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Original Research Article

Evaluation of multidrug resistant pathogens in wound infections: Bacteriological profiles and clinical outcome implication

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Abstract

Background: One of the most common presentations in the outpatient department is pyogenic or wound infections. Multidrug-Resistant (MDR) bacterial infection is considered as one of the significant risk factor for impaired wound healing. The aim of this study is analysing bacteriological profiles and antibiotic sensitivity patterns from pus samples, and examine how these organisms correlate with patient outcomes.

Materials and Methods: During the period of retrospective cross-sectional study, around 940 pus samples from wounded sites were collected and processed in Microbiology department as per the standard guidelines. Clinical data was also analysed to correlate MDRO presence with infection outcomes. All prepared biochemical and streaking media were checked for their sterility. Quality control Strains were used as reference strains for quality control of AST and biochemical tests

Results: Majority of the organisms isolated were *Pseudomonas* (23.0%), *Staphylococcus aureus* (21%), *E.coli* (17.5%), *Klebsiella* (17.2%). ESBL production was observed more in *E.coli* (58.4%) followed by *Klebsiella* (50.9%) and *Proteus* (24.2%). MBL production was noted in 27.3% isolates of *E.coli*, 21.1% of *Klebsiella* and 6.06% of *Proteus* isolates. ESBL production was observed more in *Pseudomonas* (52.1%) followed by *Acinetobacter* (51.6%). MBL production was noted in 32.2% isolates of *Pseudomonas*, and 41.7% of *Acinetobacter* isolates. MRSA was noted in 77.08% isolates of wound infections.

Conclusion: Our study highlights that MDRO-infected wound require prolonged hospital stays, intensive interventions, and alternative therapies due to limited antibiotic options. Effective antimicrobial stewardship, rapid diagnostics, and strict infection control measures are essential to curb resistance and improve outcomes.

Keywords: Multidrug resistant pathogens, Wound infections, Microbiotia

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1. Introduction

Pyogenic infections refer to infections causing pus formation. Pus is a collection of thick, white or yellow fluid, formed at the site of inflammation during infection. It is made up of dead tissue, white blood cells, and damaged cells. Invasion of external pathogens and action of their toxic metabolites and leukocidins in tissues causes formation of pus.

One of the most common presentations in the outpatient department is pyogenic or wound infections which had a wide clinical spectrum including skin, surgical site infections, soft tissue infections, diabetic wound, and abscesses. These infections are frequently caused by a diverse range of bacteria, including methicillin resistant *Staphylococcus*

aureus (MRSA), E. coli, K. pneumoniae, P. aeruginosa and many others.²

The crude mortality rate due to infectious diseases in India is approximately 417 per one lakh persons.³ The rise and spread of antibiotic-resistant bacteria have drastically limited treatment options for infectious diseases, leading to increased morbidity, mortality, and healthcare expenses worldwide. It is estimated that antimicrobial resistance kills at least 1.27 million people every year and it could increase up-to 10 million people per year by 2050.² Understanding the antibiotic sensitivity patterns of prevalent pathogenic bacteria found in pus samples can provide valuable insights into appropriate antibiotic selection, dosage optimization, and effective treatment strategies.²

*Corresponding author: Srilalitha Vishnubhatla Email: srilalithavishnubhatla@gmail.com The evaluated root cause behind the poor wound healing include age where poor wound healing increases by 34% for each additional year of age, association with injuries to the lower limbs and trunk and perineum, outdoor injuries, co morbidities like chronic diseases, immunosuppression, malnutrition, radiation therapy and vascular insufficiencies, large wounds and stress. Along with these factors a Multidrug-Resistant (MDR) infection is considered a significant risk factor for impaired wound healing, as the bacteria causing such infections are resistant to multiple antibiotics, making treatment difficult and potentially prolonging the healing process, leading to complications like chronic wounds and increased risk of further infection.

2. Aim

To investigate the impact of MDROs (MultiDrug Resistant Organisms) on Wound Infections by analysing bacteriological profiles and antibiotic sensitivity patterns from pus samples, and examine how these organisms correlate with patient outcomes.

3. Objectives

- 1. To isolate, identify and perform antibiotic sensitivity testing of bacterial pathogens from pus samples.
- 2. To correlate the presence of MDROs with clinical outcomes and assess the risk factors contributing to these infections.

