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Original Article

Examining the prevalence and antimicrobial resistance profiles of multidrug-resistant bacterial isolates in wound infections from Indonesian patients

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Abstract

The emergence of multidrug-resistant (MDR) infections in wounds is a significant public health issue. The aim of this study was to investigate the prevalence and antimicrobial resistance profiles of MDR bacterial isolates in wound infections. Through a cross-sectional study, 1,035 bacterial isolates were collected from wound infection patients at Tugurejo Hospital in Semarang, Indonesia, over a three-year period (from January 2020 to December 2022). Initial identification involved Gram staining and colony morphology assessment, followed by biochemical assays and antimicrobial susceptibility testing using the VITEK®2 Compact system. Gram-negative bacteria constituted the majority of isolates (60.77%, n=629). The predominant strains included were *Staphylococcus* spp. (30.92%, n=320), *Escherichia coli* (18.45%, n=191), and *Klebsiella pneumoniae* (13.04%, n=135). Notably, Gram-negative bacteria exhibited a significantly higher likelihood of MDR development compared to their Gram-positive counterparts ($p<0.001$), with Gram-negative bacteria having a 2.05 times higher probability of acquiring MDR. These findings underscore the urgent need for comprehensive surveillance of antimicrobial resistance patterns and the implementation of tailored antimicrobial stewardship programs to address the pressing public health challenge of MDR wound infections. Further research is warranted to elucidate the complex interplay of factors contributing to MDR development in wound infections, thereby informing targeted intervention strategies and improving patient outcomes.

Keywords: Antimicrobial resistance, health risk, multidrug-resistance, wound infections, antibiotic

Introduction

Skin functions to protect the human body from the external environment and is essential for moisturization, sensory perception, temperature control, and resistance to external pathogens [1]. As the largest sensory organ, the skin is critical to the human body's defense, protecting it from harm and microbial invasion. When the skin is damaged or a wound form, the exposed tissue provides a warm, moist environment rich in nutrients, which accelerates bacterial colonization of the skin [2]. Moreover, an open wound serves as an entry point for microorganisms to infect and multiply [3]. Bacterial colonization of the wound is a feature of chronic wounds, with infection occurring when there is more than 1×10^5 CFU/g tissue [4].

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Bacterial infection of the wound inhibits the wound-healing process. In chronic wounds, bacteria with more than one species can cause increased virulence and tissue damage [5].

Pseudomonas aeruginosa and *Staphylococcus aureus* are the most prevalent bacteria frequently discovered in wounds [6-11]. Treatment for wound infections generally utilizes antibiotics, which are supposed to suppress bacterial infections. However, prolonged and inappropriate use of antibiotics may result in the development of antibiotic resistance in bacteria and multidrug-resistance (MDR), resulting in difficulties in infection treatment [12,13]. In Indonesia, people have relatively easy access to antibiotics without a prescription [14]. This ease of access may contribute to the emergence of MDR bacterial strains in the country [15].

MDR bacterial infections pose a significant challenge to global healthcare systems [14], particularly in the context of wound infections. These infections are associated with prolonged hospital stays, increased morbidity and mortality rates, and higher healthcare costs. Understanding the prevalence and antimicrobial resistance profiles of MDR bacterial isolates in wound infections is crucial for informing effective treatment strategies and antimicrobial stewardship initiatives. Among the 15 provinces in Indonesia, Central Java Province stands out with a higher proportion of 87.10%, exceeding the national average of household-stocked antibiotics [16]. Our study location (Semarang, Central Java), in particular, has 91.00% household-stocked antibiotics [16]. Despite multiple recent reports demonstrating the prevalence and patterns of MDR in numerous diseases or medical conditions in various locations in Indonesia [14,17-19]. No study has been published on the MDR wound infection profile in Indonesia. The aim of this study was to investigate the prevalence of MDR in wound infections and to provide information for the advancement of wound infection treatment.

Methods

Study design and data collection

A cross-sectional study was conducted among patients who had their wounds swabbed to collect any wound-infecting bacteria at Tugurejo Hospital in Semarang, Indonesia. The patients' wounds ranged from burn wounds to postsurgical wounds, as well as superficial and soft tissue infections. Data were collected from January 2020 to December 2022. In total, 1,035 patients were swabbed during this period and 1,035 samples were collected. Using a standard data collection form, the age, sex, and diabetes mellitus (DM) status of patients were extracted from the microbiology laboratory unit registration records. The workflow of the study is presented in **Figure 1**.

Sample collection

Before collecting samples, the wound edges were cleaned and washed with a physiologically sterile solution to remove surface exudate. When collecting the samples, swab sampling was specifically utilized based on the type of wound and it was only used when the incision was minor. Swab specimens were collected according to the protocols, inoculated into blood agar and MacConkey agar (both from Merck, Darmstadt, Germany), and incubated overnight at 37±2°C.

Identification and antimicrobial susceptibility pattern analysis

The isolated bacteria were identified preliminary based on the type of colony, margin, elevation, size, shape, and color. Using the VITEK®2 Compact (bioMérieux, Craponne, France) equipment, all isolates were identified, and the resistance pattern was assessed. A total of 18 antibiotics were tested for Gram-negative bacteria, including aminopenicillins (AM: ampicillin, AMC: amoxicillin + clavulanic acid); 1st generation cephalosporin (CZO: cefazolin); 2nd generation cephalosporin (FAM: ampicillin + sulbactam); 3rd generation cephalosporins (CAZ: ceftazidime, CTX: cefotaxime, CRO: ceftriaxone); 4th generation cephalosporin (CEF: cefepime); aminoglycosides (AMK: amikacin, GM: gentamicin); penicillins (PIP/TAZ: piperacillin + tazobactam); monobactam (AZM: aztreonam); carbapenems (ETP: ertapenem; MEM: meropenem); fluoroquinolone (CIP: ciprofloxacin); glycylcycline (TGC: tigecycline); nitrofuran (NIT: nitrofurantoin); sulfonamides-trimethoprim (SXT: trimethoprim + sulfamethoxazole).

