








ORIGINAL

Assessing Antimicrobial Resistance in Microbial Species from Infected Wounds: A Retrospective perspective View on cancer patients

Evaluación de la resistencia a los antimicrobianos en especies microbianas de heridas infectadas: una perspectiva retrospectiva sobre pacientes con cáncer

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ABSTRACT

Introduction: conducting an infection evaluation based on microbiological scrutiny, wound evaluation, and infection indicators identification takes a great deal of time, the appropriate tools, and highly skilled personnel. The use of antibiotics and wound healing are the two main strategies required to prevent and manage infections. The medical care of wound infections has made antibiotic resistance an issue of worldwide attention. This research was conducted retrospectively to identify the microorganisms that cause wound infections and their pattern of resistance to treatment.

Method: 315 samples from burn wounds that were infected were used in this investigation. The species that had been divided apart by culture techniques were identified and evaluated using the defunct automated system. Next, several antibiotic susceptibility patterns were applied to the species.

Results: gram-negative species made up the majority of the species, among the most common are *Proteus mirabilis*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The strain *Staphylococcus aureus* accounted for the total number of gram-positive bacteria, with these samples identified isolates had a multi-drug resistance to at least five antibiotics, compared to isolates with at least one antibiotic resistance. Although individuals noted concurrent infections and multi-drug resistance organisms were not as common at the wound site.

Conclusion: these circumstances make getting rid of the germs harder. Antibiotic resistance can be stopped and wound healing can be promoted by addressing the poly-microbial infection and multidrug-resistant bacteria that were found, then treating the infection with the appropriate medication.

Keywords: Microbial Species; Infected Wounds; Antimicrobial Resistance.

RESUMEN

Introducción: realizar una evaluación de infecciones basada en el análisis microbiológico, la evaluación de heridas y la identificación de indicadores de infección requiere mucho tiempo, las herramientas adecuadas y personal altamente capacitado. El uso de antibióticos y la cicatrización de heridas son las dos estrategias

principales para prevenir y controlar las infecciones. La atención médica de las infecciones de heridas ha convertido la resistencia a los antibióticos en un problema de atención mundial. Esta investigación se realizó retrospectivamente para identificar los microorganismos causantes de infecciones de heridas y su patrón de resistencia al tratamiento.

Método: se utilizaron 315 muestras de heridas por quemaduras infectadas. Las especies que se separaron mediante técnicas de cultivo se identificaron y evaluaron utilizando el sistema automatizado obsoleto. A continuación, se aplicaron varios patrones de susceptibilidad a los antibióticos a las especies.

Resultados: las especies gramnegativas constituyeron la mayoría de las especies; entre las más comunes se encuentran *Proteus mirabilis*, *Acinetobacter baumannii*, *Escherichia coli* y *Pseudomonas aeruginosa*. La cepa *Staphylococcus aureus* representó el número total de bacterias grampositivas. En estas muestras, los aislados identificados presentaron resistencia a múltiples fármacos a al menos cinco antibióticos, en comparación con los aislados con resistencia a al menos un antibiótico. Si bien se observaron infecciones concurrentes y los microorganismos resistentes a múltiples fármacos no fueron tan comunes en la herida.

Conclusión: estas circunstancias dificultan la eliminación de los gérmenes. La resistencia a los antibióticos se puede detener y promover la cicatrización de las heridas abordando la infección polimicrobiana y las bacterias multirresistentes detectadas, y posteriormente tratando la infección con la medicación adecuada.

Palabras clave: Especies Microbianas; Heridas Infectadas; Resistencia a los Antimicrobianos.