4. Materials and Methods

4.1. Study design

A retrospective cross-sectional study (September 2023 to September 2024) was undertaken in the teaching hospital of KIMS&RF, Amalapuram.

4.2. Sample size

All the Pus samples collected in the lab from September 2023 to September 2024 were included in the study.

4.3. Ethics committee approval

Ethical approval was obtained from the Institutional Ethics Committee before conducting the study (Approval Letter No-IEC/CD/2025). Prior consent was obtained from all participants.

4.4. Sample collection

All pus samples from patients attending the OPD (outpatient department) or admitted in wards with infected wounds that were collected following standard procedures and received to the Microbiology lab were used for the study. During the period of study, around 940 pus samples from wounded sites of both diabetic and non-diabetic patients with complications like venous ulcers, superficial abscesses, and traumatic injuries were collected and included in the study. The patients

belonging to both genders and age group of 01 to 90 years were included.

4.5. Inclusion criteria

The pus samples (pus aspirate and wound swab) from patients attending the outpatient department (OPD) or patients admitted in wards (in patients) with wound infections were collected in the Microbiology lab of KIMS College Hospital, Amalapuram, India, between September 2023 and September 2024.

4.6. Exclusion criteria

Samples collected without following standard guidelines like improper sample collection and recent trauma cases were not considered as wound infections.

Pus samples were collected and bacteriological identification and antibiotic sensitivity testing was done. Clinical data was also analysed to correlate MDRO presence with infection outcomes. Risk factors would be evaluated to understand the impact of presence of MDROs.

4.7. Quality control

All prepared biochemical and streaking media were checked for their sterility. Strains of *E. coli* ATCC 25922, *E.faecalis* ATCC 29212 and *S. aureus* ATCC 25923 were used as reference strains for quality control of AST and biochemical tests. The same strain of E. coli was also considered as a negative control during the screening and phenotypic confirmation (Double Disc Synergy Testing) tests of ESBL producing Gram-negative bacilli.

4.8. Analysis of sample

- Collected samples were streaked on media such as 5% sheep blood agar, and MacConkey agar and incubated at 37 °C for 24h.
- 2. Direct microscopic examination of Gram-stained smears of isolates.
- 3. Additional tests included Coagulase test, Arabinose and other sugar fermentation tests, species specific identification tests, Optochin and Bacitracin sensitivity test, and specific biochemical tests such as indole test, citrate utilization tests, urease test to identify Enterobacteriaceae members. After 24 hours of incubation, the bacteria were identified by colony characteristics and biochemical reactions.

4.9. Antibiotic sensitivity testing

Antimicrobial susceptibility of the isolates was assessed on Muller Hinton Agar plates using Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards institute (CLSI) guidelines. The list of antibiotics tested include ampicillin (AMP-10µg), amoxyclav (AMC-20/10µg), ampicillin+sulbactum (A/S-10/10µg), ceftazidime

(CAZ-30 μg), cefoxitin (CX-30μg), cefotaxime (CTX-30 μg), ceftriaxone (CTR-30μg), cefaperazone+sulbactum (CFS-75/30μg), Ceftazidime+clavulanic acid (CAC-30/10 mcg), ciprofloxacin (CIP-5 μg), clindamycin (CD-2μg), azithromycin (AZM-15μg), gentamycin (G-10 μg), high level gentamycin (GeH-120 μg), levofloxacin (LE-5μg), linezolid (LZ-30μg), meropenem (MER-10μg), piperacillin+tazobactum (PIT -100/10 μg), tetracycline (TE-30μg), minocycline (MI – 30 mcg), tobramycin (TOB-10mcg), cotrimoxazole (COT-1.25μg), teicoplanin (TEI-30μg), and vancomycin (VA-30μg).

4.10. Phenotypic detection of multi drug resistance in pus isolates

4.10.1. Extended spectrum β lactamase

ESBL producers were identified by using Cefotaxime (30 mcg) & Ceftazidime disc (30 mcg), alone and in combination with clavulanic acid by using disc diffusion method and interpreted as per CLSI guidelines. Bacterial isolates showing ceftazidime < 22 mm, and cefotaxime < 27 mm are the possible ESBL producers. An increased difference in zone of inhibition ≥ 5mm between Cefotaxime/Ceftazidime and clavulanic acid combination (30/10 mcg) is suggestive of ESBL producers. Klebsiella pneumoniae ATCC 700603 and Escherichia coli ATCC 25922 were used as control for ESBL production. The suspected ESBL producer strains were subjected to double disc synergy test (DDST) for the confirmation of ESBL producing Enterobacteriaceae.