A total of 29 antibiotics were tested for Gram-positive bacteria, including aminopenicillins (AMC, AM, and FAM); penicillins (BENPEN: benzylpenicillin, TZP: piperacillin + tazobactam,

OXA: oxacillin); aminoglycosides (AMK); 1st generation cephalosporins (CRF: cefadroxil, CF: cephalothin); 3rd generation cephalosporins (CTX: cefotaxime, FOP: cefoperazone); 4th generation cephalosporins (CEF); aminoglycosides (GM); fluoroquinolones (CIP, LEV: levofloxacin, MXF: moxifloxacin); macrolides (ERY: erythromycin); lincosamides (DA: clindamycin); streptogramins (PR: pristinamycin); oxazolidinones (LNZ: linezolid); glycylcyclines (TGC: tigecycline); sulfonamides-trimethoprim (TMP: trimethoprim, SXT: trimethoprim + sulfamethoxazole); tetracyclines (TET: tetracycline); nitrofurans (NIT); rifamycin (RIF: rifampicin); carbapenems (MEM: meropenem, IMI: imipenem); and glycopeptides (VAN: vancomycin).

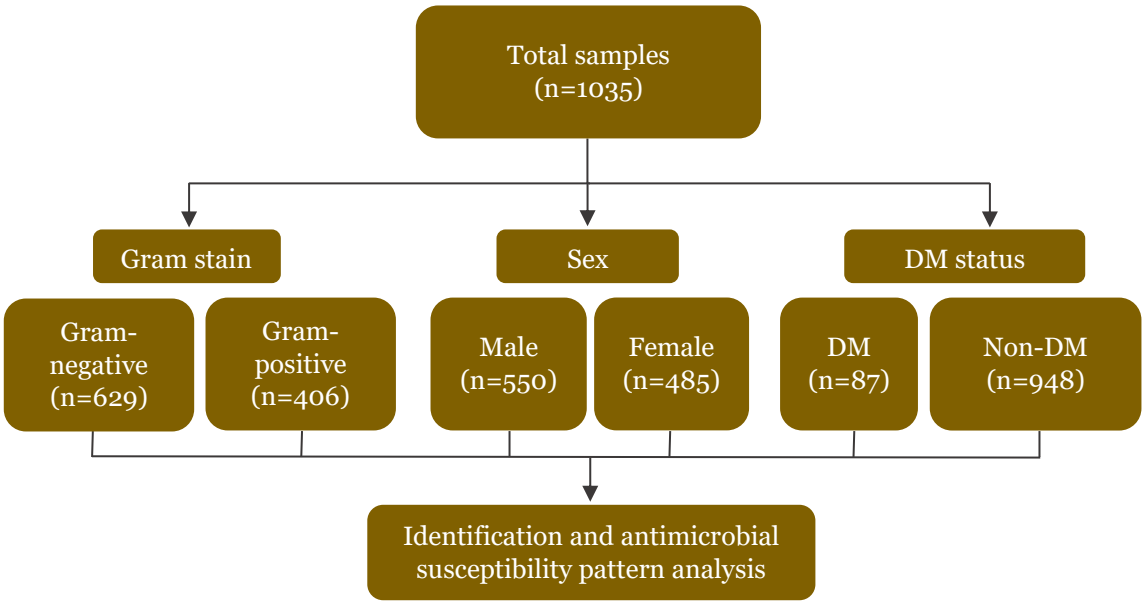

Figure 1. Workflow outline. DM: diabetes mellitus.

Statistical analysis

Descriptive statistics, such as frequencies and percentages, were used to determine the pattern of antimicrobial resistance and MDR in the discovered wound-infecting bacterial strains. Logistic regression was performed to determine the strength of the association between the observed variables (sex, DM status, and Gram staining) and bacterial MDR status. Data analyses were conducted using SPSS 25.0 (IBM, New York, USA).

Results

Distribution of wound infections

In this study, we found 60.77% (n=629) Gram-negative bacteria and 39.23% (n=406) Gram-positive bacteria. The number of bacteria found in male patients was 53.14% (n=550), while 46.85% (n=485) were found in female patients. Out of all patients, 8.40% (n=87) had DM.

Distribution of the bacteria from the wound samples

Our data indicated that *E. coli* (30.9%, n=191) and *K. pneumoniae* (21.5%, n=135) were the most common bacteria among Gram-negative bacteria (**Figure 2A**). *Staphylococcus* spp. (78.8%, n=320) was the most dominant bacteria among Gram-positive bacteria (**Figure 2B**). The dominant strains isolated overall were *Staphylococcus* spp. (30.92%, n=320), *E. coli* (18.45%, n=191), and *K. pneumoniae* (13.04%, n=135) (**Figure 2C**). We also discovered that the most prevalent bacteria in all wound samples, regardless of sex and DM status, were *Staphylococcus* spp., *E. coli*, and *K. pneumoniae* (**Figures 2D-2G**).

