

Original Article

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Microbiological Spectrum and Phenotypic Detection of Multidrug-Resistant Organisms (MDROs) Isolated from Wound Infection Patients at a Tertiary Care Hospital

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Abstract:

Background: Wound infections causes significant morbidity in addition to escalate of health expenditure and these infections typically begin with bacterial colonization that are highly resistant to drugs.

Objective: The purpose of this study was to evaluate the phenotypic detection and antimicrobial susceptibility pattern of multi-drug resistance organisms (MDROs) isolated from wound infection patients at a tertiary care hospital, Bangladesh.

Methodology: This retrospective study was conducted in the Department of Microbiology at Monno Medical College, Manikganj, Bangladesh during the period from January 2021 to December 2024 for duration of four years. Patients who were presented with wound infection at any site of body and age more than 18 years with both sexes attending at outpatient department were selected as study population. All the samples were inoculated on blood agar and MacConkey agar media and were incubated at for hour at 37°C for 72 hours. Organisms were identified by standard microbiological procedures. Antimicrobial susceptibility test was done for all isolated bacteria by disc diffusion method

Results: Total of 680 patients were recruited for this study, with male patients being predominant at 360 (52.94%). Bacterial growth was observed in 504 cases (74.11%). The predominant isolate was *Staphylococcus aureus* which was 252 (50.00%) followed by *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas* species and *Klebsiella* species which were 100(19.84%), 72(14.28%), 64(12.69%) and 16(3.17%) respectively. Most resistant antibiotic was cefixime which was 456(90.47%) followed by nitrofurantoin, ceftriaxone and sulphamethoxazole which were 444(88.09%), 432(85.71%) and 424 (84.12%) respectively. The most predominant multidrug resistance bacteria was *Pseudomonas* species which was 60(93.75%) followed by *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* which were 12(75.0%),72(72.00%),180(71.42%) and 48(66.66%) respectively.

Conclusion: In conclusion the predominant isolate is *Staphylococcus aureus*, moreover the predominant multidrug resistant bacteria was *Pseudomonas* species followed by *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Most resistant antibiotic was cefixime. Antibiotic sensitivity pattern will guide for appropriate selection of antibiotics to reduce the spread of resistant bacteria against wound infection.

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Introduction:

Wound infections remain a major public health concern worldwide due to their association with prolonged morbidity, delayed healing, increased healthcare costs, and significant risk of systemic complications.

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A wound may be defined as a disruption of the normal anatomical structure and function of the skin and underlying tissues, resulting from trauma, surgery, burns, or underlying disease conditions. Once the protective skin barrier is breached, wounds become highly susceptible to microbial colonization and subsequent infection, particularly when host immunity is compromised or hygiene conditions are suboptimal¹. Community-acquired wound infections constitute a substantial proportion of cases presenting at outpatient departments, especially in low- and middle-income countries such as Bangladesh. These infections commonly occur due to minor trauma, road traffic accidents, occupational injuries, diabetic ulcers, surgical site infections, and skin and soft tissue infections managed outside hospital settings. Poor wound care practices, delayed medical consultation, inappropriate use of antibiotics, and lack of microbiological diagnosis contribute to increased rates of infection and antimicrobial resistance². Bacterial colonization of wounds typically progresses through distinct stages, beginning with contamination, followed by colonization, local infection, and in severe cases, systemic infection. While many wounds initially harbor commensal flora, pathogenic bacteria may proliferate when favorable conditions exist, leading to clinical infection characterized by pain, erythema, swelling, discharge, delayed healing, and sometimes fever. The most frequently implicated organisms in wound infections include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, and *Streptococcus pyogenes*³. Among *Staphylococcus aureus* is consistently reported as the predominant pathogen in both community- and hospital-acquired wound infections. Gram-negative organisms such as *E. coli*, *Pseudomonas* species, and *Klebsiella* species are particularly concerning due to their intrinsic resistance mechanisms and ability to acquire resistance genes via horizontal gene transfer⁴.

