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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF WOUND ISOLATED BACTERIA

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Abstract
Antimicrobial resistance is increasing, making it more difficult to treat wound infections. Using the Antimicrobial Vitek 2 Compact System, the objective of this investigation was to determine which antibiotics were effective against wound-isolated bacteria. In Uttarakhand, we isolated MDR strains from a sample of 200 patients with wound infections. The Vitek 2 Compact System was used to determine the susceptibility of bacterial incision isolates to various antimicrobials. According to the findings, the most effective antimicrobial medications target S. aureus (42.66%), Klebsiella species (23.3%), Pseudomonas aeruginosa (19.33%), and Proteus mirabilis (14.6%). These findings emphasize the significance of constant monitoring for antibiotic resistance in bacteria isolated from wounds and have significant implications for the treatment of wound infections. The Vitek 2 Compact System was found to be a reliable instrument for determining bacterial susceptibility to various antimicrobials in this study.

Keywords: Wound İnfections, Multidrug Resistance, Vitek 2 Compact, Antimicrobial Resistance.

1. INTRODUCTION

A major global health concern that jeopardizes the effectiveness of present infection control efforts is antibiotic resistance in disease-causing microorganisms. Wound infections are a frequent healthcare issue that can be caused by a variety of microorganisms. If medical professionals have a solid understanding of the antimicrobial susceptibility profiles of the bacteria isolated from wounds [1], they can guide the proper antibiotic treatment and control drug-resistant strains.

Antimicrobial susceptibility testing has a significant role in the efficacy of antibiotics against certain bacterial isolates. It demonstrates which microorganisms are vulnerable to specific medications and which are resistant, assisting clinicians in selecting the best course of therapy. Gram-positive and Gram-negative bacteria can both infect wounds, including Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, and Enterobacter species [2].

The susceptibility patterns of bacteria isolated from wounds can vary regionally and over time due to several factors. Numerous factors, including patient demographics, local antibiotic prescribing practices, and medical conditions, have an impact on antibiotic resistance. This necessitates regular monitoring and surveillance of antimicrobial susceptibility patterns in order to spot emerging trends in resistance and inform the best possible antibiotic stewardship. By examining the antimicrobial susceptibility patterns of bacteria isolated from wounds, it is possible to discover the most effective treatments for wound infections. Methicillin-resistant Staphylococcus aureus (MRSA) & Enterobacteriaceae that produce extended-spectrum -lactamases (ESBLs) are just two examples of resistance patterns that it helps identify. Stopping

the growth of antibiotic-resistant bacteria requires both the wise use of medications and an awareness of susceptibility patterns [3].

1.1 Antimicrobial resistance in wound infections

The emergence of antibiotic resistance poses a significant threat to the effective treatment of bacterial, viral, fungal, and parasitic diseases [4, 5]. When bacteria evolve and become resistant to antimicrobial treatments, diseases become more difficult to treat and there is a greater chance of disease transmission, severe illness, and mortality. Antimicrobial resistance (AMR) is the term used to describe this phenomenon. A number of factors, including as the overuse and improper use of antibiotics in humans, animals, & agriculture, as well as the absence of effective infection prevention and control measures, have an impact on the emergence and spread of AMR [5].

As AMR has spread, it has become more challenging to treat wound infections in healthcare settings, which is a significant concern. Antimicrobial resistance (AMR) rates in wound infections are not uniform across the country or the world, but rather vary based on the character of the infecting bacteria and the patient population's location. Due to the rise of multidrug-resistant bacteria, which are resistant to a variety of antimicrobial drugs and thus more difficult to treat [6], wound infections in particular are becoming increasingly problematic. Antimicrobial susceptibility testing can assist physicians in selecting the most effective antibiotics for treating wound infections. In this assay, bacterial isolates from wound infections are used to determine the antibacterial efficacy of various medications. Clinicians can use the results of antimicrobial susceptibility testing to select the most effective antimicrobial medication for treating an infection, and antimicrobial stewardship programs can be developed using the data from these tests to reduce the likelihood of AMR arising and spreading [7].

1.2 Multidrug-resistant bacteria and its impact on wound infections

Multidrug-resistant (MDR) wound infections are a growing health concern because they are more challenging to treat and resistant to different classes of antimicrobial drugs [8]. Different kinds of bacteria can live in infectious wounds, and the frequency of multidrug-resistant bacteria varies by bacterial species and geographical location. Multidrug-resistant bacteria can infect a wound, leading to major effects such a prolonged hospital stay, increased medical costs, and even death [9].