The distribution of Gram-negative bacteria, calculated from a total of 629 isolates, is presented in **Figure 2A**. The most prevalent bacteria were *E. coli* (30.4%), *K. pneumoniae* (21.5%), and *Pseudomonas* spp. (12.2%). The distribution of the Gram-positive bacteria,

calculated from 406 isolates, with *Staphylococcus* spp. being the most common (78.8%), is presented in **Figure 2B**. The combined distribution of Gram-negative and Gram-positive bacteria is presented in **Figure 2C**. The most prevalent bacteria were *Staphylococcus* spp. (30.92%), *E. coli* (18.45%), and *K. pneumoniae* (13.04%). The distribution of bacterial wound infections in male samples, based on 550 isolates, is displayed in **Figure 2D**. The most common bacteria were *Staphylococcus* spp. (30.18%), *E. coli* (18.36%), and *K. pneumoniae* (14.91%). For female samples, calculated from 485 isolates and displayed in **Figure 2E**, the most prevalent bacteria were *Staphylococcus* spp. (31.75%), *E. coli* (18.56%), and *K. pneumoniae* (10.93%). The distribution of bacterial wound infections in DM patients, based on 87 isolates, is displayed in **Figure 2F**. The three highest observed bacteria were *Staphylococcus* spp. (19.54%), *E. coli* (18.39%), and *K. pneumoniae* (13.79%). Lastly, the distribution in non-DM patients, calculated from 948 isolates and displayed in **Figure 2G**, reveals that the highest prevalence was *Staphylococcus* spp. (31.96%), *E. coli* (18.46%), and *K. pneumoniae* (12.97%).

Antibiotic sensitivity and resistance profiles of isolates

Over half of the *E. coli* isolates indicated high resistance to aminopenicillins (ampicillin), 1st generation cephalosporins (cefazolin), 2nd generation cephalosporins (ampicillin + sulbactam), 3rd generation cephalosporins (cefotaxime, ceftriaxone), fluoroquinolones (ciprofloxacin), and sulfonamides-trimethoprim (trimethoprim/sulfamethoxazole) (**Table 1**). Conversely, *E. coli* showed low resistance (less than 5%) to glycylcyclines (tigecycline), aminoglycosides (amikacin), and carbapenems (ertapenem, meropenem) (**Table 1**). *K. pneumoniae* had high resistance (more than 50%) to aminopenicillins (ampicillin) and nitrofurans (nitrofurantoin). *K. pneumoniae* also had low resistance (less than 10%) to aminopenicillins (amoxicillin + clavulanic acid) and carbapenems (ertapenem, meropenem). *Staphylococcus* spp. had high resistance (more than 50%) to penicillin (benzylpenicillin) (**Table 2**). However, *Staphylococcus* spp. had low resistance (less than 5%) to a range of antibiotics, including aminopenicillins (amoxicillin + clavulanic acid, ampicillin, ampicillin + sulbactam), penicillins (piperacillin + tazobactam), 4th generation cephalosporins (cefepime), 3rd generation cephalosporins (cefoperazone), 1st generation cephalosporins (cefadroxil, cephalothin), streptogramins (pristinamycin), oxazolidinones (linezolid), glycylcyclines (tigecycline), tetracyclines (tetracycline), nitrofurans (nitrofurantoin), sulfonamides-trimethoprim (trimethoprim + sulfamethoxazole), carbapenems (meropenem, imipenem), glycopeptides (vancomycin) (**Table 2**).

Multidrug-resistance (MDR) profiles of isolates

MDR percentages were calculated for each bacterium based on 1,035 isolates. Overall, 67.25% (n=696) were MDR (resistant to three or more antibiotic classes), while 32.75% (n=339) had a non-MDR profile (**Table 3** and **Table 4**). Out of the total Gram-negative isolates (n=629), *E. coli* and *K. pneumoniae* had the highest MDR prevalence, contributing to 73.9% of all MDR cases (n=465). Among the total number of Gram-positive isolates (n=406), the MDR rate for *Staphylococcus* spp. was the highest at 56.9% (n=231). The overall MDR rates of *E. coli* (n=191) and *K. pneumoniae* (n=135) were 75.4% (n=144) and 57% (n=77), respectively (**Table 3**). *Staphylococcus* spp. (n=320) was found in 55.6% (n=178) of MDR isolates (**Table 4**).

Logistic regression analysis of independent variables and MDR status

To identify the risk factors for MDR bacteria, logistic regression was utilized. The odds ratio (OR) was 0.486 for Gram-positive, indicating that Gram-negative had OR 2.05 (from 1:0.486). This indicates that the Gram-negative had a two-times higher chance of being MDR. MDR isolates were also more likely to be identified as such (**Table 5**).