4.10.2. Screening and confirmation for MBL producers

All the isolates that showed resistance to Imipenem were tested for Metallo – Beta – Lactamase (MBL) production by phenotypic test, Imipenem-EDTA-combined disc test method. EDTA being a chelating agent removes Zinc ions from the active site of the MBL enzyme. This makes the enzyme inactive and thus the organism becomes sensitive to Carbapenems. The difference of 7mm between the inhibition zone diameter of the IPM-EDTA disc and that of IPM only disk was considered to be a positive for the presence of MBLs.¹⁶

4.11. Statistical analysis

Data was analysed by SPSS software version 21. Calculation of Mean, Standard Deviation was done for Quantitative data and calculation of frequency, percentages and Odds ratio was calculated to compare two groups, the p value <0.05 is considered as statistically significant. Inter Quartile Range (IQR) was calculated for the necessary parameters.

5. Results

In this study, we have projected the data of multidrug resistant organisms in wound infections and the outcome of patients affected by MDROs. Total pus samples collected and processed for analysis were 940 and the positive cultures were noted in 604 (64.2%). Majority of the organisms isolated were *Pseudomonas* (23.0%), *Staphylococcus aureus* (21%), *E.coli* (17.5%), *Klebsiella* (17.2%) [**Figure 1**].

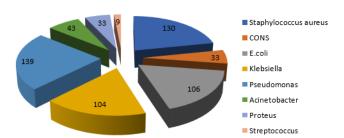


Figure 1: Distribution of pathogens in wound infections

Based on the antibiotic susceptibility testing the Enterobacteriaceae family isolates were highly susceptible to meropenem, piperacillin+tazobactum, gentamycin, followed by cefaperazone+sulbactum, ceftazidime, levofloxacin, tetracycline. ESBL production was observed more in *E.coli* (58.4%) followed by *Klebsiella* (50.9%) and *Proteus* (24.2%). MBL production was noted in 27.3% isolates of *E.coli*, 21.1% of *Klebsiella* and 6.06% of *Proteus* isolates.(**Table 1**)

Non fermenters were highly susceptible to meropenem, tobramycin, piperacillin+tazobactum, gentamicin, cotrimoxazole followed by cefaperazone + sulbactum, ceftazidime, tetracycline. ESBL production was observed more in *Pseudomonas* (52.1%) followed by *Acinetobacter* (51.6%). MBL production was noted in 32.2% isolates of *Pseudomonas*, and 41.7% of *Acinetobacter* isolates.(**Table 2**)

S.aureus was highly susceptible to linezolid, minocycline (95%), teicoplanin (86%), vancomycin (83%), doxycycline (82%). MRSA was noted in 77.08% isolates of wound infections. Similar susceptiblity range was noted in *Streptococcus* and *Enterococcus*. No vancomycin resistant isolates were observed.(**Table 3**)

On assessing the outcome of MDR and NON MDR bacteria infected patients in wound infections, we noted MDR (Multidrug resistant group) patients' length of stay, readmissions and mortality rate is high when compared to Non MDR group.(**Table 4**)

Table 1: Antibiotic susceptibility pattern of Enterobacteriaceae family

Organism	No. of	AMC	AS	CAZ	CTR	LE	TE	G	COT	PIT	CFS	CAV	MRP	MI
	isolates													
E.coli	106	22%	20%	27%	25%	29%	45%	-	59%	46%	51%	58%	68%	63%
Klebsiella	104	IR	21%	46%	19%	47%	58%	75%	33%	77%	48%	47%	82%	38%
Proteus	33	44%	47%	42%	45%	24%	IR	-	43%	74%	63%	64%	79%	40%

*AMC - Amoxiclav, AS - Ampicillin+Sulbactum, CAZ - Ceftazidime, CTR - Ceftriaxone, LE - Levofloxacin, Tetracycline, G-Gentamicin, COT - Cotrimoxazole, PIT - Piperacillin+ tazobactum, CFS - Cefaperazone+sulbactum, CAV - Ceftazidime+Avibactum, MRP - Meropenem, MI-Minocycline.