MDR bacteria are mostly to blame for the introduction & spread due to misuse & overuse of antimicrobial drugs in humans, animals, & crops, as well as inadequate infection prevention & control methods. Since MDR bacteria can be found in the general populace, their spread is not limited to institutional settings. Community-acquired diseases brought on by bacteria with multiple medication resistance have become more common.

There is evidence from numerous research projects that MDR bacteria have an impact on wound infections. Researchers in Ethiopia discovered that many wound infections were brought on by various bacteria and showed resistance to numerous drugs in a retrospective investigation [10]. The most prevalent MDR bacteria were found to be Staphylococcus aureus & Pseudomonas aeruginosa in a wound infection investigation carried out in South-West Ethiopia [11].

Antimicrobial susceptibility testing is necessary for the efficient management of wound infections brought on by MDR bacteria. To do this, we test the ability of several antimicrobials to stop the spread of bacteria on bacterial isolates from wound infections. Antimicrobial stewardship initiatives can be based on the results of antimicrobial susceptibility testing to stop the spread of multidrug-resistant bacteria. Clinicians can use these data to choose the appropriate antimicrobial medication for treating a patient's infection.

2. RELATED STUDIES

Antimicrobial resistance is on the rise in hospital settings, making it more challenging to treat wound infections. Antimicrobial susceptibility testing is a crucial instrument for the treatment of wound infections. The aim of this research is to examine the antibiotic susceptibility profile of bacteria isolated from wound infections.

In a cross-sectional study conducted in Ethiopia, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* were the most frequently recovered bacteria from wound infections. Numerous polymicrobial and multidrug-resistant wound infections have been identified [12]. In another Indian study, the most commonly isolated bacteria from wound infections were *Staphylococcus aureus*, *Escherichia coli*, & *Pseudomonas aeruginosa*. Numerous bacterial isolates were resistant to conventional antimicrobials, and the investigation confirmed the prevalence of multidrug-resistant bacteria [13].

Over a period of three years, the antibiotic susceptibility profile of bacterial isolates from infected lesions in Italy was retrospectively examined. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were the microbes most commonly isolated from wound infections. An additional finding of the study [14] was that numerous bacterial isolates were resistant to a variety of antimicrobials.

In China, individuals with chronic cutaneous wounds were evaluated for patterns of pathogenic microorganisms and antibiotic resistance. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were the most commonly isolated bacteria from chronic cutaneous lesions. Numerous bacterial isolates were found to be resistant to conventional antimicrobials, and the investigation confirmed the pervasive occurrence of multidrug-resistant bacteria [15].

It is crucial to prevent the development and dissemination of antibiotic resistance that wound care incorporate antimicrobial stewardship. According to a position paper from the British Society for Antimicrobial Chemotherapy & the European Wound Management Association, antibiotic treatment for incisions should only be given in instances of clinical infection. In addition, the report recommends that clinicians base their empirical antibiotic therapy decisions on local antibiotic resistance data and treat for the most probable bacteria based on patient presentation [16].

3. MATERIAL AND METHODS

Collection site: With the approval of the ethical committee (ECR/710/Inst/UK/2015/RR-21), clinical samples were taken from suspected patients at Shr Mahant Indresh Hospitals in Doon Valley in 2020 and 2021**.**

3.1 Sample collection: Samples will be taken from various types of wounds, including pus, wounds, blood, & ascetic/plural fluids. A total of 200 wound samples will be collected with sterile cotton swabs or other appropriate collection methods.

3.2 Isolation: Pus, blood, urine, and ascetic fluids swab specimens were inoculated on Blood agar, MacConkey agar, Nutrient agar, and Potato Dextrose agar plates, per clinical laboratory guidelines. On the basis of colony form, size, shape, pigmentation, margin, & elevation, the bacteria were initially verified. The isolated organisms were identified using a variety of biochemical assays and Gram staining techniques. Then, testing for antibiotic susceptibility was conducted. After appropriate incubation (overnight at 37 degrees Celsius), each culture plate was carefully examined for microbial growth. In order to identify bacterial isolates, sterile media were used to conduct biochemical tests [17].

