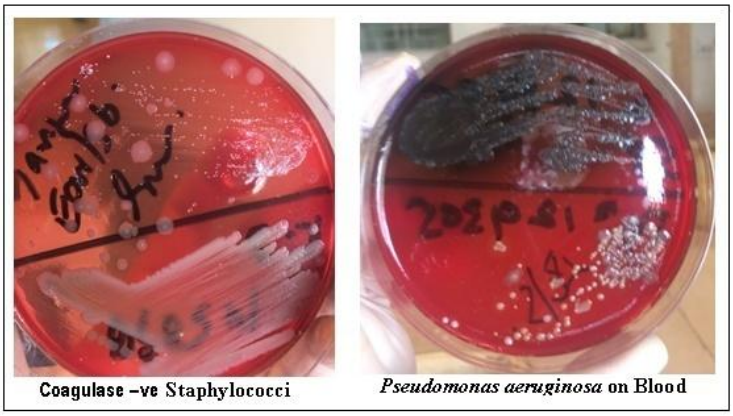

Fig. 4. Representative picture showing a pure culture of selected wound pathogens isolated in the present study

FREQUENCY OF BACTERIAL ISOLATES OF WOUND INFECTIONS

Cultivation of the wound specimens for bacteriological analysis demonstrated twelve different types of bacteria belonging to both Gram-positive and Gram-negative groups of bacteria. The majority of

bacteria belonged to MRSA which accounted for 31.33% (47/150) of all bacterial isolates of wounds, followed by *P. aeruginosa* (18%), then MSSA 15.33% (13/150). Other isolates of wounds included *E. coli* (8%), *Enterobacter* spp. (4.67%), β-hemolytic Group A Streptococci (4.67%), *K. pneumoniae* (2.67%), *P. mirabilis* (1.33%), *P. vulgaris* (2%), *Acinetobacter* spp. (0.67%) and mixed cultures (7.33%) (Fig. 5).

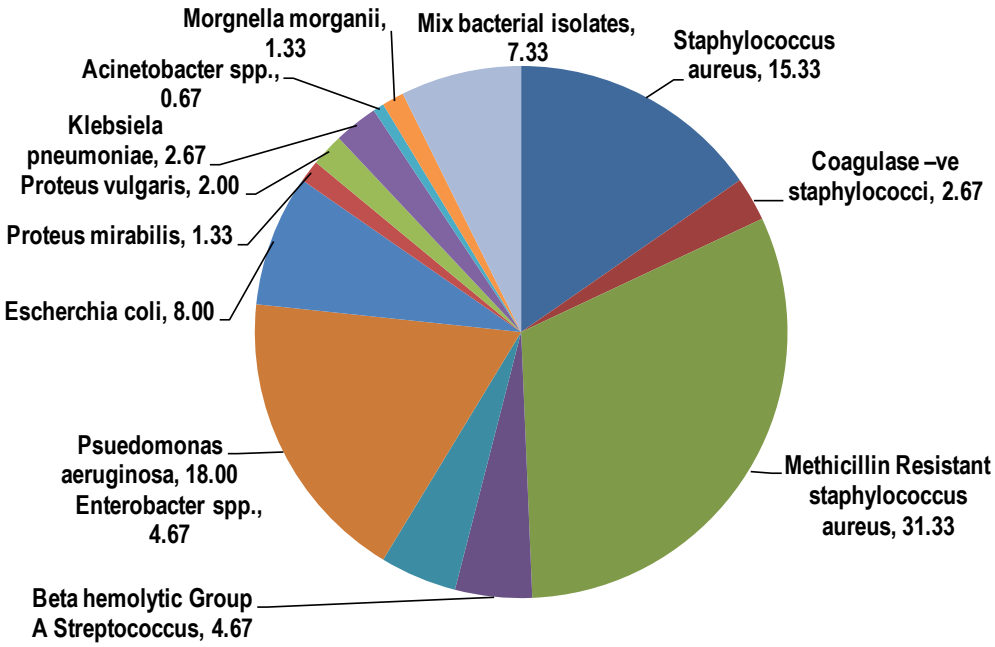

Fig. 5. The overall prevalence of bacteria isolated from wounds

EFFECT OF DIFFERENT ANTISEPTICS ON WOUND ISOLATES

In-vitro antibacterial activity of acetic acid and Benzalkonium chloride was found highly effective against both Gram-positive and Gram-negative bacteria. Boric acid showed antibacterial effects against *E. coli*, however, no zone of inhibition was observed against other bacteria tested (Table I).