INTRODUCTION

The successful management of infections caused by bacteria is threatened by Antimicrobial Resistance (AMR), a worldwide health problem. The appearance and spread of bacteria species resistance to antibiotics has become an urgent concern in the context of infected wounds, a frequent medical occurrence.⁽¹⁾ The skin acts as a barrier of protection to prevent pathogen infection. Therefore, a wound comes about a change in the normal anatomy brought by procedures or physical, chemical, mechanical, or thermal processes, in addition to a modification in the way the skin functions. Because skin comes into direct contact with the outside world, and gets scrapes and bruises easily, therefore, it is more vulnerable to pathogen colonization.⁽²⁾ There are two kinds of wounds: acute and chronic. Acute injuries, such as cuts, burns, abrasions, and surgical wounds, are caused by outside forces and heal through the standard stages of wound healing. In underdeveloped countries, bacterial infections have been linked to increased rates of patient morbidity and mortality, regardless of the kind of wound.⁽³⁾

The assessment process for infections is time-consuming and depends on wound examination, the identification of infection biomarkers and microbiological investigations, appropriate apparatus along skilled personnel. Two essential components of managing infections are the use of antibiotics and wound healing. Antibiotics are administered empirically, without the benefit of microbiological examination.⁽⁴⁾ Aspects that are overlooked yet crucial for preventing the spread of isolated resistance to antibiotics include the identification of microbial species, the distribution of pathogens, and indicators of antimicrobial responsiveness. Specifically, determining which types of bacteria are colonizing a wound and how sensitive they are to antibiotics can help to determine which therapy is better for the patient, hence lowering the cost of treatment.⁽⁵⁾

The body's defenses and antimicrobial medications are defended against by the exopolysaccharide (EPS) bacterial structure. As a result, bacteria that co-infect can form poly-microbial bio-films that are typified by phenotypic resistance or tolerance to antibiotics linked to the bio-film matrix, in addition to intrinsic biological resistances.⁽⁶⁾ To eradicate a biofilm formed by aggregating bacteria cells, antimicrobial doses as high as four times the Minimal Inhibitory Concentration (MIC) are needed. Many different bacterial pathogens can cause wound infections, which vary in severity from surface wounds to the skin to fundamental infections at the surgical site.⁽⁷⁾

Antimicrobial substances, such as antibiotics, are used and occasionally misused, which has led to the emergence of resistance in microbial species. When bacteria develop defenses against medications meant to eradicate them, it's known as resistance to antimicrobial agents. The emergence of antibiotic resistance in the conditions of wounds that are infected makes handling patients even more difficult, creating difficulties for medical professionals and perhaps reducing available treatments.⁽⁸⁾ Developing successful treatment plans, putting preventative measures in place, and addressing the larger problem of antibiotic stewardship depends on an understanding of the mechanisms of resistance to medicines in bacteria species from wounds that are infected. Using bacteria species collected from infected wounds, the overview explores the present status of antimicrobial resistance and looks at the mechanisms that have led to its growth, the consequences for patient care, and possible strategies for addressing the evolving problem.⁽⁹⁾

Also intend to add to the existing discussion about antimicrobial resistance and raise awareness of the importance of responsible and environmentally conscious use of antibiotics by illuminating the complex interactions between bacteria and drugs in the environment of infections of wounds.⁽¹⁰⁾

To highlight the known gaps in epidemiological data, evidence-based preventative interventions for Surgical Site Infections (SSI), monitoring methods, and ways for estimating the financial burden of SSI associated with AMR bacteria were examined.⁽¹¹⁾ The facts were nonetheless generalizable and similar, despite the subject being handled without the backing of a global coalition. Several ailments included bloodstream infections in infants, endometritis, tonsillitis, pneumonia, burn infections, and urinary tract infections.⁽¹²⁾ Numerous bacterial infections were identified, and their medication resistance was noted. Following data analysis, significant patterns of treatment resistance were observed in the identified bacterial infections.