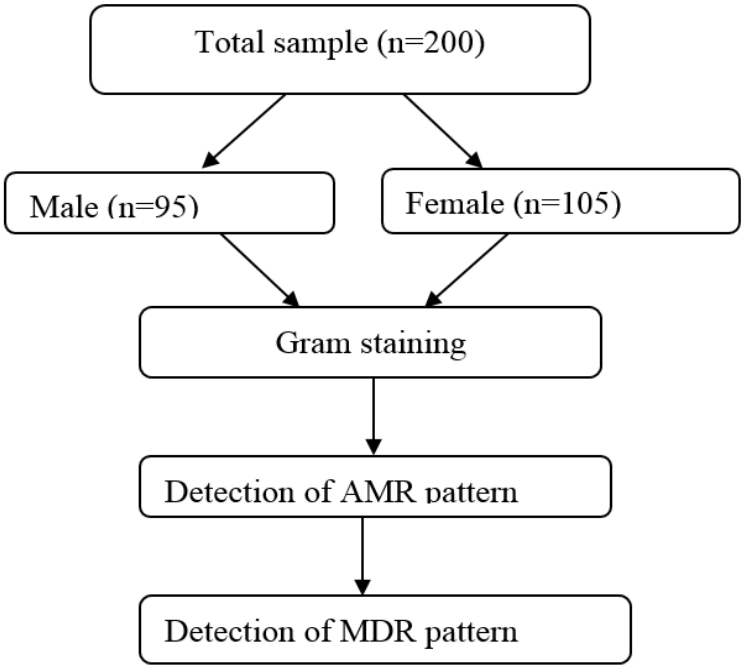


Fig 1: Workflow outline

3.3 Identification from Viteck 2 compact system

The Vitek 2 Compact System is a widely utilized automated platform for bacterial identification and susceptibility testing. It employs cutting-edge technology to accurately & rapidly identify wound-isolated microorganisms. Several biochemical tests and algorithms are utilized to determine the bacterial species present in the lesion sample during the identification process.

The lesion specimen is initially processed and cultured on appropriate agar plates to permit bacterial growth. After observing colonies, a subset of the isolated colonies is chosen for further analysis with the Vitek 2 Compact System. The chosen communities are transferred into the Vitek 2 system, which consists of identification cards or panels with specialized information. These cards include metabolic, enzymatic, and growth-related biochemical analyses. The Vitek 2 system inoculates the cards automatically with the bacterial suspension and incubates them under controlled conditions.

The Vitek 2 system monitors the growth and biochemical reactions of bacterial isolates during the incubation period. It measures various parameters, including pH changes, gas production, and color changes, in order to generate a metabolic fingerprint for each bacterial species. Within the Vitek 2 system, the generated data is then compared to an extensive database of reference profiles. This database comprises a wide variety of bacterial species as well as their biochemical profiles. The system employs sophisticated algorithms to match the observed metabolic fingerprint of the test isolate to the profiles contained in the database.

The Vitek 2 system provides a probable identification of the wound-isolated bacteria based on the comparison results. Typically, the identification is reported as the most probable species alongside a confidence level. Notably, the system may occasionally provide multiple prospective identifications or indicate an uncertain identification, necessitating additional testing for confirmation.

The identification provided by the Vitek 2 system is a valuable resource for clinicians in determining the most effective antibiotic treatment. Different bacterial species may exhibit varying susceptibilities to antimicrobial agents; therefore, accurate identification is essential for choosing the most effective treatment.

Fig 2: Isolation of Bacteria Using Vitek 2 Machine

3.3.1 Sealing and Incubation of Cards

After placing the selected colonies into the Vitek 2 system, the identification cards are secured and incubated. To prevent contamination & optimize bacterial growth & metabolic processes, it is necessary to seal identification card wells. Vitek 2 technology automates the sealing process. The technique uses specific films or covers to seal identification cards. The sterility of the bacterial isolates is maintained by the close seal of these films.

After being sealed, the identification cards are placed in the incubation module of the Vitek 2 system, where 30 to 60 cards are arranged in a line at 36+1.0C. The incubation module regulates temperature, humidity, and oxygen concentration for bacterial growth and metabolism. During incubation, which can last anywhere from a few hours to a day, the bacterial isolates endure metabolic processes. These reactions result in byproducts, pH alterations, and additional effects.

Vitek 2 monitors and analyzes these metabolic responses continuously. It records biochemical test data and interprets it in real time. The identity cards remain enclosed within the incubation module for the duration of the incubation and metabolic reactions. The cards are withdrawn from the module for evaluation and interpretation following incubation.