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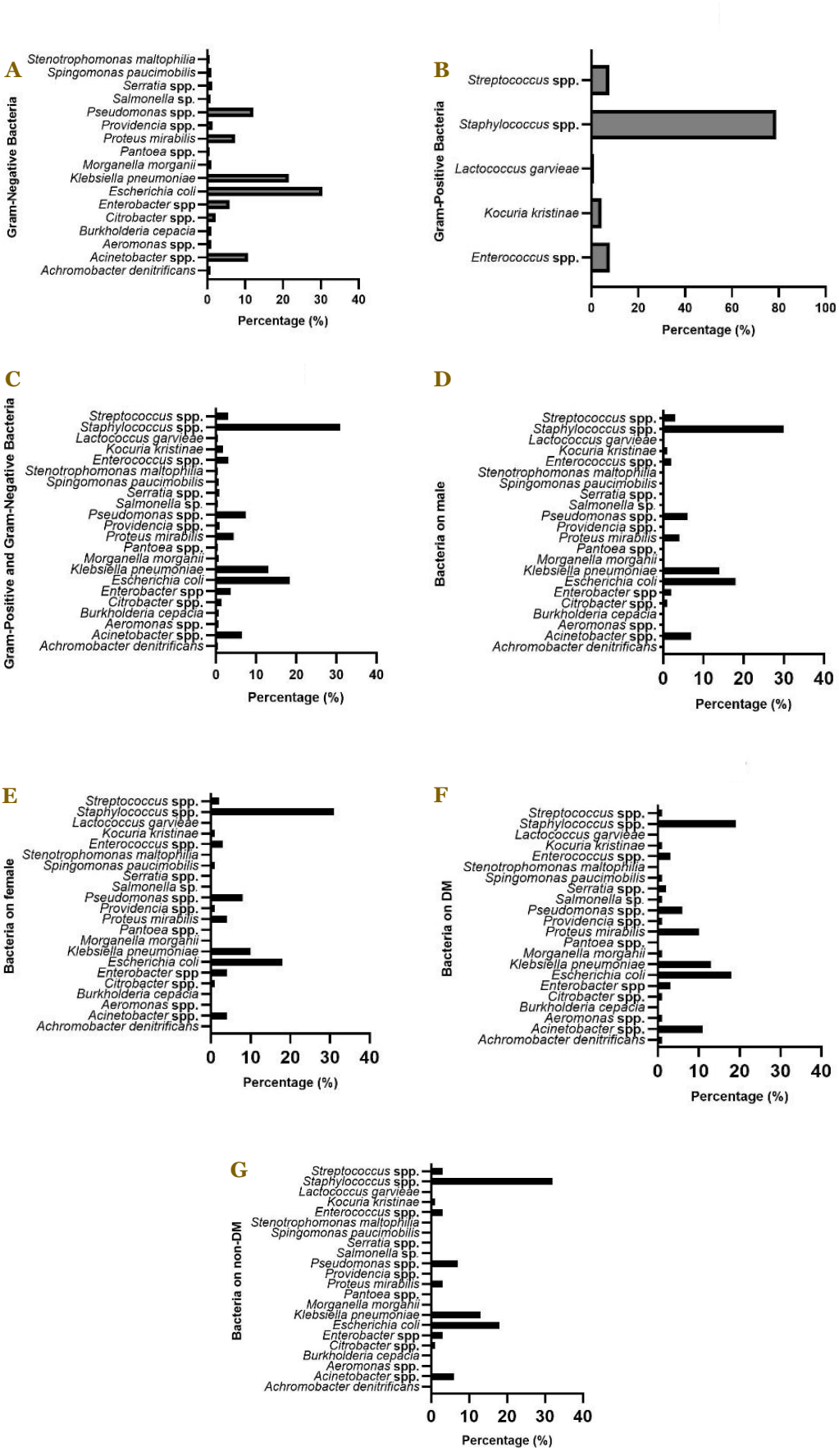

Figure 2. Distribution of the bacteria from 1,035 wound samples: (A) Gram-negative bacteria (n=629); (B) Gram-positive bacteria (n=406); (C) Gram-positive and Gram-negative bacteria (n=1,035); (D) bacteria on male (n=550); (E) bacteria on female (n=485); (F) bacteria on diabetic patients (n=87); and (G) bacteria on non-diabetic patients (n=948).

Table 1. Antibiotic resistance pattern of Gram-negative bacteria

Bacteria, n	AM	FAM	AMK	TZP	CAZ	CZO	CTX	CRO	CEF	AZM	AMC	ETP	MEM	GM	CIP	TGC	NIT	SXT
Achromobacter denitrificans (n=5)	-	-	2	2	0	5	5	5	5	5	5	-	1	5	5	3	-	0
Percentage	-	-	40.0	40.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	-	20.0	100.0	100.0	60.0	-	0.0
Acinetobacter spp. (n=67)	-	29	40	47	49	65	57	60	47	3	12	-	28	45	50	15	-	13
Percentage	-	43.3	59.7	70.1	73.1	97.0	85.1	89.6	70.1	4.5	17.9	-	41.8	67.2	74.6	22.4	-	19.4
Aeromonas spp. (n=6)	-	4	2	4	4	4	3	3	2	2	2	2	2	3	3	1	-	4
Percentage	-	66.7	33.3	66.7	66.7	66.7	50.0	50.0	33.3	33.3	33.3	33.3	33.3	50.0	50.0	16.7	-	66.7
Burkholderia cepacia (n=7)	-	-	4	5	5	7	2	6	5	3	0	-	3	5	4	3	-	2
Percentage	-	-	57.1	71.4	71.4	100.0	28.6	85.7	71.4	42.9	0.0	-	42.9	71.4	57.1	42.9	-	28.6
Citrobacter spp. (n=14)	13	13	8	2	3	14	1	2	2	3	0	0	0	1	4	2	3	4
Percentage	92.9	92.9	57.1	14.3	21.4	100.0	7.1	14.3	14.3	21.4	0.0	0.0	0.0	7.1	28.6	14.3	21.4	28.6
Enterobacter spp. (n=37)	37	37	19	17	21	37	20	20	10	21	3	12	3	7	14	3	28	12
Percentage	100.0	100.0	51.4	45.9	56.8	100.0	54.1	54.1	27.0	56.8	8.1	32.4	8.1	18.9	37.8	8.1	75.7	32.4
Escherichia coli (n=191)	163	122	86	16	76	106	116	117	42	90	2	5	3	69	142	0	10	116
Percentage	85.3	63.9	45.0	8.4	39.8	55.5	60.7	61.3	22.0	47.1	1.0	2.6	1.6	36.1	74.3	0.0	5.2	60.7
Klebsiella pneumoniae (n=135)	132	64	42	27	36	51	43	44	14	39	2	5	3	32	65	23	94	49
Percentage	97.8	48.5	31.8	20.5	27.3	37.8	32.6	32.6	10.6	29.5	1.5	3.7	2.3	24.2	49.2	17.0	69.6	37.1
Morganella morganii (n=7)	7	5	4	1	1	7	1	1	1	1	0	1	0	3	5	7	7	4
Percentage	100.0	71.4	57.1	14.3	14.3	100.0	14.3	14.3	14.3	14.3	0.0	14.3	0.0	42.9	71.4	100.0	100.0	57.1
Pantoea spp. (n=4)	-	1	-	1	1	3	1	1	1	-	1	-	1	0	0	0	-	0
Percentage	-	25.0	-	25.0	25.0	75.0	25.0	25.0	25.0	-	25.0	-	25.0	0.0	0.0	0.0	-	0.0
Proteus mirabilis (n=46)	31	23	23	4	8	23	10	10	1	5	1	2	2	17	29	44	41	31
Percentage	67.4	50.0	50.0	8.7	17.4	50.0	21.7	21.7	2.2	10.9	2.2	4.3	4.3	37.0	63.0	95.7	89.1	67.4
Providencia spp. (n=9)	9	8	6	1	6	8	6	6	0	1	2	1	0	9	7	9	7	7
Percentage	100.0	88.9	66.7	11.1	66.7	88.9	66.7	66.7	0.0	11.1	22.2	11.1	0.0	100.0	77.8	100.0	77.8	77.8
Pseudomonas spp. (n=77)	-	-	42	35	31	70	4	5	29	43	13	-	23	30	36	75	-	3
Percentage	-	-	54.5	45.5	40.3	90.9	5.2	6.5	37.7	55.8	16.9	-	29.9	39.0	46.8	97.4	-	3.9
Salmonella sp. (n=5)	1	1	4	0	2	4	3	2	1	1	3	0	0	5	5	0	3	2
Percentage	20.0	20.0	80.0	0.0	40.0	80.0	60.0	40.0	20.0	20.0	60.0	0.0	0.0	100.0	100.0	0,0	60.0	40.0
Serratia spp. (n=8)	6	5	3	0	2	5	1	3	2	5	0	0	1	2	3	2	4	1
Percentage	75.0	62.5	37.5	0.0	25.0	62.5	12.5	37.5	25.0	62.5	0.0	0.0	12.5	25.0	37.5	25.0	50.0	12.5
Spingomonas paucimobilis (n=7)	-	1	2	3	4	3	4	5	2	4	2	0	2	5	3	1	-	3
Percentage		14.3	28.6	42.9	57.1	42.9	57.1	71.4	28.6	57.1	28.6	0.0	28.6	71.4	42.9	14.3	-	429
Stenotrophomonas maltophilia (n=4)	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	0
Percentage	-	-	25.0	-	-	-	-	-	-	25.0	-	-	-	-	-	-	-	0.0