The microbiological profile of Diabetes Foot Infection (DFI) in Lebanon was equivalent to other countries in the Middle East and North Africa (MENA) region, with significant variations from Western countries, when selecting empiric antibiotics keep in mind the maximum prevalence of Gram-Negative Rods (GNR) in DFI as well as the significant fluoroquinolone resistance.⁽¹³⁾ The common and natural type of bacterial life has not been taken into consideration when designing measures to combat antibiotic resistance at medical facilities were evaluated, which found capacity to boost bacterial survival, persistence, as well as promote antibiotic resistance.⁽¹⁴⁾ The analysis focused on the bacterial makeup and drug susceptibility profiles of patients identified with surgical site infections at Jimma Medical Center in Ethiopia.⁽¹⁵⁾ Because of the high level of antibiotic resistance, specimens demanded regular microbiological investigation and antibiograms. In addition to stressing the role silver plays in wound healing, sought to give the reader an overview of the most recent advancements in silver nanotechnologies.⁽¹⁶⁾ Silver nanotechnology has generated a lot of attention in the field of better healthcare in recent years, and antimicrobial medications and anti-biofilm techniques were required.

The current state of knowledge regarding the functional range of Antimicrobial Peptides (AMPs) and the challenges encountered in the development process were compiled.⁽¹⁷⁾ Developments in the current summary's investigation on the co-administration of conventional antibiotics and AMPs also included information on contemporary clinical studies.

The development report concurrently emphasized the approaches based on nanotechnology, which were the investigated.⁽¹⁸⁾ Novel and exciting techniques such as photo-thermal and photodynamic therapies, in conjunction with cold atmosphere medicines, might be viewed as supplementary strategies to counteract antimicrobial resistance and attain synergistic enhanced antibacterial effectiveness. In vivo outcomes were the focus of the research, which addressed non-antibiotic antibacterial therapies based on biomaterials for treating infectious infections that were contracted in clinics and the general public.⁽¹⁹⁾ While stressing the possibility of conversion, they gave a summary of the challenges associated with applying different biomaterial-based approaches in clinical settings.

Antibiotic sensitivity of microorganisms was measured without taking these interactions into account, according investigation.⁽²⁰⁾ From their vantage point, they argue that resistance and the ability of bacteria to adapt to antibiotic treatments should be viewed as both unique microbe traits and emergent features of the microbial community in which the illnesses originated. Physicochemical approaches, drug delivery systems, combination therapy, strategies like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and novel approaches like focusing on enzymes or proteins that produce resistance to antimicrobial-resistant bacteria were all included.⁽²¹⁾ These different approaches could change the way multi-drug-resistant germs are handled in human hospitals.

The distribution, age, gender, and location-specific antibiotic resistance trends of bacterial pathogens from injury, plasma, and urine illnesses were assessed.⁽²²⁾ More than 90 % of the *Escherichia coli* isolate in plasma, urine, and wound swabs were ampicillin-resistant. The logical prediction and development of new, effective antimicrobial nanomaterials, examined, which combined chemically and photo dynamically induced antimicrobial treatment with multifunctional nanoparticles.⁽²³⁾ Mostly, it might represent a productive class of antimicrobial strategies for potential medical application. Finding replacement drugs has proven to be a significant undertaking.⁽²⁴⁾ Due in large part to their richness, insects were the largest class of animals. Humoral AMP was produced as a part of the resistant reaction and dissolved into the insect plasma vessels upon microbial infection. Their focus was on the immunological reactions of insects, with a particular focus on the characteristics, modes of action, and applications of antimicrobial drugs AMPs, particularly within the biomedical field. Bacteriophages demonstrated remarkable efficacy in Multidrug-Resistant (MDR) bacterial pathogens and wound infections, while also expediting the healing process.⁽²⁵⁾ The outcomes demonstrated that phages were highly effective at preventing various MDR bacterial wound infections, healing wounds more quickly, posing less of a risk of side effects and toxicity, eliminating bacterial biofilm, and regulating immune responses.

This retrospective research aimed to identify the microorganisms causing wound infections and their mechanism of resistance to treatment.

METHOD

Infected wound samples were obtained for this retrospective investigation. Standard methods of microbiology were employed to identify the various types of microbes. Testing for antibiotic susceptibility was done by the diffusion technique. To establish a relationship between resistance patterns and clinical outcomes, patient records were examined in retrospective. Throughout the research period, changes in antibiotic resistance were evaluated using statistical analysis.