Fig 3: identification cards are used for isolation and identification of bacteria in vitek 2 machine

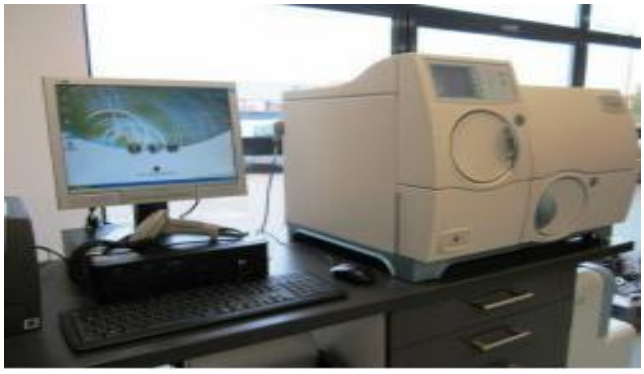


Fig 4: VITEK 2 Compact Instruments and Workstation

3.4 Antimicrobial susceptibility pattern analysis

To prepare inoculums, pure-growth colonies isolated after 18 to 24 hours of culture were suspended in half-strength saline and adjusted to a turbidity equivalent to 5macfarland index; this suspension must be used within 15 minutes. In order to culture Gram-negative bacteria, 3 ml of half-strength saline should be added to a polysterinzviteck tube. Insects and a vitek GN card Transfer the suspension of organisms from 145 ml to 3 ml of saline. Using GP cards, Gram-positive bacteria were grown by adding 280micro Itr of bacterial suspension to 3 ml of saline. Inventory from the correct Viteck tube card was polluted into the suspension tube. The card must be activated within two hours of suspending liquid. To submit data, launch the immusion light vitek. Enter the predicted identification number and assertion number in addition to the test specimen number and date. Simply position the caste in the filler box, wait for the filling cycle to complete, unlock the loading door, and then scan the barcode on the virtual caste to initiate the automatic filling and sealing of the vacuum device. In order to allow sufficient time for examination & organism identification, card processing typically takes between seven and twelve hours. Tomorrow, you will learn the outcome. Each ATCC batch's QC strains' instrument results must lie within the acceptable range. Compare a transcript to the biochemical outcomes, morphological

characteristics, and growth characteristics. Based on an analysis of the zones of inhibition, two distinct levels have been identified: sensitive and resistant.

The following are common methods to characterize the outcomes:

- **Susceptible**: The treatment that was tried slowed or killed the bacteria that was causing your sickness. The medication could be an effective therapy option.
- **Intermediate**: A greater dose of the medication may be effective.
- **Resistant**: Antibiotic did not stop the infection from spreading or destroy the bacteria that was causing it. It would not be a good therapeutic option.

4. STATISTICAL ANALYSIS

The analysis was conducted using statistical software such as SPSS & MS-Excel, descriptive statistics such as frequency distribution, cross-tabulation, & pie charts, & descriptive statistics were used to determine the patterns of AMR and MDR.

5. RESULTS

A total of 200 samples are chosen to isolate the wound associates bacteria. 191 samples out of 200 were confirmed as highly pathogenic strain and 82 as MDR. In pathogenic strain, 50 S. aureus, 27 Klebsiella species, 45 Pseudomonas aeruginosa, 20 Proteus mirabilis, 17 Acinetobacter baumannii 18 E.coli, 08 Enterobacter cloacae and 06 Enterococcs faeclis. The information can be obtained by analyzing the antibiotic susceptibility patterns of clinical sample isolates of bacteria. S. aureus was isolated the most frequently (32.46%), followed by Klebsiella species (16.23%), Proteus mirabilis (7.85%), Pseudomonas aeruginosa (24.08%), Acinetibacter baumanni (8.37%), E.coli (6.80%), Enterobacter cloacae (2.61) and Enterococs faeclis (1.57%). Table 1 presents the gender and age distribution of clinical samples (n = 200). Table 2. Distribution of clinical samples according to pathogenic and non pathogenic strains. Table 3 consists distribution of various bacterial isolates grown in clinical samples as well,Tables 4-10 show visual representations of isolated microorganisms.

