Microbiology Research Journal International

Volume 33, Issue 5, Page 26-34, 2023; Article no.MRJI.103678
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Prevalence of Bacterial Species in Traumatic, Burns and Post-Surgical Wounds: Focus on Emerging Drug Resistance

Partapbir Singh [a*] and Tejinder Kaur [a]

[a] Khalsa College of Pharmacy and Technology, Amritsar, 143001, Punjab, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2023/v33i51383

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/103678

Original Research Article

Received: 20/05/2023
Accepted: 22/07/2023
Published: 31/07/2023

ABSTRACT

Introduction: Resistance of bacteria to antibiotics poses a serious challenge in managing and treating wound-related complications. Determining the antibiotic resistance patterns is crucial for guiding appropriate antibiotic therapy to avoid their overuse and emergence of deadly superbugs.
Aim: The study was aimed to isolate and detect bacterial species responsible for causing infection in different types of traumatic, burns and post-surgical wounds and antibiotic resistance profile of isolated microorganisms.
Methodology: The bacterial load and isolation of microbes was determined by plating of samples onto duplicate blood and MacConkey agar plates. The morphologically distinct bacterial isolates were identified by biochemical tests and susceptibility of bacteria against selected antibiotics was determined by Kirby-Bauer disc diffusion method.
Results: The major bacterial strains isolated were *Staphylococcus aureus*, *E. coli*, *Klebsiella* sp. and *Pseudomonas aeruginosa*. The most prominent bacterial strain found was *Staphylococcus*

**Corresponding author: E-mail: pbsjosan@gmail.com;*

aureus, present in more than 54% of collected samples, followed by *E. coli*, *Klebsiella sp.*, and *Pseudomonas aeruginosa*, present in 17%, 11%, and 10% of collected samples, respectively. The study further explored that *Pseudomonas* spp. and *Acinetobacter* spp. showed resistance against various non-identical antibiotics such as Amikacin (aminoglycosides), Azithromycin (macrolides), Levofloxacin & ciprofloxacin (fluoroquinolones) and Cefazolin (cephalosporins). However, Doxycycline (tetracycline) was the only antibiotic that inhibited the growth of all the bacterial species isolated from the infected wounds. levofloxacin was also one of the effective antibiotics in restricting the growth of *Staphylococcus aureus, E. coli* and *Klebsiella* spp.
Consequently, the study suggests that Doxycycline followed by levofloxacin may be the most suitable antibiotics for controlling wound infection in this specific geographic region.

Keywords: Antibiotics; resistance; infections; wounds.

1. INTRODUCTION

There are various pathogenic bacterial strains explored causing skin infections from which the *Staphylococcus* species has emerged as a dominant human pathogen that has over the last few decades been a leading cause of community-acquired and nosocomial infections [1]. This bacterium may or may not contain a polysaccharide capsule, non-motile, non-sporing, and facultative anaerobes grows in clusters which is a normal flora of the human nose, skin and mucous membranes [2,3].

Further, these *Staphylococcus* and many other skin residing bacteria may directly transfer from an infected person with the usage of contaminated objects such as clothes, towels, gym equipment, telephones, public transportation, door knobs, remote controls, elevator buttons etc. Skin infections can spread through the bloodstream and may reach distant organs of the body specifically bones and heart valves. However, risk of getting infection after surgery, burns or in open wounds excelled and the severity of complication is largely based on the virulence of the infecting pathogen and the site of infection [4,5]. The incidence of hospital-based postoperative infection varies from 10%–25% in India [6]. Apart from *Staphylococcus aureus* the most common bacteria causing infections of wound include, *E. coli*, *Streptococcus pyogenes, Klebsiella pneumonia, Enterococci* and *Pseudomonas aeruginosa* [2]. These microbes have a remarkable ability of evolving different mechanisms of resistance to antimicrobial agents. Annually >700,000 deaths are being caused due to multidrug resistant organisms and this number is projected to surpass the number of deaths caused from cancer by 2050 as it may reach up to 10 million deaths annually [7]. The aim of the present study is to isolate and identify common bacterial species capable of causing infection in different types of traumatic, burns and post-surgical wounds and to explore the resistance and sensitivity profile of isolated microbes against non-identical antibiotics. This investigation may provide essential information for selection of appropriate antibiotic for treatment of wound infection and contribute to global efforts in combating antibiotic resistance.