Antibiotic classes and each of the class's respective tested antibiotics are as follows: aminopenicillin (AM: ampicillin); 2nd generation cephalosporin (FAM: ampicillin + sulbactam); aminoglycosides (AMK: amikacin); penicillin (TZP: piperacillin + tazobactam); 3rd generation cephalosporin (CAZ: ceftazidime); 1st generation cephalosporin (CZO: cefazolin); 3rd generation cephalosporin (CTX: cefotaxime); 3rd generation cephalosporin (CRO: ceftriaxone); 4th generation cephalosporin (CEF: cefepime); monobactam (AZM: aztreonam); aminopenicillin (AMC: amoxicillin + clavulanic acid); carbapenem (ETP: ertapenem; MEM: meropenem); aminoglycoside (GM: gentamicin); fluoroquinolone (CIP: ciprofloxacin); glycylcycline (TGC: tigecycline); nitrofuran (NIT: nitrofurantoin); sulfonamide-trimethoprim (SXT: trimethoprim-sulfamethoxazole)

Table 2. Antibiotic resistance pattern of Gram-positive bacteria

Bacteria, n	AMC	BENPEN	AM	FAM	TZP	AMK	OXA	CTX	CEF	FOP	CFR	CF	GM	CIP	MXF
Enterococcus spp. (n=32)	1	3	2	-	-	7	-	-	-	-	-	0	1	5	-
Percentage	3.1	9.4	6.3	-	-	21.8	-	-	-	-	-	0.0	3.1	15.6	-
Kocuria kristinae (n=18)	-	14	-	-	-	5	10	4	-	-	-	-	6	7	5
Percentage	-	77.8	-	-	-	27.8	55.6	22.2	-	-	-	-	33.3	38.9	27.8
Lactococcus garvieae (n=5)	-	5	-	-	-	2	3	3	-	-	-	-	5	0	0
Percentage	-	100.0	-	-	-	40.0	60.0	60.0	-	-	-	-	100.0	0.0	0.0
Staphylococcus spp. (n=320)	7	278	4	9	18	83	113	81	9	7	8	6	79	128	101
Percentage	2.2	86.9	1.3	2.8	5.6	25.9	35.5	25.3	2.8	2.2	2.5	1.9	24.7	40.0	32.6
Streptococcus spp. (n=31)	-	7	0	-	-	6	5	3	-	1	-	-	1	2	0
Percentage	-	22.6	0.0	-	-	19.4	16.1	9.7	-	3.2	-	-	3.2	6.5	0.0

Antibiotic classes and each of the class's respective tested antibiotics are as follows: aminopenicillin (AMC: amoxicillin + clavulanic acid); penicillin (BENPEN: benzylpenicillin); aminopenicillin (AM: ampicillin); aminopenicillin (FAM: ampicillin + sulbactam), penicillin's (TZP: piperacillin + tazobactam); aminoglycosides (AMK: amikacin); penicillin (OXA: oxacillin); 3rd generation cephalosporin (CTX: cefotaxime); 4th generation cephalosporin (CEF: cefepime); 3rd generation cephalosporin (FOP: cefoperazone); 1st generation cephalosporin (CRF: cefadroxil); 1st generation cephalosporin (CF: cephalothin); aminoglycosides (GM: gentamicin); fluoroquinolone (CIP: ciprofloxacin); fluoroquinolones (MXF: moxifloxacin)

Table 2. Antibiotic resistance pattern of Gram-positive bacteria (continued)