Table 2: Antibiotic susceptibility pattern of Non fermenters

Organism	No. of isolates	ТОВ	AS	CAZ	CTR	LE	TE	G	COT	PIT	CFS	CAV	MRP	MI
Pseudomonas	322	74%	22%	52%	IR	29%	IR	79%	IR	81%	53%	47%	80%	35%
Acientobacter	151	96%	49%	11%	12%	28%	46%	38%	69%	40%	37%	37%	40%	63%

*TOB - Tobramycin, AS - Ampicillin+Sulbactum, CAZ - Ceftazidime, CTR - Ceftriaxone, LE - Levofloxacin, Tetracycline, G-Gentamicin, COT - Cotrimoxazole, PIT - Piperacillin+ tazobactum, CFS - Cefaperazone+sulbactum, CAV - Ceftazidime+Avibactum, MRP - Meropenem, MI-Minocycline.

Table 3: Gram positive isolates susceptibility pattern

Organism	No. of	AMP	A	ΑZ	CT	CT	CI	C	C	G	LE	H	C	D	V	LZ	MI	TE
	isolates		M	M	R	X	P	D	X			L	О	O	A			I
			C									G	T					
S.aureus	484	21%	61	29	35	31	25	68	23	72	30	-	70	82	83	95	95	86
			%	%	%	%	%	%	%	%	%		%	%	%	%	%	%
CONS	141	-	46	32	42	36	25	44	32	74	-	-	-	72	66	93	62	-
			%	%	%	%	%	%	%	%				%	%	%	%	
Streptococci	95	70%	48	24	52	57	32	77	18	-	-	-	-	79	64	88	83	-
			%	%	%	%	%	%	%					%	%	%	%	
Enterococci	42	40%	IR	IR	IR	IR	13	IR	IR	IR	-	53	IR	72	75	92	61	-
							%					%		%	%	%	%	

*AMP-Ampicillin, AMC - Amoxiclav, AZ - Azithromycin, CTR - Ceftriaxone, CTX - Cefotaxime, CIP - Ciprofloxacin, CD - Clindamycin, CX - Cefoxitin, G - Gentamicin, LE - Levofloxacin, HLG-High Level Gentamicin, COT - Cotrimoxazole, DO - Doxycycline, VA-Vancomycin, LZ-Linezolid, MI-Minocycline, TE-Tetracycline.

Table 4: Outcome analysis of MDR and Non MDR group in wound infections

Outcome feature	MDR group (n=604)	Non MDR group (n=336)	OR	P value
Recurrence	124 (20.5%)	55 (16.3%)	1.3198	0.1202
Length of stay, Median (IQR)	10 (6 to 12)	5 (2 to 7)	-	< 0.001
Readmission	243 (40.2%)	67 (19.9%)	2.7026	< 0.001
Mortality	72 (11.9%)	12 (3.57%)	3.6541	0.0001

*OR - Odds Ratio

6. Discussion

Antimicrobial resistance is currently one of the most important public health problems. The irrational use of broad spectrum antibiotics for wound infections is responsible for increase in antibiotic resistance. Delayed wound healing leads to increased hospital visits, risk of acquiring infections and also patients become vulnerable to complications like decreased mobility, low immunity and reduced quality of life. Usage of expensive antibiotics during the hospital stay causes increased healthcare costs, numerous side effects, and financial loss to families.

6.1. Key points about MDR infections and wound healing:

6.1.1. Impaired healing process

MDR bacteria can disrupt the normal stages of wound healing by producing toxins, creating biofilms that impede cell migration and tissue regeneration, and causing inflammation that further hinders the healing process.⁵

6.1.2. Treatment challenges

Due to their resistance to multiple antibiotics, treating MDR infections often requires broad-spectrum antibiotics with potentially higher side effects, further impacting the healing process.⁵

6.1.3. Prolonged healing time

Wounds infected with MDR bacteria tend to take significantly longer to heal compared to wounds with susceptible bacteria, increasing the risk of complications like tissue necrosis and further infection.⁶