Table I. In vitro antibacterial effects of selected disinfectants on wound pathogens using well diffusion method

S. No.	Wound pathogen	Antiseptic (1%)	Antibacterial efficacy
1	*P. aeruginosa*	Acetic acid	+++
		Boric acid	-
		Benzalkonium chloride	++
2	*E. coli*	Acetic acid	+++
		Boric acid	++
		Benzalkonium chloride	+++
3	*K. pneumoniae*	Acetic acid	+++
		Boric acid	-
		Benzalkonium chloride	+++
4	MSSA	Acetic acid	+++
		Boric acid	-
		Benzalkonium chloride	+++
5	MRSA	Acetic acid	+++
		Boric acid	-
		Benzalkonium chloride	++++

DISCUSSION

The present research study focuses on the bacteriological analysis of wound infections in Hyderabad, Sindh. Skin serves as the protective barrier, the outside of which is secured by a slim layer that is created by skin glands (sebaceous glands). This layer is effective and acts as a first line of defense by maintaining the pH of the skin and it also prevents harmful bacteria from entering the body. The harmful bacteria will frequently replace the normal skin flora and occupy some areas of the skin. Many times, this will not result in infection and does not induce an immune response. However, if the immune system is

weak or if the skin is damaged, microorganisms that are present on the skin may enter the wound and result in infection. Bacteria that usually cause infection in wounds are primarily those bacteria that are found on the skin, in addition to the extent and site of the wound. Infection in wounds caused by different microorganisms contributes to significant mortality and morbidity. It is a major problem in healthcare settings that not only increases the treatment cost and longer hospital stay but it also makes surgical procedures difficult in which sterile sites can easily be contaminated and become infected. Moreover, nowadays emerging resistance to different types of antibiotics makes it extremely difficult to treat infected wounds. (13). Therefore, the identification of bacteria and their antibiotic sensitivity profile will be helpful in the management of wound infections.

Infection in the wound is indicated by the exhuming of the skin or basic delicate tissues in which harmful bacteria have attacked tissues around the wound. The body's immune system is initiated by wound infection and also a cascade of reactions followed by inflammation and tissue damage & resulting in a delay in the repair or healing of the wound. In the present study, bacterial species were recovered from different wound samples. Of these samples, the ratio of samples collected from male patients was recorded as 54.67%, while a 45.33% sample ratio was recorded from female patients. Among all these samples, 92.67% of wound samples yielded single bacterial isolate, as they were infected with a single type of pathogen. While, 7.33% of samples showed mixed growth of two bacterial isolates showing that they were infected with more than one type of microorganism. Most of the time infected wounds are self-limiting and do not require any treatment and recovery. For example, abrasions and folliculitis. But some infections if not treated properly become severely infected and may require surgical procedures. Mainly, chronic wounds or open wounds are infected with mixed microorganisms, while surgical incision or superficial wound infections are nonbacterial (14, 15). The results of Gram's staining showed that the Gram-positive bacteria were more prevalent and were recorded as 54%, while the Gram-negative bacteria were recorded as 38.67%. Principally, *S. aureus* was the predominant among all of the Gram-positive bacteria, as they are commonly present on the skin so they can easily get entrance in the wound. Previous studies have also shown *S. aureus* as the most common pathogen frequently found in wound infections (16, 17).

The findings of the current study have demonstrated that twelve different types of bacteria belonging to both major groups were recovered from wound samples. The majority of bacteria belonged to MRSA which accounted for 31.33% of all bacterial isolates of wounds, followed by *P. aeruginosa* (18%), then MSSA (15.33%). Other isolates of wounds included *E. coli* (8%), Enterobacter spp. (4.67%), beta hemolytic Group A Streptococci (4.67%), *K. pneumoniae* (2.67%), *P. mirabilis* (1.33%), *P. vulgaris* (2%), Acinetobacter spp. (0.67%) and mixed cultures (7.33%). Most dermal wounds are populated by microorganisms though it does not mean that all dermal wounds are infected. Inflammatory processes happen in all wounds during the repair of the wound whether it is infected or not and perhaps some level of edema, redness, and increase in temperature at the site occur, which is a normal inflammatory reaction, and it does not indicate signs of clinical infection (18). When the dermis of the skin is breached, the defensive system becomes compromised, and conditions become more favorable for bacteria to grow and multiply (10). Microorganisms that infect the wounds are primarily those bacteria that come from different sources such as the environment (dirt, air pollution, use of infected equipment), the surrounding dermis (normal resident bacteria), and the epithelial mucous membrane of (GIT tract, urinary tract). The findings of present study demonstrated that the acetic acid and Benzalkonium chloride exhibited strong efficacy against both Gram-positive and Gram-negative bacteria while the Boric acid was only effective against Gram-negative particularly the E. coli strains isolated in this study.