Data collection

A collection of 315 samples from infected burnt wounds has been obtained for the current retrospective analysis. A total of 115 males and 200 females provided these samples. The patient's age ranged from 1 year to 79 years, with an average age of 35. Among the total number of wound swabs obtained, 58,7 % (n = 185) showed evidence of an infection with bacteria on cultures; of these, 112 patients were female, and 73 male are shown in table 1. The age range of individuals with positive infections caused by bacteria revealed that, respectively, 16,76 %, 35,68 %, 22,7 %, 15,14 %, and 9,73 % of those infected were between the ages of 0 and 16 years, 17 and 31 years, 32 and 46 years, 47 and 61 years as well as more than 62 years are shown in table 2. For females, the largest percentage of infections caused by bacteria (41,96 %; 47/112) observed in the 16-30 age group, while for males, the greatest percentage of bacterial diseases (26,03 %; 19/73) observed in the 16-30 and 31-45 age groups. An evaluation of the relationship between infection, age, and sex was done using a logistic regression approach.

Table 1. Information on infected wounds

Samples	Patients with infections N (%)	Patients with Non-infections N (%)
Patients	200 (63,50)	115 (36,50)
Female	120 (38,09)	60 (19,04)
Male	80 (25,39)	55 (17,46)
Median	30 years	32,06 years
Range	1-75 years	1-80 years
Average	33,72 years	35,53 years

Table 2. Age and gender distribution of infected wounds

Age (year)	Female (N)	Male (N)	Overall (N)
0-16	35	28	63
17-31	27	32	59
32-46	43	20	63
47-61	70	15	85
>62	25	20	45

Determining the Samples and Antimicrobial Susceptibility Pattern

Following the VersaTREK incubation, the useful samples were placed on Agar from blood agar medium, mannitol salt, Mc Conkey, and sabourad. Apart from sabourad agar plates, which were incubated for 49 hours at 31 °C in an aerobic environment, the plates were thereafter incubated at 38 °C for 19 to 25 hours. The process of identifying the microorganisms involved examining the morphology and characteristics of colonies and utilizing the micro-Scan walk-away structure. This approach makes use of specialized components to determine antimicrobial susceptibility patterns and biochemical reactions to identify microbiological species. Involuntary fast-growing gram-positive cocci and aerobic cocci have been identified using Pos the breakpoint Combo Panel Type 33, A few careful aquatic gram-positive cocci as well as *Listeria monocytogenes*. It has been used to determine exposure to 31 antimicrobial agents, of which those indicated. Applying negative barriers combo panels 47 and 48, the level of species detection of willingly and aerobic gram-negative bacteria that are aerobic as well as susceptibility to 28 antibiotics were carried out. To identify aerobic streptococci, such as pneumonia, and assess their response to 28 antimicrobial drugs, Micro-strep plus panels have been employed. A quick yeast ID panel has been developed for mushrooms, enabling quick detection of yeasts. Since treatment is standardized and the practitioner makes the decision regarding which medication to use, the recommendations do not specify how the antifungal susceptibility test should be performed on the analyzed samples. The manufacturer's instructions were adhered to in every experimental technique.

Statistical analysis

The use of percentages and numbers is used to represent categorical variables, whereas average and inter-quartile range (IQ) are used to communicate continuous variables. To determine if the percentage of infections caused by the most common bacteria throughout the research period showed an independently significant linear trend, the Analysis of Variance (ANOVA), t-test for trend was employed. A significant p-value for analyses was defined as $< 0,05$. The statistical studies were carried out by IBM® SPSS Statistics v 20.0 along with Prism software's graph pad version 7.