The emergence and spread of multidrug-resistant organisms (MDROs) pose a serious threat to effective wound management. MDROs are defined as microorganisms, primarily bacteria, that are resistant to at least one agent in three or more antimicrobial classes. The increasing prevalence of MDROs in community settings has blurred the

traditional distinction between hospital-acquired and community-acquired infections⁵. Inappropriate antibiotic prescribing, self-medication, availability of over-the-counter antibiotics, and incomplete treatment courses significantly contribute to this growing problem in developing countries⁶. Bangladesh faces a particularly high burden of antimicrobial resistance due to widespread misuse of antibiotics in human medicine. Several studies from different regions of the country have reported alarming resistance rates among wound pathogens, including resistance to commonly prescribed antibiotics such as cephalosporins, fluoroquinolones, and sulfonamides⁷. However, resistance patterns may vary across geographical regions, emphasizing the importance of local surveillance data to guide empirical therapy.

Phenotypic detection of bacteria through standard microbiological techniques remains the cornerstone of diagnosis in most clinical microbiology laboratories in resource-limited settings. Culture-based identification, followed by antimicrobial susceptibility testing using the disc diffusion method, provides essential information for appropriate antibiotic selection. Despite the availability of molecular diagnostic tools, phenotypic methods remain practical, cost-effective, and widely applicable in routine clinical practice⁸. Community-acquired wound infections in district-level settings are often managed empirically without microbiological confirmation, increasing the risk of treatment failure and further resistance development. Manikganj district, a semi-urban area of Bangladesh, represents a typical community setting where wound infections are frequently encountered in outpatient departments. However, limited published data are available regarding the bacterial profile and antimicrobial resistance patterns of wound pathogens in this region. Understanding the local epidemiology of wound infections, particularly the prevalence of MDROs and their antibiotic susceptibility patterns, is crucial for optimizing treatment strategies, improving patient outcomes, and curbing the spread of resistant bacteria.

Methodology:

Study Settings and Population: This retrospective study was conducted in the Department of Microbiology at the Monno

Medical College, Manikganj from June 2020 to July 2021 for a period of one year. Wound swabs were collected from patients attending at outpatient and inpatient department of Monno Medical College and Hospital. The patients attending at outpatient department were represented as community acquired infection and patient who admitted at least 48 hours were represented as hospital acquired infection. Wound infection was suspected if a wound was not healing well, getting bigger, exuding pus or fluid. Very ill patients and those undergoing antibiotic therapy two weeks prior to the study were excluded.

Sample Collection Procedure: Open wound swabs were aseptically obtained after the wound immediate surface exudates and contaminants were cleansed off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with sterile normal saline after removing the dressing. The specimen was collected on sterile cotton swab by rotating with sufficient pressure. Double wound swabs were taken from each wound at a point in time to reduce the chance of contamination. The samples were transported to the laboratory after collection with minimum delay.

Isolation and Identification of Bacteria: All the samples were streaked on blood agar and MacConkey agar media by sterile inoculation loop and incubated 24 to 48 hours at 35 to 37°C. Organisms were identified by standard microbiological procedures including colony characters, haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Gram staining and biochemical reactions tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests namely: Kligler's Iron Agar (KIA), Indole, Simon's citrate agar, urea and motility. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results. Susceptibility to antimicrobial agents of all isolates was done by Kirby Bauer modified disc diffusion technique using Mueller Hinton agar plates and zones of inhibition were interpreted according to CLSI guidelines (2015). Antibiotic discs such as cefotaxime(30µg), ceftriaxone(30µg),

amoxicillin (20µg), ciprofloxacin(5µg), cefuroxime (30µg), amikacin(30µg), azythromycin(15µg), gentamicin(10µg), doxycycline(30µg), nalidixicAcid(30µg), tetracycline(30µg), cefixime(30µg), imipenum(10µg), nitrofurantoin(300µg), vancomycin (30µg/disc), linezolid (30µg/disc), sulphamethozazole with trimethoprim (25 µg).

Statistical Analysis: All data were checked and were analyzed after collection. Then the data were entered into a computer and were analyzed using simple statistical methods. Some statistical calculation and percentage were done by using a scientific calculation, and the finding was arranged in tabular form. Descriptive analyses of frequencies, median, mean, minimum and maximum for continuous variables and percentages for categorical variables were performed using SPSS version 27.0 (Windows Based Software). Chi-square test was used for comparison of categorical variables. Every effort was made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Ethical Clearance: Formal ethics approval was granted by the IRB of Monno Medical College. IRBMemo No:IRB/MoMC/2025/009. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Result

A total number of 680 wound infection patients were recruited in this study among them male patients were predominant 360 (52.94%) (figure-I). About 504 (74.11%) yielded growth of different bacteria (figure-II).