Bacteria, n	LEV	ERY	DA	PR	LNZ	TGC	TMP	TET	NIT	RIF	SXT	MEM	IMI	VAN
Enterococcus spp. (n=32)	4	22	2	25	7	2	21	-	1	-	-	1	-	2
Percentage	12.5	68.8	6.3	78.1	21.9	6.3	65.5		3.1			3.1		6.3
Kocuria kristinae (n=18)	6	7	14	10	8	0	6	-	-	-	0	-	-	2
Percentage	33.3	38.9	77.8	55.6	44.4	0.0	33.3	-	-	-	0.0	-	-	11.1
Lactococcus garvieae (n=5)	0	3	3	3	0	0	5	-	-	-	-	-	-	0
Percentage	0.0	60.0	60.0	60.0	0.0	0.0	100.0	-	-	-	-	-	-	0.0
Staphylococcus spp. (n=320)	141	75	62	4	12	5	153	3	21	23	6	8	8	14
Percentage	44.1	23.4	19.4	1.3	3.8	1.6	47.8	0.9	6.6	7.2	1.9	2.5	2.5	4.4
Streptococcus spp. (n=31)	1	3	5	3	2	0	15	1	1	0	-	-	-	5
Percentage	3.2	9.7	16.1	9.7	6.5	0.0	48.4	3.2	3.2	0.0	-	-	-	16.1

Antibiotic classes and each of the class's respective tested antibiotics are as follows: fluoroquinolone (LEV: levofloxacin); macrolides (ERY: erythromycin); lincosamides (DA: clindamycin); streptogramins (PR: pristinamycin); oxazolidinones (LNZ: linezolid); glycylcycline (TGC: tigecycline); sulphonamides-trimethoprim (TMP: trimethoprim); tetracycline (TET: tetracycline); nitrofuran (NIT: nitrofurantoin); rifamycin (RIF: rifampicin); sulphonamides-trimethoprim (SXT: trimethoprim/sulfamethoxazole); carbapenem (MEM: meropenem); carbapenem (IMI: imipenem); glycopeptides (VAN: vancomycin)

Table 3. Multidrug-resistance (MDR) pattern of Gram-negative bacteria isolated from wound swabs

Bacteria	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	MDR	Non-MDR
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Achromobacter denitrificans (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)
Acinetobacter spp. (n=67)	0 (0.0)	6 (9.0)	12 (17.9)	2 (3.0)	6 (9.0)	8 (11.0)	8 (11.9)	16 (23.9)	9 (13.4)	0 (0.0)	0 (0.0)	49 (73.1)	18 (26.9)
Aeromonas spp. (n=6)	0 (0.0)	0 (0.0)	0 (0.0)	1(16.7)	2 (33.3)	0 (0.0)	0 (0.0)	1 (16.7)	2 (33.3)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)
Burkholderia cepacia (n=7)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	5 (71.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (100.0)	0 (0.0)
Citrobacter spp. (n=14)	0 (0.0)	0 (0.0)	5 (35.7)	4 (28.6)	3 (21.4)	1 (7.1)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	9 (64.3)	5 (35.7)

Bacteria	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	MDR	Non-MDR
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Enterobacter spp. (n=37)	0 (0.0)	0 (0.0)	5 (13.5)	9 (24.3)	2 (5.4)	5 (13.5)	5 (13.5)	5 (13.5)	1 (2.7)	2 (5.4)	3 (8.1)	32 (86.5)	5 (13.5)
Escherichia coli (n=191)	9 (4.7)	17 (8.9)	21 (11.0)	21 (11.0)	30 (15.7)	53 (27.7)	31 (16.2)	6 (3.1)	2 (1.0)	1 (0.5)	0 (0.0)	144 (75.4)	47 (24.6)
Klebsiella spp. (n=135)	3 (2.2)	21 (15.6)	34 (25.2)	11 (8.1)	14 (10.4)	16 (11.9)	10 (7.4)	10 (7.4)	9 (6.7)	6 (4.4)	1 (0.7)	77 (57.0)	58 (43.0)
Morganella morganii (n=7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)	2 (28.6)	2 (28.6)	0 (0.0)	1 (14.3)	0 (0.0)	7 (100.0)	0 (0.0)
Pantoea spp. (n=4)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	3 (75.0)
Proteus mirabilis (n=46)	0 (0.0)	0 (0.0)	4 (8.7)	8 (17.4)	5 (10.9)	7 (15.2)	10(21.7)	7 (15.2)	3 (6.5)	1 (2.2)	1 (2.2)	42 (91.3)	4 (8.7)
Providencia spp. (n=9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)	1 (11.1)	5 (55.6)	0 (0.0)	0 (0.0)	9 (100.0)	0 (0.0)
Pseudomonas spp. (n=77)	0 (0.0)	3 (3.9)	16 (20.8)	14 (18.2)	7 (9.1)	5 (6.5)	4(5.2)	8 (10.4)	15 (19.5)	4 (5.2)	0 (0.0)	58 (75.3)	19 (24.7)
Salmonella sp. (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	2 (40.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)
Serratia spp. (n=8)	0 (0.0)	0 (0.0)	0 (0.0)	4 (50.0)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)
Spingomonas paucimobilis (n=7)	0 (0.0)	1 (14.3)	0 (0.0)	3 (42.9)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	1 (14.3)	0 (0.0)	0 (0.0)	1 (14.3)	6 (85.7)
Stenotrophomonas maltophilia (n=4)	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100,0)

R0: sensitive against all selected antibiotic classes; R1: resistant to at least one antibiotic class; R2 to R10: resistant to the number of antibiotic classes (two to ten); MDR: resistant to at least three antibiotic classes; MDR (%): number of strains and percentage of multidrug-resistant bacteria strains found; non-MDR (%): number of strains and percentage of non-multidrug-resistant bacteria strains found