RESULTS

Table 3. Microorganism distribution sorted by category and year of survey

Samples	Values (%)			
	2020	2021	2022	Overall
	N	N	N	N
<i>Staphylococcus lugdunensis</i>	50	-	50,2	1,02
<i>Staphylococcus aureus</i>	-	-	99,2	1,2
<i>Enterococcus avium</i>	99	-	-	0,9
<i>Staphylococcus haemolyticus</i>	40,1	20,2	40,2	2,9
<i>Staphylococcus aureus</i>	30,3	31,7	30,3	92,3
<i>Morganellamorganii</i>	23,4	36,3	43,6	1,03
<i>Providencia sp.</i>	-	100,5	-	1,5
<i>Proteus vulgaris</i>	-	100,2	-	1,2
<i>Pseudomonas aeruginosa</i>	20,6	35,4	46,8	1,02
<i>Acinetobacter</i>	-	61,2	42,2	1,03

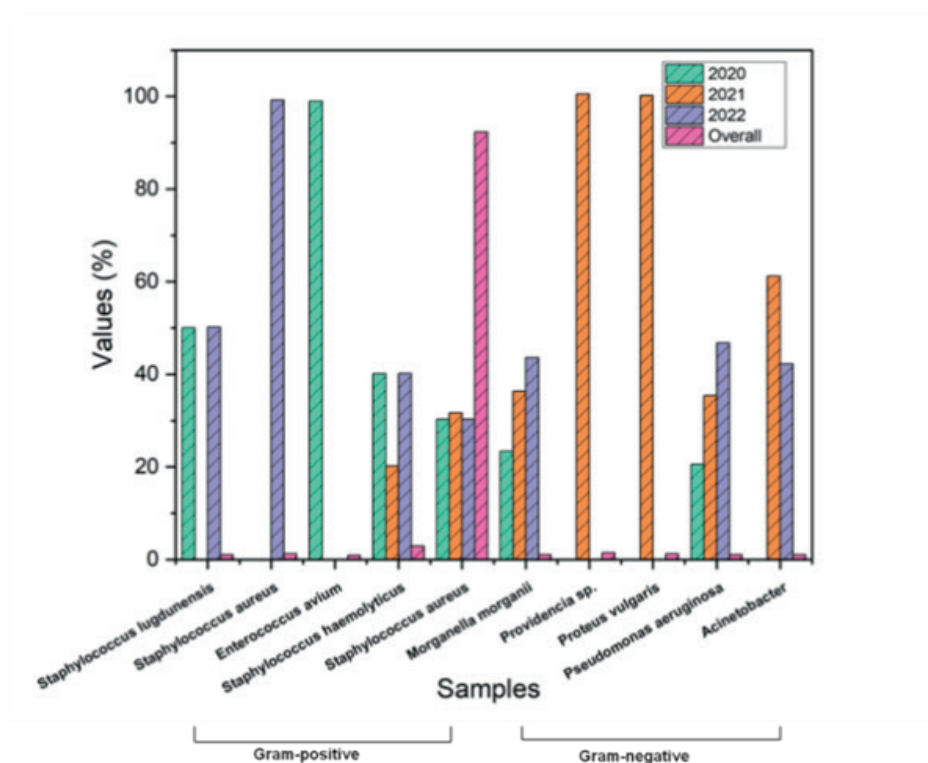


Figure 1. Distribution of microorganisms by year in percentage terms

The total number of wound samples included in this research work came from 315 patients between the years 2020 and 2022, with a median age of 75. Specifically focusing on Gram-positive and Gram-negative species, table 3 displays statistics on bacterial samples that were taken during three years in 2020, 2021, and 2022. For a given bacterial species, the amounts in each cell indicate the percentage of that species of samples in that year. Regarding the Gram-positive group, the incidence was constant between 2020 and 2022

for *Staphylococcus lugdunensis* and between 30,3 % and 31,7 % for *Staphylococcus aureus*. It is noteworthy that in 2020, *Enterococcus avium* has been found in 99 % of samples, but that's not found in any samples in the following years. Over three years, there was an overall rise in prevalence with the Gram-negative group. *Proteus vulgaris*, *Providencia* sp, and *Morganella morganii* all had different patterns; *Morganella morganii* has the most common, rising from 23,4 % in 2020 to 43,6 % in 2022. With a starting point of 20,6 % in 2020 and a peak of 46,8 % in 2022, *Pseudomonas aeruginosa* likewise showed an increasing tendency. In 2020, *Acinetobacter* was absent; in 2021, it has been more prevalent (61,2 %) and in 2022, it has slightly less prevalent (42,2 %). These outcomes imply dynamic changes in the composition of bacteria throughout the investigation. The microbiological research yielded 6 species as shown in figure 1.