2. MATERIALS AND METHODS

2.1 Study Design and Area

A descriptive study was designed and carried out to determine the load of different types of bacteria in infectious wounds and activities of different antibiotics against the isolated microbes. The wound swab samples were obtained from wounds of patients came to receive treatment at Sri Guru Ram Das Charitable Hospital, Amritsar and Ivy Hospital, Amritsar, Punjab, India. These samples were tested in Khalsa Diagnostic Laboratory, Amritsar, Punjab, India. The majority of patients were either laborer or farmers.

2.2 Inclusion and Exclusion Criteria

Patients with non-healing wounds suspected with infection showing exuding fluid and pus were included in the study. Those have interrupted the antibiotic course before the full recovery were also included in the study. On the other hand, patients undergoing antibiotic therapy at the time of sample collection were excluded. The patients with diabetes, cancers and autoimmune disorders were also excluded from the study.

2.3 Sample Collection

Sample from the wounds were collected using sterile swabs by laboratory workers and medical officers of the mentioned hospitals in the out-

patient department (OPD) while maintaining the aseptic conditions as per the hospital guidelines. The samples were collected by medical officers of the hospital after carefully cleaning the wound surface with sterile water to prevent surface contamination.

Thus, the study was conducted on already available biological material obtained from the hospital laboratories with the written consent from the patients which is ethically approved. The study protocol was approved by institutional ethics committee. The swab samples were transferred under aseptic conditions into a vial containing 0.8% sterile saline and then transported to the Khalsa Diagnostic laboratory within one hour of collection.

2.4 Microbiological Techniques

The determination of bacterial load and isolation of microbes in each sample was done using serial dilutions (1:10 and 1:100) of saline containing the swabs in 0.8% sterile saline followed by their plating onto duplicate blood and MacConkey agar plates (HiMedia, Maharashtra, India). The plates were incubated at 37°C under aerobic conditions for 24-48 hours. The morphologically distinct bacterial colonies were counted separately as colony forming units/swab [8]. The bacterial colonies were then purified by repeated transfers on fresh media plates. The morphologically distinct bacterial isolates were identified based upon their various biochemical characteristics such as Catalase production, citrate utilization, Coagulase production, Hydrogen sulfide production, Oxidase production, MR-VP, presence/absence of motility and gram staining test [9].

The susceptibility of isolated bacteria against selected antibiotics was determined by using Kirby-Bauer disc diffusion method and the outcomes were interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards [10,11]. The antibiotics used for this study were Amikacin (30 µg), Ciprofloxacin (30 µg), Azithromycin (15 µg), Levofloxacin (5 µg), Cefazolin (30 µg) and Doxycycline (30 µg) impregnated in the discs (Thermo Fisher Scientific, Mumbai, India). The concentrations were selected based upon their sensitivity within therapeutic limits. An inoculum of pure culture for each bacterial isolate was grown overnight in Mueller Hinton broth and then mixed in sterile saline (0.85%) with turbidity adjusted to 0.5 McFarland standard [12]. The suspension was spread plated uniformly on Mueller Hinton agar plates using sterile swabs and then different antibiotic discs were placed in the center of the plates [13]. The plates were incubated at 37 °C for 24 h, and the diameters of zone of inhibition were measured.

2.5 Statistical Analysis

The statistics were used to interpret the data in which Pearson chi square test was used for categorical variables whereas mean and standard deviation (SD) were given to describe continuous variables. Minitab-15 of Minitab LLC, Pennsylvania was used to analyse the data. A *p*-value <0.05 was considered statistically significant. The regression models were used to determine 95% confidence intervals.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacteria from Wounds

A total of 80 swab samples were collected over the period of study and among these 67 samples were found infected as observed by growth of microbes after their plating onto duplicate blood and MacConkey agar plates (Fig. 1).

The samples were collected from wounds of 48 male and 32 female subjects. Their age varied from 1 year to 76 years with mean age of 42.47 years. These samples were spread plated on sterile blood agar and MacConkey's agar for screening and isolation of different bacteria. Among 80 samples 67 showed positive bacterial growth whereas 13 samples showed no bacterial growth which depicts that even the interrupted antibiotic course have inhibited the bacterial growth in some patients. However, such a practice should be strongly discouraged as left-over bacteria "if any" may more likely to develop resistance to the antibiotics. The bacteria isolated from various wounds were identified by analyzing their colony morphology and testing biochemical characteristics.