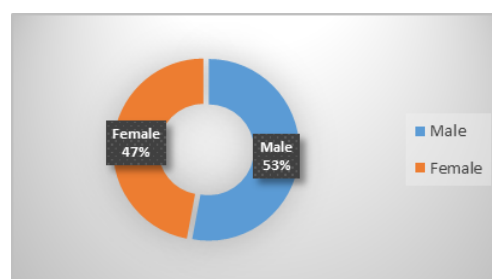


Figure I: Pie chart showing distribution of participants according to gender (n=680)

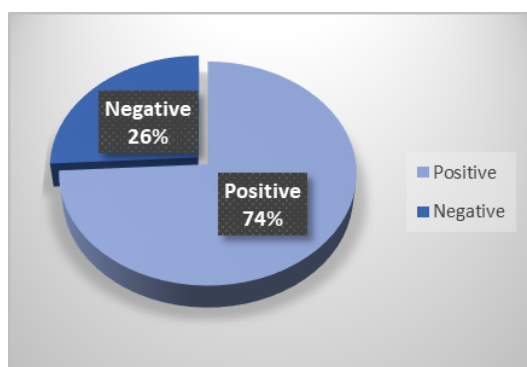


Figure II: Pie chart showing distribution of participants according to culture positivity (n=680)

The predominant isolate was *Staphylococcus aureus* which was 252 (50.00%) followed by *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas* species and *Klebsiella* species which were 100(19.84%), 72(14.28%), 64(12.69%) and 16(3.17%) respectively. (Table-1)

Table -1: Distribution of Isolated Bacteria

Name of Bacteria	Frequency	Percent
<i>Staphylococcus aureus</i>	252	50.0
<i>Streptococcus pyogenes</i>	72	14.28
<i>Escherichia coli</i>	100	19.84
<i>Pseudomonas</i> species	64	12.69
<i>Klebsiella</i> species	16	3.17
Total	504	100.0

Out of 252 isolates of *Staphylococcus aureus* majority were found in the MDRO than Non MDRO which was 180 (71.42%) and 72 (28.58%) respectively. The difference between the isolation rate in MDRO & Non-MDRO was not statistically significant ($p=0.26$). Out of 72 isolates of *Streptococcus pyogenes* majority were found in the MDRO than Non MDRO which was 48 (66.66%) and 24(33.34%) respectively. The difference between the isolation rate in MDRO & Non-MDRO was not statistically significant ($p=0.17$). Out of 100 isolates of *Escherichia coli* majority were found in the MDRO than Non MDRO which was 72(72.0%) and 28(28.0%) respectively. The difference between the isolation rate in MDRO

& Non-MDRO was not statistically significant ($p=0.73$). Out of 64 isolates of *Pseudomonas* species majority were found in the MDRO than Non MDRO which was 60(93.75%) and 4(6.25%) respectively. The difference between the isolation rate was statistically significant ($p=0.00$). Out of 16 isolates of *Klebsiella* species majority were found in the MDRO than Non MDRO which was 12 (75.0%) and 4(25.0%) respectively. The difference between the isolation rate was not statistically significant ($p=1.00$). (Figure III) and (Table 3)

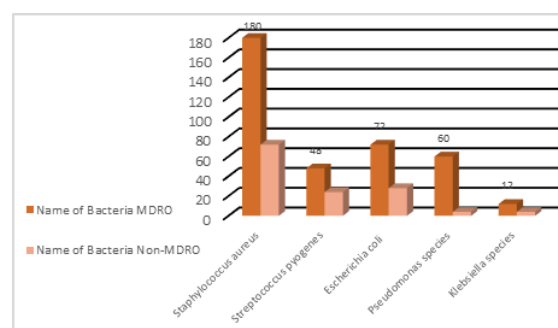


Figure III: Bar diagram showing distribution of Isolated Bacteria according to MDRO & Non-MDRO Groups (n=680)

Table 3: Isolated Bacteria in MDRO & Non-MDRO Groups

Name of Bacteria	Group		Total	P value
	MDRO	Non-MDRO		
<i>Staphylococcus aureus</i>	180(71.42%)	72(28.58%)	252(50.0%)	0.26
<i>Streptococcus pyogenes</i>	48(66.66%)	24(33.34%)	72(14.28%)	0.17
<i>Escherichia coli</i>	72(72.00%)	28(28.00%)	100(19.84%)	0.73
<i>Pseudomonas</i> species	60(93.75%)	4(6.25%)	64(12.69%)	0.00
<i>Klebsiella</i> species	12(75.0%)	4(25.00%)	16(3.17%)	1.00

Chi- Square (X^2) test was performed to see the association. $P \leq 0.05$ was determined as level of significance.