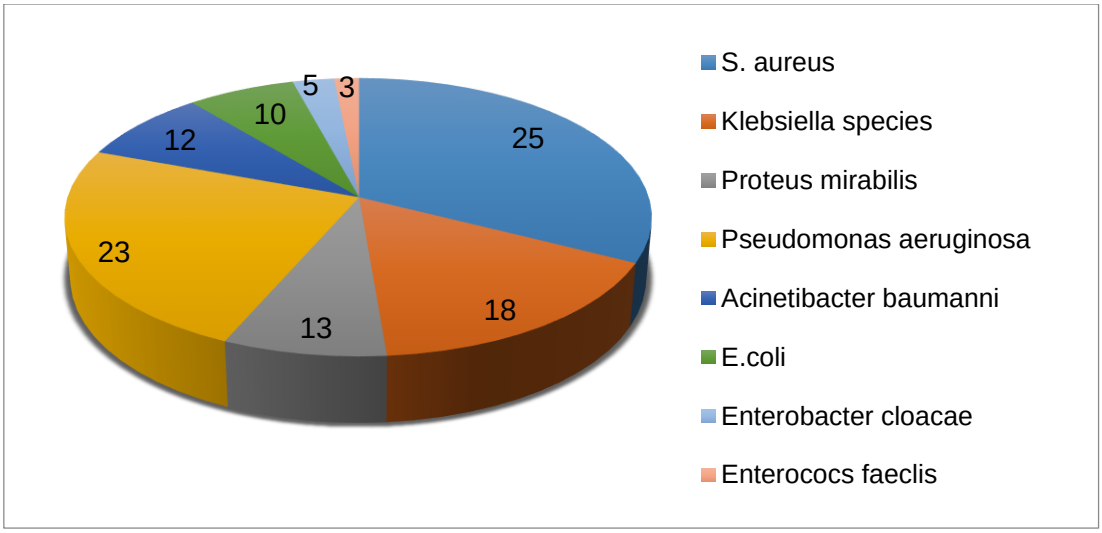
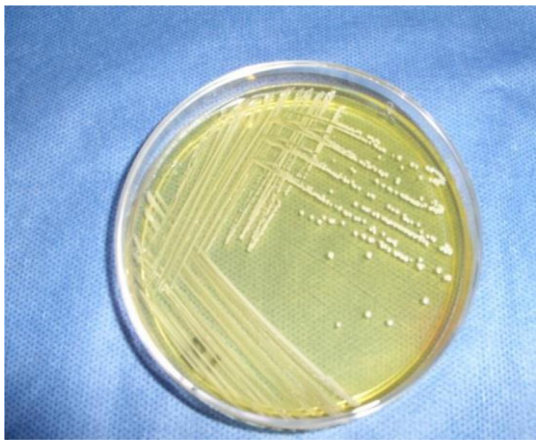
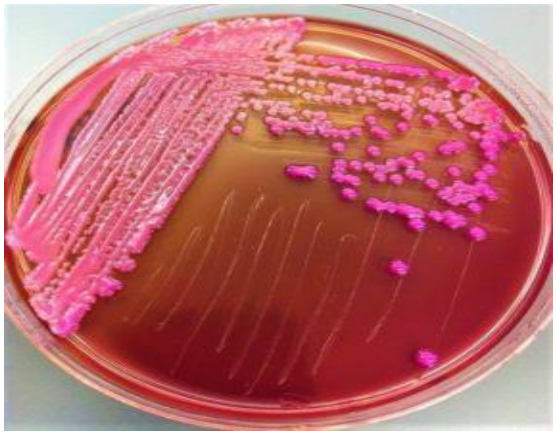


Fig 5: Distribution of various isolates grown in clinical samples (n= 200)