The following table shows the number of different bacterial species and the makeup of microorganisms in a certain environment. An analysis of several microbiological samples is shown in the sample's column. The total number of each type of bacteria in the entire microbial community is represented by its abundance. The percentage of bacteria that are resistant to primary and extended-spectrum medications is shown by Pan-Drug Resistant (PDR) and extensive drug resistance (EDR), correspondingly. 3,19 of the microbial population are made up of *Pseudomonas luteola*, of which 26,0 are resistant to extended-spectrum antibiotics and 51,3 to basic medicines. With a 7,60-sensitivity rate, *Escherichia coli* exhibit resistance to 59,34 of extended-spectrum antimicrobial agents. *Enterobacter* sp. accounts for 3,3 of the sample and has an astounding 98 efficacy against extended-spectrum antibiotics. The population is made up of *Pseudomonas aeruginosa* (45,2), which is resistant to antibiotics *Klebsiella pneumoniae* (2,97). With a composition of (16,85), *Acinetobacter baumannii* exhibits an (8,42) resistance to basic treatments and a high (93,60) sensitivity to extended-spectrum medicines. To comprehend and manage microbial communities in a variety of environments, it is crucial to know the frequency and patterns of resistance to medicines shown by these bacteria. The outcomes of the microorganisms from infected wounds are shown in table 4 and figure 2.

Table 4. Dispersion of isolated microorganisms from infected wounds			
Samples	Abundance N	(PDR) N	(EDR) N
<i>Pseudomonas luteola</i>	3,19	51,3	26
<i>Escherichia coli</i>	7,6	-	59,34
<i>Enterobacter</i> sp.	3,3	-	98
<i>Pseudomonas aeruginosa</i>	45,2	16,29	73,7
<i>Klebsiella pneumoniae</i>	30,2	2,97	69,65
<i>Acinetobacter baumannii</i>	16,85	8,42	93,6