3.2 Micro-Organisms Isolated

Among 67 infected subjects around 54% load was of *Staphylococcus aureus* which was found in around 36 samples (Fig. 2) and hence become the most prevalent and responsible microbe for wound infection in this study whereas *Serratia sp.* was the least detected i.e.,

only 2%. However, 17% load was of *E. coli, Klebsiella sp.* (11%), *Pseudomonas aeruginosa* (10%), *Acinetobacter Spp.* (4%) and *Morganella Sp.* (2%) *Serratia sp.* (2%). Hence, the major infection causing bacteria was *Staphylococcus aureus* and interestingly was the only gram-positive bacteria among all the isolated microorganisms (Fig. 2).

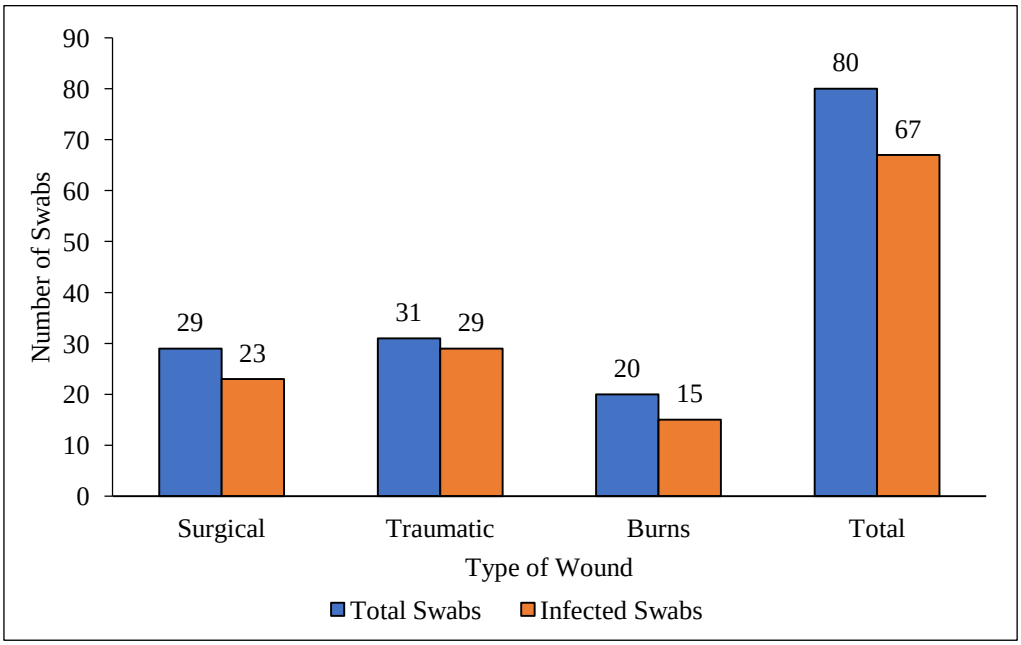

Fig. 1. Describe the number of swab samples collected from different types of wounds and number of infected samples observed during the study