Table 4. Multidrug-resistance (MDR) pattern of Gram-positive bacteria isolated from wound swabs

Bacteria	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	MDR	Non-MDR
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Enterococcus spp. (n=32)	0 (0.0)	2 (6.3)	6 (18.8)	13 (40.6)	6 (18.8)	3 (9.4)	2 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (75.0)	8 (25.0)
Kocuria spp. (n=18)	0 (0.0)	2 (11.1)	0 (0.0)	0 (0.0)	5 (27.8)	4 (22.2)	4 (22.2)	1 (5.6)	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	16 (88.9)	2 (11.1)
Lactococcus garvieae (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)
Staphylococcus spp. (n=320)	11 (3.4)	57 (17.8)	74 (23.1)	54 (16.9)	28 (8.8)	20 (6.3)	22 (6.9)	19 (5.9)	19 (5.9)	10 (3.1)	4 (1.3)	2 (0.6)	178 (55.6)	142 (44.4)
Streptococcus (n=31)	7 (22.6)	12 (38.7)	4 (12.9)	2 (6.5)	0 (0.0)	3 (9.4)	1 (3.2)	1 (3.2)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	8 (25.8)	23 (74.2)

R0: sensitive against all selected antibiotic classes; R1: resistant to at least one antibiotic class; R2 to R11: resistant to the number of antibiotic classes (two to eleven); MDR: resistant to at least three antibiotic classes; MDR (%): number of strains and percentage of multidrug-resistant bacteria strains found; non-MDR (%): number of strains and percentage of non-multidrug-resistant bacteria strains found

Table 5. Logistic regression analysis of independent variables and multidrug-resistance (MDR) pattern

Variables	Category	Frequency	MDR		Non-MDR		Odds ratio	*p*-value
			Frequency	Percentage	Frequency	Percentage		
Sex	Female	485	327	67.4	158	32.6	0.962	0.856
	Male	550	369	67.1	181	32.9	Reference	
Diabetes mellitus	Yes	87	61	70.1	26	29.9	0.932	0.812
	No	948	635	67.0	313	33.0	Reference	
Gram stain	Negative	629	**465**	**73.9**	**164**	**26.1**	Reference	<0.001
	Positive	406	**231**	**56.9**	**175**	**43.1**	0.486	

Discussion

Antibiotic overuse has resulted in a broad occurrence of antimicrobial resistance. Bacterial pathogens will eventually resist every antibacterial option, making containment extremely difficult. As a result, the World Health Organization (WHO) has designated it as a major international health issue [20]. Comprehensive antibiotic stewardship in developing countries is crucial for controlling this growing crisis. However, there is insufficient data on antibiotic resistance to assess the scope of the problem accurately.

Our findings revealed that Gram-negative isolates (60.77%, n =629) were more common compared to Gram-positive isolates (39.23%, n=406). Similar findings have been published, showing that Gram-negative bacteria are at a higher risk of causing wound infections than Gram-positive bacteria [21–23]. However, previous studies found that the ratios of Gram-positive and Gram-negative bacteria were nearly identical [24,25]. Variations in participant demographics may be the cause of the inconsistent outcomes.

E. coli was the most prevalent Gram-negative bacterium (30.4%), followed by *K. pneumoniae* (21.5%), and *Pseudomonas* spp. (12.2%). On the other hand, *Staphylococcus* spp. (78.8%) dominated among Gram-positive pathogens. Among all bacteria, *Staphylococcus* spp. represented the majority (30.92%), followed by *E. coli* (18.45%) and *K. pneumoniae* (13.04%). These three bacteria were the most common in all types of wound samples (across sexes and DM status of patients). This is consistent with findings from Ahmed *et al.* [6], who identified *S. aureus* in 61% of wound specimens, including 59% of surgical site infections, 65% of abscesses, and 52% of burn infections. Another study by Puca *et al.* [26] found that 85.2% of Gram-positive isolates and 62.5% of all isolates were *S. aureus* from various wound infection samples. One prevalent Gram-positive bacterium discovered in wound infections among people with DM is *S. aureus* [27]. This is unsurprising given that *S. aureus* is a common commensal on the skin and can cause infections externally or from endogenous sources.

In this study, among all Gram-negative isolates, the most prevalent pathogen was *E. coli*, followed by *K. pneumoniae*. These results are in line with a previous research report investigating bacterial strains from wound infections in Southwest Ethiopia [28]. In contrast, the study by Trivedi *et al.* [27] showed different results; *P. aeruginosa* was identified as the most common Gram-negative bacterium causing wound infections in people with DM. According to Shebl and Mosaad [29], differences between research could be related to factors such as participant numbers, healthcare service delivery, and personal healthcare conditions. Among Gram-negative bacteria, *E. coli* is known as a general contaminant in Indonesia's sanitary system [30], which may contribute to high rates of *E. coli* infection in wounds.

In the current study, high resistance was reported among *E. coli* isolates to aminopenicillin (ampicillin), 1st generation cephalosporin (cefazolin), 2nd generation cephalosporin (ampicillin + sulbactam), 3rd generation cephalosporin (cefotaxime, ceftriaxone), fluoroquinolone (ciprofloxacin), and sulfonamides-trimethoprim (trimethoprim + sulfamethoxazole). Cefazolin, ampicillin, cefuroxime, ciprofloxacin, mezlocillin, moxifloxacin, piperacillin, tetracycline, and trimethoprim + sulfamethoxazole were ineffective against more than half of the *E. coli* isolates from wound infections [31]. In general, *E. coli* showed significant resistance rates to trimethoprim + sulfamethoxazole (47.46%), cefazolin (52.36%), and ampicillin (82.00%) [32]. Glycylcycline (tigecycline), aminoglycosides (amikacin), and carbapenems (ertapenem, meropenem) performed well against *E. coli* in our study, consistent with findings from Bessa *et al.* [33]. Aminopenicillin (ampicillin) and nitrofuran (nitrofurantoin) resistance were observed in over half of *K. pneumoniae* isolates, aligning with previous studies from Myanmar where *Klebsiella* spp. showed 100% resistance to ampicillin. This finding is in line with the review by Effah *et al.* [34], which underlined the growing threat of MDR *Klebsiella* spp. During our investigation, we discovered aminopenicillin (amoxicillin + clavulanic acid) and carbapenems (ertapenem, meropenem) were the most effective antibiotics against *Klebsiella* spp., consistent with a previous study in wound cases in New Delhi [35].