6.1.4. High-risk patient populations

Patients with chronic conditions like diabetes, compromised immune systems, or those undergoing extensive surgery are particularly vulnerable to MDR infections and associated wound healing complications.⁷

The culture positivity in this study is 64.2% which is similar to other studies, noted around 60%. ^{8,9} A Study done in Nepal on wound infections showed similar culture positivity. ¹⁰ Majority of the organisms isolated were *Pseudomonas* (23.0%), *Staphylococcus aureus* (21%), *E.coli* (17.5%), *Klebsiella* (17.2%) in the present study. A study from western Rajasthan in India conducted a similar study where 75.53% of wound swabs and 24.47% of pus samples were collected from various pyogenic infections of patients. They observed Staphylococcus aureus (30.9%) as a predominant pathogen followed by *Escherichia coli* (24.76%), *Pseudomonas aeruginosa* (16.68%), and *Klebsiella* (14.4%). ⁹

A study conducted by Rijal BP et al¹⁰ documented that Staphylococcus aureus (412, 49.28%), Escherichia coli (136, 16.27%), Klebsiella spp. (88, 10.53%), and Pseudomonas spp. (44, 5.26%) were the common pathogens isolated. Upreti N et al¹¹ showed among 116 bacterial isolates, Staphylococcus aureus was the most predominant bacteria (56.9%) followed by Escherichia coli (8.6%), Coagulase negative staphylococci (7.8%), Acinetobacter spp. (5.2%), Klebsiella pneumoniae (5.2%), Pseudomonas aeruginosa (4.3%), Enterococcus spp. (4.3%), Citrobacter freundii (2.6%), Proteus vulgaris (1.6%) and P. mirabilis (0.9%). A study by Ahmed EF et al¹² noted Gram positive isolates predominance in pyogenic infections caused due to accidents, in which 71.8% were Staphylococcus aureus. Rasmi AH et al concluded that Staphylococcus aureus was the most prevalent bacteria, followed by Pseudomonas aeruginosa. MRSA isolates accounted for 91.5%, whereas MSSA isolates accounted for 8.5%. The multidrug resistance (MDR) percentage in S. aureus isolates was 54.2%.13

In our study, Gram negative isolates were highly susceptible to meropenem, piperacillin + tazobactum, gentamycin, tobramycin, followed by cefaperazone + sulbactum, ceftazidime, levofloxacin, tetracycline in this study. *S.aureus* was highly susceptible to linezolid, minocycline (95%), teicoplanin (86%), vancomycin (83%), doxycycline (82%). Kalita JM et al noted⁹ that most of the Gram negative isolates showed high resistance towards cephalosporin, cotrimoxazole and quinolones and Gram positive cocci showed high resistance towards penicillin and quinolone group of drugs. Among Gram negative bacterial

isolates, 74.79% were multidrug resistant *Klebsiella* and 74.32% were MDR *Acinetobacter spp*. Methicillin resistant *Staphylococcus aureus* percentage was 13.26%, inducible clindamycin resistance among *S.aureus* isolates was 16.19%. 16.98% of total *Enterococci* isolates were Vancomycin resistant. Rijal BP et al¹⁰ studied microbiota in pyogenic infection and noted 51.9% and 48.7% of high levels of drug resistance among Gram positive bacteria and Gram negative bacteria respectively. Gram positive isolates were resistant to ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, and cloxacillin. Gram negative isolates were resistant to cephalosporins but were well susceptible to amikacin and imipenem.

As per this study ESBL production was observed more in E.coli (58.4%) followed by Klebsiella (50.9%) and Proteus (24.2%). MBL production was noted in 27.3% isolates of E.coli, 21.1% of Klebsiella and 6.06% of Proteus isolates. ESBL production was observed more in Pseudomonas (52.1%) followed by Acinetobacter (51.6%). MBL production was noted in 32.2% isolates of Pseudomonas, and 41.7% of Acinetobacter isolates. MRSA was noted in 77.08% isolates of wound infections. Upreti N et al11 specially focused on MRSA, MDR and ESBL producing Gram negative bacilli causing wound infections. Noted among S. aureus isolates, 60.6% were MRSA strains, whereas 40% of K. pneumoniae and 33.3% of C. freundii were ESBL producing bacteria followed by E. coli (25%). Both Gram positive (73.3%) and negative (78.8%) isolates showed high frequency of sensitivity to gentamycin.