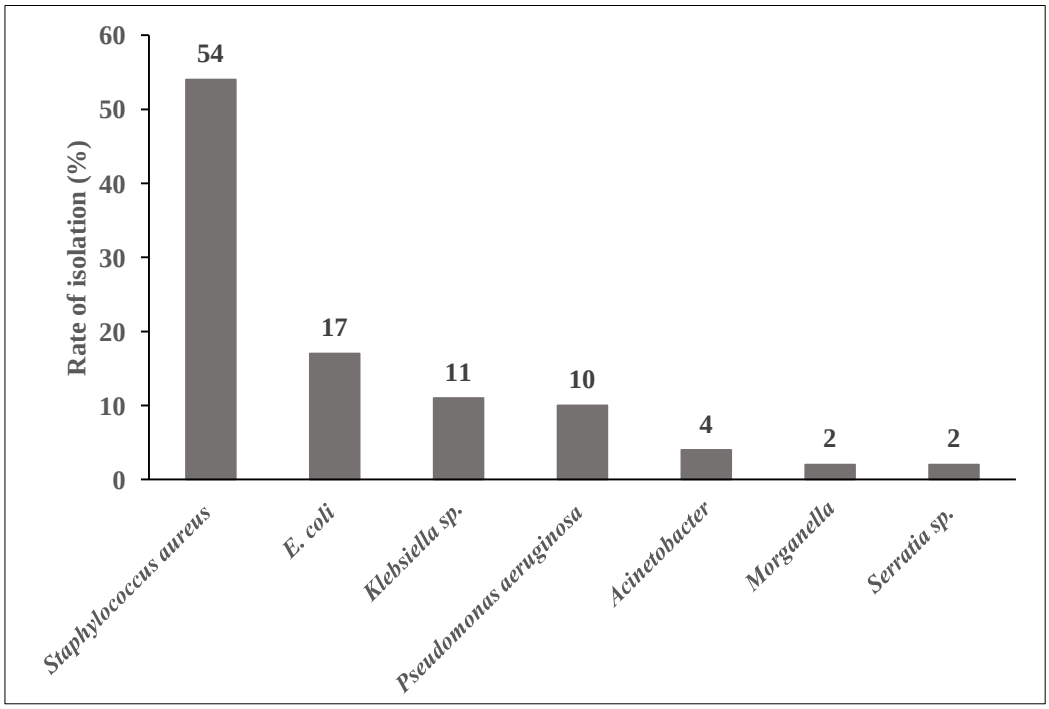

Fig. 2. Describe the percentage of different bacterial species isolated from different types of infected wounds

There was no specific type of microorganism special to any specific type of the wound. However, overall rate of infection by all the microbes was found higher in traumatic injuries followed by injuries caused due to surgery and then by burns (Fig. 3). This might be due to the involvement of antibiotic course before and after the planned surgeries and normal tendencies of patients to get treatment/fast relief for the highly painful burns. Such practice is not usually observed by patients after minor to moderate traumatic injuries until these gets painfully infected.

The number of samples collected in age group of 21-40 years were more (i.e., 28) as compared to samples collected in other age groups. However, number of infected samples in this age group (21-40 years) were less i.e. 71% (22) as compared to other age groups e.g. all the 12 samples (100%) in age group of 1-20 years were infected whereas in 41-60 years and more than 60 years of age group 82% and 83% samples were found infected (Fig. 4 and Table 1). The number of surgical injuries were highest in age group of 21-40 years however, the *p* value of 0.36 indicated no significant relation between age groups with intensity of infection. The wounds due to burn were more in age group of 1-20 years whereas no burn wound observed in higher age group of >60 years but their number of traumatic injuries were more as compared to other age groups (Fig. 4).

3.3 Relation of Infection with Type of Wound, Age and Gender of the Subjects

The infection in wounds of subjects from post-surgery, burns and various other non-traumatic reasons was observed. The number of male patients having infection in their wounds were slightly more (58%) and number of infected patients were fractionally more in the age group of 21-40 years (Table 1). However, there was no significant association found between the infection of the wound with gender ($p = 0.62$) and age ($p = 0.71$) of the patient.

3.4 Antibiotic Resistance

Notably, the isolated bacteria exposed to the selected antibiotics showed varying percentages of resistance (Fig. 5). The test was executed using agar-well diffusion method and degree of susceptibility of isolated micro-organisms (in percentage resistance) was observed based upon their diameters of zones of inhibition and resistance/sensitivity pattern against these antibiotics [14].

Among all the bacterial species isolated from the infectious wounds *Klebsiella* was the most sensitive bacteria as 80% of its growth remain restricted against most of the antibiotic used in this study. However, *Pseudomonas* and *Acinetobacter* were found highly resistant against all the selected antibiotics except doxycycline. Pseudomonas is inherently resistant to many antibiotics due to its natural efflux pumps and impermeable outer membrane, which limit drug penetration. Additionally, Pseudomonas aeruginosa can acquire resistance genes through horizontal gene transfer, leading to resistance against multiple classes of antibiotics, including beta-lactams, aminoglycosides, fluoroquinolones, and carbapenems [15]. Acinetobacter species possess intrinsic resistance mechanisms and can also acquire resistance genes, leading to MDR and extensively drug-resistant (XDR) strains. The most concerning resistance mechanism in Acinetobacter is the production of carbapenemases, particularly the class D carbapenemase known as OXA-type enzymes, which can inactivate carbapenem antibiotics [16].