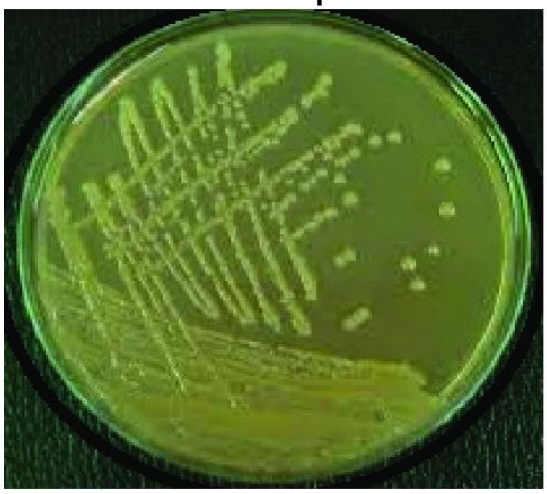


Staphylococcus aureus

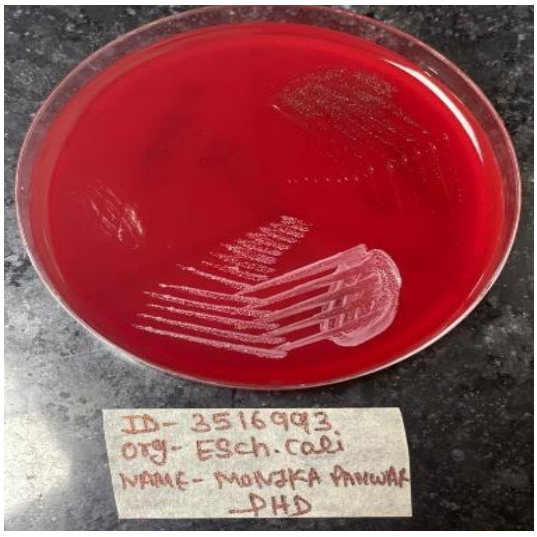


Klebsiella species

Proteus mirabilis



Pseudomonas aeruginosa



E.coli

Acinetibacter baumanni

Table 1: Age-wise and gender distribution of clinical samples (n=200)

Age group	Male	Female	TOTAL
0-10	13	10	23
11-20	11	15	26
21-30	14	19	33
31-40	16	22	38
41-50	15	14	29
51-60	18	21	39
61-70	08	04	12

Table 2: distribution of clinical samples according to pathogenic and non-pathogenic strains.

Age	Pathogenic	Non Pathogenic	MDR
0-10	21	2	10
11-20	26	1	12
21-30	32	-	14
31-40	35	3	11
41-50	29	2	13
51-60	37	-	15
61-70	11	1	07

Table 3: Distribution of microorganism grown in clinical samples (n=191)

Bacteria isolates from wound sample	Total
S. aureus	50
Klebsiella species	27
Proteus mirabilis	20
Pseudomonas aeruginosa	45
Acinetobacter baumannii	17
E.coli	18
Enterobacter cloacae	08
Enterococcus faecalis	06

Table 4: Visual representation of isolated bacteria (*S. aureus*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
S. aureus		
Cefoxitin Screen	POS	+
Benzylpenicilin	≥0.5	R
Oxacillin	≥4	R
Gentamicin High Level	-	-
Gentamicin	≥16	R
Ciprofloxacin	≥8	R
Levofloxacin	≥8	R
Inducible clindamycin	NEG	-
Erythromycin	≥8	R
Clindamycin	≥4	R
Linezolid	≥8	R
Daptomycin	≥8	-
Teichoplanin	4	S
Vancomycin	2	S
Tetracycline	≥16	R
Tigecycline	1	
Nitrofurantoin	64	I
Rifampicin	≥4	R
Trimethoprim/ sulfame	≤10	S

Table 4: Visual representation of isolated bacteria (*Klebsiella species*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Klebsiella species		
Ampicillin	≥32	R
Amoxicillin/ clavularic	≥32	R
Piperacillin/ Tazobactam	≥120	R
Cefuroxime	≥64	R
Cefuroxime Axebl	≥64	R
Ceftriaxone	≥64	R
Cefoperazone/ sulbac……	≥64	R
Cefepime	≥64	R
Ertapenem	≥8	R
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	4	R
Gentamicin	≤1	R
Nalidixic acid	≥32	R
Ciprofloxacin	≥4	R
Tigecycline	≤0.5	R
Nitrofurantoin	128	R
Colistin	≤0.5	S
Trimethoprim/ sulfame	≥320	R

Table 5: Visual representation of isolated bacteria (*Proteus mirabilis*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Proteus mirabilis		
Ampicillin	≥32	R
Amoxicillin/ clavularic	≥32	R
Piperacillin/ Tazobactam	≤4	R
Cefuroxime	≥64	R
Cefuroxime Axebl	≥64	R
Ceftriaxone	8	R
Cefoperazone/ sulbac……	32	R
Cefepime	32	R
Ertapenem	2	R
Imipenem	≥16	R
Meropenem	1	S
Amikacin	4	S
Gentamicin	≥16	R
Nalidixic acid	≥32	R
Ciprofloxacin	≥4	R
Tigecycline	4	R
Nitrofurantoin	128	R
Colistin	≥16	R
Trimethoprim/ sulfame	≥320	R