CONCLUSION

The present study demonstrated the frequency distribution of bacteria causing wound infections. The analysis of wounds revealed 12 bacterial genera belonging to both Gram-negative and Gram-positive bacteria. Among Gram-positive bacteria, MRSA was the leading wound pathogen, followed by MSSA, Coagulase-negative Staphylococci, and beta hemolytic streptococci. Moreover, acetic acid and

Benzalkonium chloride were highly effective antiseptics against both Gram-positive and Gram-negative bacteria while the boric acid was only effective against *E. coli*. In summary, the knowledge regarding the prevalence of bacteria causing wound infections is pertinent in the selection and commencing of adequate treatment for the management of these infections.

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Ndip RN, Takang A, Echakachi CM, Malongue A, Akoachere J, Ndip LM, Luma HN. In-vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. African health sciences. 2007;7(4).
2. Ohalete C, Obi R, EmeaKoroha M. Bacteriology of different wound infection and their antimicrobial susceptibility patterns in Imo state Nigeria. World J Pharm Pharm Sci. 2012;1(3):1155-72.
3. Memon FS, Bano S, Tunio SA. Patterns of antibiotic susceptibility and resistance in some common wound pathogens from Sukkur, Pakistan. Rawal Medical Journal. 2020;45(2):287-90.
4. Dai T, Huang Y-Y, K Sharma S, T Hashmi J, B Kurup D, R Hamblin M. Topical antimicrobials for burn wound infections. Recent patents on anti-infective drug discovery. 2010;5(2):124-51.
5. Collier M. Recognition and management of wound infections. World wide wounds. 2004;7(1):8-14.
6. Bano S, Tunio S, Mal S, Jatt A. Frequency of Methicillin Resistant Staphylococcus aureus Among Isolates of Wound Infections from Hyderabad. Sindh University Research Journal-SURJ (Science Series). 2015;44(4):683-6.
7. Williams M. Wound infections: an overview. British Journal of Community Nursing. 2021;26(Sup6):S22-S5.
8. Manikandan C, Amsath A. Antibiotic susceptibility of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnadu, India. Int J Curr Microbiol App Sci. 2013;2(6):195-203.
9. Rubin RH. Surgical wound infection: epidemiology, pathogenesis, diagnosis and management. BMC infectious diseases. 2006;6:1-2.
10. Akinkunmi EO, Adesunkanmi A-R, Lamikanra A. Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. African health sciences. 2014;14(4):802-9.
11. Bathija SK, Bano S, Tunio SA. Bacteriological analysis and antibiotic resistance trends in bacteria isolated from cerebrospinal spinal fluid of suspected bacterial meningitis patients at Hyderabad, Sindh. Pakistan Journal of Biotechnology. 2024;21(2):387-91.
12. Barrigah-Benissan K, Ory J, Sotto A, Salipante F, Lavigne J-P, Loubet P. Antiseptic agents for chronic wounds: a systematic review. Antibiotics. 2022;11(3):350.
13. Detho H, Bano S, Tunio SA, Abbasi SM, Ahmed M. Biofilm formation and antibiotic resistance in uropathogenic Escherichia coli: The quest for effective treatment of urinary tract infections. Pure and Applied Biology (PAB). 2023;13(1):38-46.
14. Kingsley A. A proactive approach to wound infection. Nursing Standard (through 2013). 2001;15(30):50.
15. Cutting KF, White R. Defined and refined: criteria for identifying wound infection revisited. British journal of community nursing. 2004;9(Sup1):S6-S15.
16. Bitew A, Molalign T, Chanie M. Species distribution and antibiotic susceptibility profile of bacterial uropathogens among patients complaining urinary tract infections. BMC infectious diseases. 2017;17:1-8.
17. Urooj SU, Bano S, Tunio SA, Memon BA, Abbasi SM, Rajput Z. High Frequency of Gram-negative Bacilli (GNB) Pathogens in Wounds and Other Clinical Specimens: A Grave Public Health Concern. BioScientific Review. 2024;6(2):20-30.
18. Ho JK, Hantash BM. The principles of wound healing. Expert Review of Dermatology. 2013;8(6):639-58.