In this study, species of *Pseudomonas* and *Acinetobacter* showed 50% to 90% resistance against most of the antibiotics used whereas doxycycline manage to inhibit 80% to 90% growth of both *Pseudomonas* and *Acinetobacter*. Thus, doxycycline was the only antibiotic that inhibited the growth of all the bacterial species isolated from the infected wound in this study hence can be effectively used in controlling the wound infection. Moreover, doxycycline is less toxic than its former generations that act by inhibiting the protein synthesis in bacterial cell and can be consumed orally or given intravenously to control the infection [17]. However, this study showed that levofloxacin was also one of the most effective antibiotics in controlling the growth of *Staphylococcus aureus, E. coli* and *Klebsiella sp.* Levofloxacin which was found as the second-best antibiotic in this study, belongs to third generation of fluoroquinolones that act by directly inhibiting the bacterial DNA synthesis and can be used as oral and intravenous (IV) drug [18].

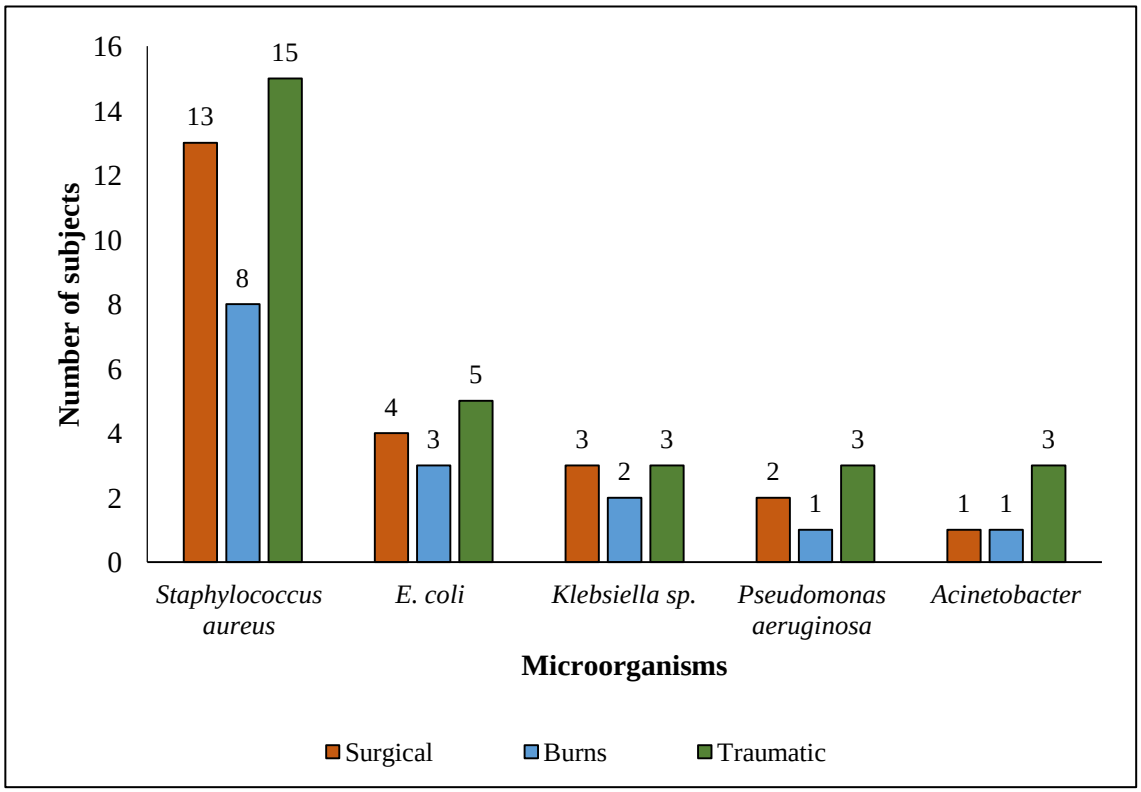

Fig. 3. Showing the comparative load of different bacteria in various types of examined wounds