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Table 6: Visual representation of isolated bacteria (*Pseudomonas aeruginosa*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Pseudomonas aeruginosa		
Ticarcillin/ clavularic acid	64	I
Piperacillin/ Tazobactum	≤4	S
Ceftazidime	4	S
Cefoperazone/ sulbac……	≤8	S
Cefepime	4	S
Aztronam	16	I
Doripenem	4	I
Imipenem	8	R
Meropenem	2	S
Amikacin	8	S
Gentamicin	8	I
Ciprofloxacin	≤0.25	S
Levofloxacin	1	S
Minocycline	-	-
Tigecycline	≥8	R
Colistin	2	S
Trimethoprim/ sulfame	-	-

Table 7: Visual representation of isolated bacteria (*E.coli*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
E. coli		
Ampicillin	≥32	R
Amoxicillin/ clavularic acid	≥32	R
Piperacillin/ Tazobactum	≥128	R
Cefuroxime	≥64	R
Cefuroxime Axetil	≥64	R
Ceftriaxone	≥64	R
Cefoperazone/ sulbac……	≥64	R
Cefepime	≥64	R
Ertapenem	≥8	R
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	≥64	R
Gentamicin	≥16	R
Nalidixic acid	≥32	R
Ciprofloxacin	≥4	R
Tigecycline	1	S
Nitrofurantoin	64	I
Colistin	≤0.5	S
Trimethoprim/ sulfame	≥320	R

Table 8: Visual representation of isolated bacteria (*Acinetibacter baumanni*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Acinetibacter baumanni		
Ticarcillin/ clavularic acid	≥128	R
Piperacillin/ Tazobactum	≥128	R
Ceftazidime	≥64	R
Cefoperazone/ sulbac……	≥64	R
Cefepime	≥64	R
Aztronam	-	-
Doripenem	≥8	R
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	≥64	R
Gentamicin	≥16	R
Ciprofloxacin	≥4	R
Levofloxacin	≥8	R
Minocycline	≥16	R
Tigecycline	1	S
Colistin	≤0.5	S
Trimethoprim/ sulfame	≥320	R

Table 9: Visual representation of isolated bacteria (*Enterobacter cloacae*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Enterobacter cloacae		
Ticarcillin/ clavularic acid	≥128	R
Piperacillin/ Tazobactum	≥128	R
Ceftazidime	≥64	R
Cefoperazone/ sulbac……	≥64	R
Cefepime	≥64	R
Aztronam	32	R
Doripenem	≥8	R
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	≥64	R
Gentamicin	≥16	R
Ciprofloxacin	≥4	R
Levofloxacin	≥8	R
Minocycline	≥16	R
Tigecycline	2	R
Colistin	≤0.5	S
Trimethoprim/ sulfame	≥320	R

Table 10: Visual representation of isolated bacteria (*Enterococs faeclis*)

Antibiotic	MIC value (ug/ml)	Resistance/ Sensitive
Entero Faecium		
Cefoxitin Screen	-	-
Benzylpenicilin	8	S
Oxacillin	-	-
Gentamicin High Level	SYN- R	R
Gentamicin	-	-
Ciprofloxacin	≥8	R
Levofloxacin	≥8	R
Inducible clindamycin	-	-
Erythromycin	≥8	R
Clindamycin	-	-
Linezolid	2	S
Daptomycin	2	S
Teichoplanin	≤0.5	S
Vancomycin	2	S
Tetracycline	≥16	R
Tigecycline	≤0.12	S
Nitrofurantoin	64	I
Rifampicin	-	-
Trimethoprim/ sulfame	-	-

7. CONCLUSION

Our study's findings emphasize the importance of selecting the appropriate antibiotics for a patient based on the results of susceptibility testing. More specifically, we examined how various bacteria isolated from wounds responded to various antibiotics. The findings have significant repercussions for clinical practitioners since they will assist them in providing more effective treatment for wound infections while simultaneously regulating and preventing antibiotic resistance. The investigation led to the identification of 191 pathogenic bacteria from which 87 were MDR strains out of a total of 200 samples. These findings highlight the relevance of antibiotic stewardship as well as the necessity of selecting the appropriate antibiotic to treat wound infections.

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