Penicillin (benzylpenicillin) resistance was present in more than half of the isolates of *Staphylococcus* species. *S. aureus* isolates from wounds revealed over 80% resistance to benzylpenicillin. Staphylococci, initially sensitive to benzylpenicillin, are now considered highly resistant [36]. The data also revealed that the most effective antibiotics against *Staphylococcus*

spp. were aminopenicillin (amoxicillin + clavulanic acid), aminopenicillin (ampicillin, ampicillin + sulbactam), penicillin (piperacillin + tazobactam), 4th generation cephalosporin (cefepime), 3rd generation cephalosporin (cefoperazone), 1st generation cephalosporin (cefadroxil, cephalothin), streptogramins (pristinamycin), oxazolidinones (linezolid), glycylcycline (tigecycline), tetracycline (tetracycline), nitrofuran (nitrofurantoin), sulfonamides-trimethoprim (trimethoprim + sulfamethoxazole), carbapenem (meropenem, imipenem), and glycopeptides (vancomycin). Penicillin (90.0%) and oxacillin (64.0%) had the highest resistance rates, as reported by Hove *et al.* [37]. According to Shebl and Mosaad [29], the rates of resistance to vancomycin and linezolid were 10.8% and 11.3%, respectively, which contrasts with our study. Nevertheless, earlier studies on linezolid and vancomycin supported our study, showing that vancomycin is effective against all *S. aureus* isolates [38]. Furthermore, Basak *et al.* [39] found complete susceptibility to linezolid and vancomycin. These differences between research could be related to inappropriate use of antibiotics, regional and socioeconomic variations, and different patient characteristics [40].

The investigation, conducted in Semarang, Indonesia, yielded a list of common microorganisms associated with wound infections and assessed the frequency of MDR bacteria in wound infections. Three independent variables were considered: sex, DM status, and Gram staining. Nevertheless, no other factor was shown to be a significant predictor of multidrug resistance in bacterial isolates in the absence of Gram staining. Our study showed that Gram-negative bacteria are significantly more likely to develop MDR than Gram-positive bacteria ($p<0.001$). Gram-positive bacteria had an odds ratio (OR) of 0.486, while Gram-negative bacteria had an OR of 2.05 (from 1:0.486). This indicates that Gram-negative bacteria have more than twice the likelihood of developing MDR.

MDR is characterized as resistance to three or more antibiotic classes in both Gram-negative [41,42] and Gram-positive bacteria [43,44]. Our study found that the rate of MDR was higher in Gram-negative bacteria (73.9%) compared to Gram-positive bacteria (56.9%) in samples obtained from wound infections. Among the bacterial isolates, *E. coli* had the highest prevalence of MDR at 75.4%, followed by *Klebsiella* spp. (57%) and *Staphylococcus* spp. (55.6%). Our investigation of Gram-negative bacteria showed a higher overall MDR rate than reported in previous studies. For instance, in two studies conducted in Ethiopia, the initial study reported a 51% MDR rate among bacteria isolated from open fracture sites [45]. Subsequent research found an MDR phenotype in 59.3% of bacteria from wound infections [46]. This degree of resistance may have been caused by a variety of circumstances, such as the overuse of antibiotics by healthcare professionals and the public. In Indonesia, where antibiotics are readily available without a prescription, this overuse exacerbates the development and spread of antibiotic resistance [14]. Despite some significant findings, our study had several limitations, including reliance on data from a diagnostic laboratory in Semarang, Indonesia, and the exclusion of anaerobic bacteria due to inadequate growth conditions. Addressing these limitations is crucial for future research focused on combating MDR in wound infections.

Conclusion

In Indonesia, the rising incidence of MDR bacterial infections from wound sources is increasingly alarming. These findings highlight the critical need for continuous monitoring of antibiotic resistance patterns and the implementation of effective antimicrobial governance strategies to tackle this pressing public health issue. More investigation is necessary to understand the risk factors contributing to the emergence of multidrug-resistant wound infections. In the future, there is an urgent need to enhance surveillance systems for monitoring antimicrobial resistance in wound infections. This involves implementing comprehensive surveillance programs capable of detecting emerging resistance trends in real-time, facilitating timely intervention strategies. Additionally, the development of rapid diagnostic tools will be essential for promptly identifying multidrug-resistant bacteria in wound infections, enabling healthcare professionals to initiate targeted treatment approaches. Strengthening antimicrobial stewardship programs is crucial to promoting responsible antimicrobial use and minimizing resistance development. By educating healthcare providers and implementing guidelines for antimicrobial prescribing, these programs can help preserve the effectiveness of available treatment options. Furthermore, investing in

research on alternative therapies such as phage therapy and immunotherapy offers promising avenues for combating multidrug resistance and reducing reliance on conventional antibiotics. Finally, fostering collaboration and knowledge-sharing among researchers, healthcare professionals, policymakers, and industry stakeholders is vital to accelerating progress in addressing multidrug resistance in wound infections, thereby enhancing patient outcomes and public health overall.

Ethics approval

This study was accepted under Ethical Clearance No. 082/KEPK.EC/VI/2022 from Tugurejo Hospital Ethics Committee.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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