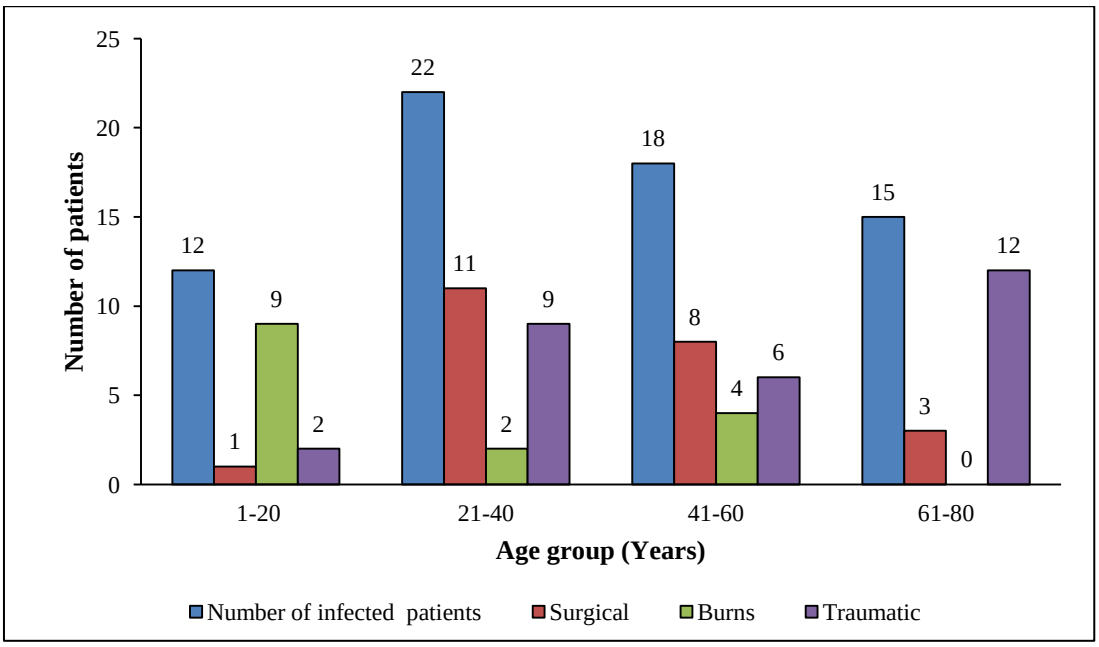

Fig. 4. Describe total number of infected patients and different types of infected wounds in each age group

Table 1. Distribution of patients with respect to their age, gender and wound infection

Age group (years)	Number of patients	Males	Females	Infected male samples	Infected female samples
1-20	12	7	5	7	5
21-40	28	16	12	12	10
41-60	22	12	10	9	9
61-80	18	13	5	11	4
Total	80	48	32	67	

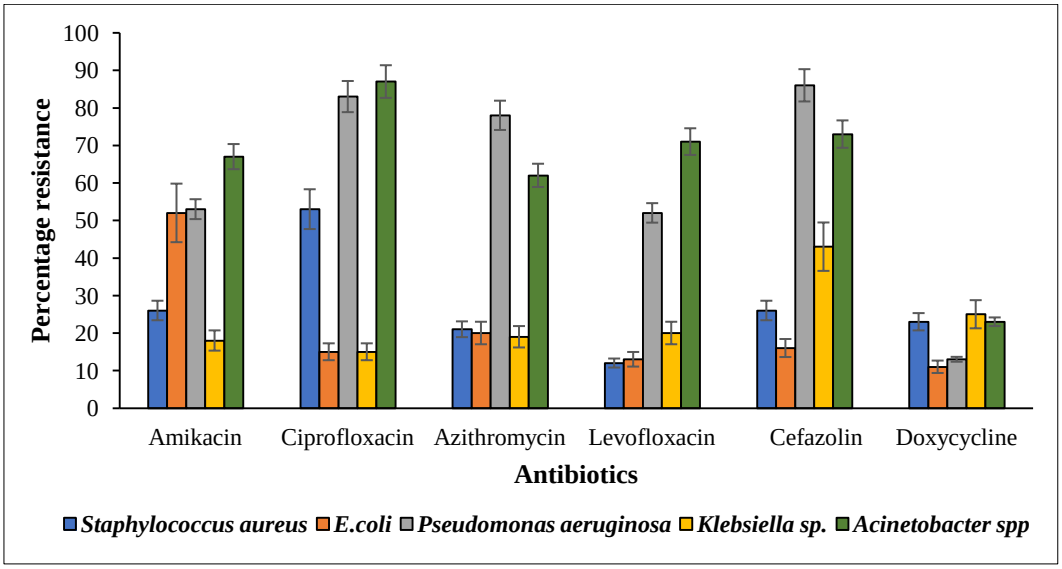

Fig. 5. The antibiotics resistance profile of bacterial species isolated from infected wounds

4. CONCLUSION

The present study provides an insight regarding different bacterial species responsible for causing infection in various types of wounds in this part of the geographical region and efficacy of different antimicrobial available to control the infection. The study also concluded the antimicrobial resistance profile for bacterial species isolated from various infected wounds. *Staphylococcus* was the most prevalent and majorly responsible for causing wound infection and interestingly was the only gram-positive bacteria among the isolated species in this study followed by *E. coli, Klebsiella sp.* and *Pseudomonas sp.* whereas *Serratia sp.* was the least detected. The rate of infection was found higher in traumatic injuries followed by injuries due to surgery and burns.