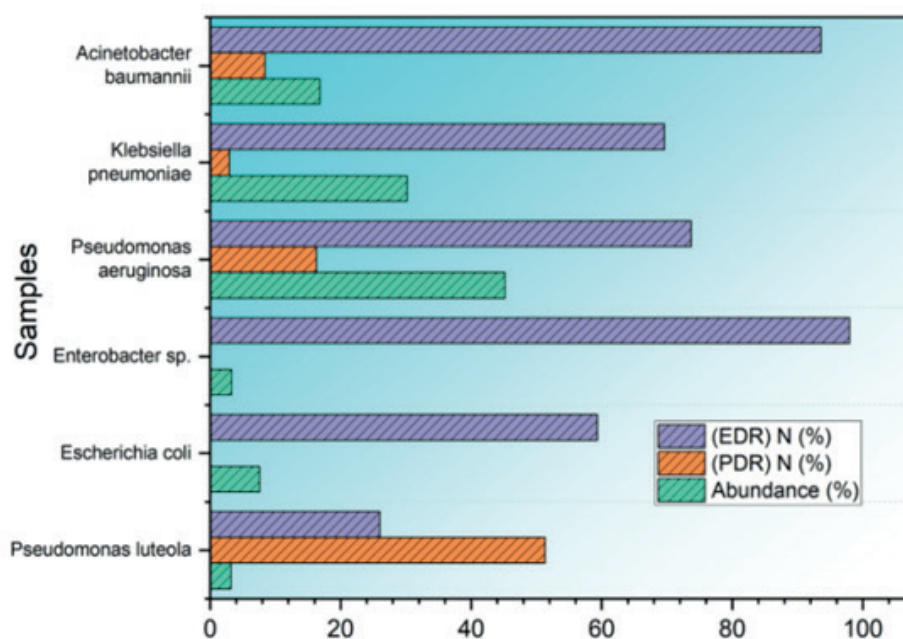


Figure 2. Outcomes of the microorganisms from infected wounds

For a variety of microorganisms, such as *Pseudomonas luteola*, *Escherichia coli*, *Enterobacter sp.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*, the both of antimicrobial resistances are shown in the following table against 6 different antibiotics represented by columns 1 through 6. Table 5 displays the proportion of resistances based on the total number of resistances observed for every combination of bacterium and drug. The data provides information on the frequency of antibiotic resistance in this particular context by indicating varied degrees of protest among the bacteria indicated.

No. of Resistances	Microorganisms					
	<i>Pseudomonas luteola</i>	<i>Escherichia coli</i>	<i>Enterobacter sp.</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Acinetobacter baumannii</i>
1 N (%)	2 (4,7)	2 (3,5)	5 (15,8)	3 (7,5)	4 (13,5)	4 (15,3)
2 N (%)	5 (16,6)	4 (7,6)	4 (13,4)	7 (20,4)	4 (17,7)	5 (20,0)
3 N (%)	2 (4,7)	3 (5,6)	3 (7,2)	2 (4,2)	3 (6,1)	4 (16,2)
4 N (%)	13 (47,2)	7 (12,6)	4 (13,4)	4 (8,9)	7 (26,0)	3 (9,7)
5 N (%)	-	-	-	-	2 (5,6)	-
6 N (%)	2 (4,9)	-	2 (6,6)	2 (4,3)	-	-

DISCUSSION

In microbiology labs, routine testing has relied on culture techniques that facilitate the process of identifying species and calculating antibiotic susceptibilities as a basis for treatment. These methods make it feasible to identify and separate infectious agents from swabs, pus, or biopsy specimens. International surveillance to stop the development of antibiotic resistance is made possible by standardized procedures. In this retrospective study, 6 microbiological species were found in wounds displaying indications of infection. Gram-negative bacteria are more common than Gram-positive bacteria, according to the data, which supported those of other studies. It was more difficult to eradicate bacteria when poly-microbial illnesses are present. Chronic wounds were poly-microbial, which means that they were likely to offer an environment that was favorable for the horizontal gene transfer of bacteria. When poly-microbial infections are present, eliminating bacteria becomes more challenging. Multiple targets or combinational treatments were necessary due to the existence of strains that were resistant to multiple drugs, the composition of the bio-films varies, the bio-films were resistant to antibiotics and the bio-films could be poly-microbial. Scheduling the isolates according to year was a crucial component as well.

CONCLUSION

To address wound infections and prevent the emergence of multidrug-resistant strains, physicians can find it useful to utilize the data presented in this study to produce guidelines for the selection of an appropriate treatment regimen based on microbiological examination a problem that is overlooked yet leads to chronic illness is wound infections. Over three years, there has been an increase in resistance to drugs Gram-negative bacteria, which highlights the need for continued, meticulous surveillance of the spread of multidrug-resistant strains throughout the nation's diverse regions. The results of this work are significant against particular bacteria species can lead physicians to prescribe some antimicrobials over others, which can reduce the amount of less effective medications used to treat infections in wounds. Findings of anaerobic microorganisms and functional but non-culturable bacteria are another crucial factor to consider. The greater than of wounds were shown to be poly-microbial by the molecular method, indicating that the microbial makeup with a wound is complicated. As a result, to comprehend the relationships and mutualism of the many microorganisms, a deeper examination of every microbial species found with an infected wound is required. To determine if one species predominates over another or whether there is some equilibrium between the many species, it can be necessary to assess the concentration of the various species with a poly-microbial illness. In the future, studies on resistance to antibiotics in microorganisms from wounds that are infected should concentrate on creating novel treatments, individualized regimens, and new diagnostic instruments. To address new issues as well as to maximize patient-specific treatments for improved wound care combined with infection control, it will be essential to integrate interdisciplinary attempts, such as genomes and nanomaterials.

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CONFLICT OF INTEREST

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