Antimicrobial susceptibility testing revealed high resistance rates among the isolates. The most resistant antibiotic was cefixime, with resistance observed in 456 (90.47%) isolates. This was followed by nitrofurantoin 444 (88.09%), ceftriaxone 432 (85.71%), and sulphamethoxazole 424 (84.12%). (Table 4)

Table 4: Antibiotic sensitive pattern of the isolated bacteria

Name of Antibiotics	Frequency	Percent
Vancomycin	412	(81.74%)
Sulphamethoxazole	424	(84.12%)
Imipenem	92	(18.25%)
Amikacin	369	(78.57%)
Azithromycin	180	(35.71%)
Ciprofloxacin	212	(42.06%)
Levofloxacin	396	(78.57%)
Tetracycline	416	(82.53%)
Doxycycline	324	(64.28%)
Cefotaxime	384	(76.19%)
Ceftriaxone	432	(85.71%)
Gentamycin	108	(21.42%)
Cefixime	456	(90.47%)
Linezolid	85	(16.80%)
Nitrofurantoin	444	(88.09%)
Ceftazidime	336	(66.66%)
Total	504	100.0%)

Discussion:

The present study provides a comprehensive overview of the bacteriological spectrum and antimicrobial susceptibility patterns of multidrug-resistant organisms isolated from wound infection patients attending the outpatient department of Monno Medical College, Manikganj, Bangladesh. The findings of this study reflect the real epidemiological scenario of wound infections in a district-level healthcare setting and highlight important challenges in the management of such infections.

In this study, a total of 680 patients with clinically suspected wound infections were included over a four-year period. Among them, bacterial growth was observed in 504 cases, yielding an overall culture positivity rate of 74.11%. This relatively high positivity rate indicates that bacterial infection is a predominant cause of wound-related complications in community-acquired cases. The culture positivity rate observed in this study is consistent with reports from similar settings, where rates exceeding 70% have been documented, underscoring the clinical relevance of microbiological evaluation in wound management⁹.

The gender distribution of patients revealed a male predominance, with males accounting for

52.94% of cases. Another study in Bangladesh was showed that the incidence of wound infection was higher in males (60.52%) than in females (39.47%)¹⁰. A study conducted in India on Post-operative wound infection supporting the fact that gender difference is not significant.¹¹ This finding may be attributed to greater exposure of males to trauma and injuries due to occupational and outdoor activities. In rural and semi-urban areas such as Manikganj, males are more frequently involved in agriculture, construction work, transportation, and other labor-intensive occupations, which increase the risk of accidental injuries and subsequent wound infections. Additionally, behavioral factors such as neglect of minor injuries and delayed medical consultation may further contribute to the higher prevalence of wound infections among males.

Analysis of the bacteriological profile revealed that *Staphylococcus aureus* was the most predominant isolate, accounting for 50.00% of all culture-positive cases. Several studies had reported that *Staphylococcus aureus* was the common isolate of purulent wound infections worldwide with the prevalence rate ranging from 4.6% to 54.4%.^{12,13} The predominance of *S. aureus* in wound infections is well recognized and can be attributed to its status as a common commensal organism of the skin and nasal mucosa. Once the integrity of the skin is disrupted, *S. aureus* can readily invade underlying tissues and establish infection¹⁴. Among Gram-negative bacteria, *Escherichia coli* was the second most common isolate, accounting for 19.84% of cases. The presence of *E. coli* in wound infections suggests possible contamination from the environment, fecal matter, or poor personal hygiene practices¹⁵. *Streptococcus pyogenes* was isolated in 14.28% of cases, making it another significant contributor to wound infections in the study population. *S. pyogenes* is a well-known pathogen associated with skin and soft tissue infections, including cellulitis, impetigo, and necrotizing fasciitis¹⁶. Although its isolation rate was lower than that of *S. aureus* and *E. coli*, the presence of *S. pyogenes* remains clinically significant due to its aggressive nature and potential for serious complications. *Pseudomonas* species accounted for 12.69% of the isolates and represented one of the most concerning

findings of the study. *Pseudomonas* species are opportunistic pathogens frequently associated with chronic, non-healing, and contaminated wounds¹⁷. Their ability to survive in environmental conditions, form biofilms, and exhibit intrinsic resistance to multiple antibiotics makes them particularly difficult to eradicate. *Klebsiella* species were the least frequently isolated organisms, accounting for 3.17% of cases. Despite their lower prevalence, *Klebsiella* species are clinically important due to their association with severe infections and high rates of antimicrobial resistance.