Further, antibiotic profiling in this study suggests that Doxycycline could be the most promising antibiotic that may be used for treatment of various infected wounds whereas levofloxacin can also be used effectively in specially those cases where the infection causing agents are *Staphylococcus aureus, E. coli* and *Klebsiella sp*.

Overall, the study emphasizes the significance of understanding the microbial profile and susceptibility pattern of wound infection which will help in selection of appropriate antibiotic for effective treatment and combat the emergence of antibiotic resistant strain.

CONSENT

As per international standard patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Medical Laboratory Sciences and the Khalsa College of Pharmacy and Technology, Amritsar, for the technical facilities that made this research possible. The team did not receive any financial

support for the research and writing of this article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3).
2. Rasigade JP, Vandenesch F. *Staphylococcus aureus*: A pathogen with still unresolved issues. Infect Genet Evol. 2014;21:510-4.
3. Karmakar A, Dua P, Ghosh C. Biochemical and Molecular Analysis of Staphylococcus aureus Clinical Isolates from Hospitalized Patients. Can J Infect Dis Med Microbiol. 2016;9041636.

   DOI: 10.1155/2016/9041636.
4. Berríos-Torres SI, Umscheid CA, Bratzler DW. et.al. Healthcare Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. JAMA Surg. 2017;152(8):784-791.
5. Larry M, Bush MD. *Staphylococcus aureus* infection' FACP, Charles E. Schmidt College of Medicine, Florida Atlantic University; 2019.
6. Mohan N, Gnanasekar D, Tk S, Ignatious A. Prevalence and Risk Factors of Surgical Site Infections in a Teaching Medical College in the Trichy District of India. Cureus. 2023;15(5):25.
7. O'Neill, J. Tackling Drug-resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance; Wellcome Trust Collection: London, UK; 2016.
8. Garre A. et al. Tail or artefact? Illustration of the impact that uncertainty of the serial dilution and cell enumeration methods has on microbial inactivation. Food Res. Int. 2019;119:76-83.
9. Aslanzadeh J. Biochemical profile-based microbial identification systems. In: Advanced Techniques in Diagnostic Microbiology. Springer, Boston, MA; 2006.

   Available:https://doi.org/10.1007/0-387-32892-0_6
10. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement. Clinical Laboratory Standards Institute; Wayne, PA, USA: CLSI Document M100-S18; 2014.
11. Edelmann A, Pietzcker T, Wellinghausen N. Comparison of direct disk diffusion and standard microtitre broth dilution susceptibility testing of blood culture isolates. J Med Microbiol. 2007; 56:202–7.
12. Montes-Duarte GG, Tostado-Blázquez G. et al. Key parameters to enhance the antibacterial effect of graphene oxide in solution. RSC Adv. 2021;5: 11(12).
13. Tebano G, Mouelhi Y, Zanichelli V, Charmillon A, Fougnot S, Lozniewski A, Thilly N, Pulcini C. Selective reporting of antibiotic susceptibility testing results: A promising antibiotic stewardship tool. Expert Rev. Anti. Infect. Ther. 2020; 18:251–262.
14. Huang MB, Baker CN, Banerjee S, Tenover FC. Accuracy of the E test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. J Clin Microbiol. 1992;30:3243-8.
15. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 2016; 3(1):15-21.

   DOI: 10.1177/2049936115621709.
16. Lorusso AB, Carrara JA, Barroso CDN, Tuon FF, Faoro H. Role of Efflux Pumps on Antimicrobial Resistance in *Pseudomonas aeruginosa*. Int J Mol Sci. 2022;13;23(24):15779.

   DOI: 10.3390/ijms232415779.

17. Nelson ML, Levy SB. The history of the tetracyclines. Ann N Y Acad Sci. 2011; 1241:17-32.

18. Vila J. Fluoroquinolone resistance. In: White D.G., Alekshun M.N., McDermott P.F., editors. ASM Press. 2005;52–41.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/103678