A study from Vietnam¹⁴ stated that Gram negative bacteria isolated from wound infections are highly worrisome as the MDR rate is 63.6% and the highest being *Acinetobacter baumanii* (88.0%). The most promising effects of antibiotics noted in this study were carbapenems. In Gram positive bacteria the promising antibiotics noted were teicoplanin and vancomycin with the resistance percentage of 0% and 3.3% respectively. Clindamycin and tetracycline showed decreasing effectiveness.

Ramsi AH et al¹³ did an intense research on virulence genes among *Staphylococcus aureus* isolates from wound infections and concluded that sea was the most predominant gene (72.9%), followed by icaA (49.2%), hla (37.3%), and fnbA (13.6%). sea was the commonest virulence gene among MRSA isolates (72.2%), and a significant difference in the distribution of icaA was found.

MDR (Multidrug resistant group) patients' length of stay (P <0.001), readmissions (OR, 2.7026; P <0.001) and mortality rate (OR, 3.6541; p 0.0001) is high when compared to Non MDR group in this study. A study by Barshes NR et al¹⁵ on diabetic foot pyogenic infections noted substantial increase in healthcare costs. Oriyan Henig et al¹⁶ did a study on MDRO infections in diabetic foot wound infection (DFI) patients observed patients with DFI-MDRO were more likely to have recurrent DFI (OR, 2.34; 95% confidence interval

[CI], 1.53–3.58), readmission within 1 year (OR, 1.46; 95% CI, 1.06–2.0), and longer duration of hospitalization during index admission, median duration [IQR], 9 [6 to 13] days for DFI- MDRO and 7 [5 to 11] days for DFI-non-MDRO (P < 0.001). There was no difference in frequency of less extensive amputations or in all-cause mortality between the groups. Delay in wound healing by any factor and presence of MDROs in the wound increases morbidity, impaired quality of life, prolonged hospital stay, financial loss to patient, and even can lead to serious complications like acute kidney injury, septicaemia, loss of limbs, and depression.

Most of the pyogenic infections may contain either community or hospital acquired pathogens so there is a greater chance to get transmitted to other patients. Robust infection control measures including aseptic dressings practices, effective sterilization of instruments, hand hygiene and patient hygiene and a strong commitment towards implementation of antimicrobials stewardship protocols can definitely help to prevent the spread of MDRO's and break the chain of transmission of infections. Outbreak surveillance and institutional antibiotic policies provide clear empirical treatment options to clinicians and help in effective pyogenic infections. management of Appropriate antimicrobial treatment against the pathogen on one hand and instructions to patients on wound care, adherence to prescribed treatments on another hand play key roles in Wound infections. Other than the antibiotic therapies, developments in research like bacteriophage therapy may become one of the most promising options in near future.

7. Limitations of this Study

- The genotypic confirmation of MDROs in the pus isolates by molecular techniques such as PCR or sequencing was not done due to lack of necessary infrastructure at our centre. This may limit the precision in characterising resistance mechanisms and detection of specific resistance genes. However phenotypic detection was done based on CLSI guidelines.
- Multi regression analysis behind the risk factors of wound infections was not being projected when analysing the outcome factors, because it is out of our scope of the research work. But analysing those can improvise the management of the patient during hospital stay.

8. Conclusion

This hospital based study done over a year depicts good number of pus samples showing *Pseudomonas, S.aureus, E.coli and Klebisella* as predominant pathogens. These pathogens were highly susceptible to carbapenems, betalactam and beta lactamase inhibitors, aminoglycosides and broad-spectrum antibiotics. MRSA was noted in high percentage of wound infection population. MDR group significantly showed high mortality rate and increase in

readmission rate and length of hospitalization stay when compared to non MDR group.

Our study highlights that MDRO-infected wound patients suffer from more complications, require intensive interventions and alternative therapies due to limited antibiotic options. Effective antimicrobial stewardship, rapid diagnostics, and strict infection control measures are essential to curb resistance and improve outcomes. Future research should focus on novel antimicrobials and personalised treatment strategies. By implementing evidence based practices, healthcare providers can mitigate the impact of MDRO's, enhance wound healing and improve patient safety.

9. Conflict of Interest

None

10. Source of Funding

None

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