Antimicrobial susceptibility testing revealed an alarming pattern of resistance among the isolated organisms. Cefixime was identified as the most resistant antibiotic, with 90.47% of isolates showing resistance which was similar to the study done by Albumani et al¹⁸. This finding is particularly concerning, as cefixime is commonly prescribed in community settings due to its oral formulation, broad-spectrum activity, and perceived safety. The high resistance rate observed in this study suggests extensive misuse and overuse of cefixime in the community, often without proper microbiological guidance. Such practices exert selective pressure on bacterial populations, leading to the emergence and spread of resistant strains.

High resistance rates were also observed for nitrofurantoin (88.09%), ceftriaxone (85.71%), and sulphamethoxazole (84.12%) This finding is in agreement with the study of Mama M et al¹⁹, Bibi S. et al²⁰ Gautam R et al²¹. The resistance to ceftriaxone, a widely used third-generation cephalosporin, is particularly alarming, as this antibiotic is often considered a mainstay for the treatment of serious infections. The high resistance to sulphamethoxazole may be attributed to its long-standing use in the treatment of a variety of infections in resource-limited settings. Over time, extensive use of this drug has led to widespread resistance, reducing its clinical utility. Similarly, the resistance observed to nitrofurantoin may reflect its inappropriate use beyond its recommended indication for urinary tract infections. These findings highlight the consequences of irrational antibiotic prescribing practices and underscore the

urgent need for antimicrobial stewardship interventions²².

One of the most striking findings of this study is the high prevalence of multidrug-resistant organisms among the isolates. *Pseudomonas* species exhibited the highest rate of multidrug resistance, with 93.75% of isolates classified as MDROs. *Klebsiella* species also demonstrated a high rate of multidrug resistance (75.00%), despite their relatively low prevalence. This finding is clinically significant, as multidrug-resistant *Klebsiella* infections are associated with increased morbidity, prolonged treatment, and higher healthcare costs. *Escherichia coli* showed a multidrug resistance rate of 72.00%, indicating that a substantial proportion of isolates were resistant to multiple antibiotic classes. This high rate of resistance limits treatment options and increases the likelihood of treatment failure, particularly when empirical therapy is initiated without susceptibility data. The multidrug resistance observed in *E. coli* also suggests widespread circulation of resistant strains in the community²³. Similarly, 71.42% of *Staphylococcus aureus* and 66.66% *Streptococcus pyogenes* isolates were found to be multidrug resistant. This finding has important clinical implications, as multidrug-resistant *S. aureus* infections are more difficult to manage and are associated with prolonged healing times and increased risk of complications. The high prevalence of multidrug-resistant *S. aureus* in wound infections indicates that resistant strains are no longer confined to hospital settings but are increasingly encountered in outpatient populations²⁴.

The high prevalence of multidrug-resistant organisms and the widespread resistance to commonly used antibiotics underscore the urgent need for coordinated efforts to address antimicrobial resistance. By guiding appropriate antibiotic selection and promoting rational use, the findings of this study can contribute to improved patient outcomes and help mitigate the growing threat of resistant bacteria in wound infections.

Conclusion:

This study demonstrated that *Staphylococcus aureus* was the most frequently isolated pathogen from wound infections, while *Pseudomonas* species showed the highest

prevalence of multidrug resistance, followed by *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. A high level of resistance was observed against commonly used antibiotics, particularly cefixime. The findings highlight the growing burden of multidrug-resistant organisms in wound infections and emphasize the need for continuous microbiological surveillance and rational antibiotic use. Regular monitoring of antimicrobial susceptibility patterns is essential to guide effective empirical therapy and to help reduce the spread of resistant pathogens in tertiary care settings.

Author's contribution: Conceptualization, methods and literature reviews: Jahan T, Mimi SA, Mahmud S; Data collection: Jahan T; Islam N; Statistical analysis: Jahan T, Neeva NI; Draft manuscript: Jahan T, Mimi SA. All the authors work and approved the final manuscript.

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