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SUSCEPTIBILITY TREND OF MDR GRAM NEGATIVE ISOLATES FROM INFECTED WOUNDS OF HOSPITALIZED PATIENTS IN TERTIARY CARE HOSPITAL SOUTH PUNJAB

Original Research

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ABSTRACT

Background: Wound infections remain a significant cause of delayed healing and increased morbidity in hospitalized patients. The presence of devitalized tissue and moist wound environments provide an ideal niche for microbial colonization and infection. Gram-negative bacteria are particularly associated with hospital-acquired infections, and their growing resistance to multiple antibiotics has become a critical concern in clinical management.

Objective: To isolate Gram-negative bacterial strains from infected wounds of hospitalized patients and determine their antibiotic susceptibility patterns.

Methods: This cross-sectional study was conducted at the Department of Pathology, Combined Military Hospital (CMH) Multan. A total of 170 wound swab samples were collected aseptically using sterile cotton swabs in Amies transport medium. All samples were cultured on Blood agar and MacConkey agar and incubated at 37°C for 24 hours. Gram-negative isolates were further identified using standard biochemical tests including oxidase, indole, MR-VP, citrate, urease, and TSI. Antibiotic susceptibility testing was performed using six commonly prescribed antibiotics. Data were entered and analyzed using SPSS version 25. Frequencies and percentages were calculated, and chi-square was applied where appropriate with $p \leq 0.05$ considered significant.

Results: Out of 170 samples, 135 (79.4%) were culture positive, with 110 (81.4%) yielding Gram-negative rods and 25 (18.5%) Gram-positive cocci. The most frequently isolated pathogen was *Pseudomonas aeruginosa* (33.3%), followed by *E. coli* (14.07%), *Proteus* (13.3%), *Enterobacter* (11.8%), *Citrobacter* (5.18%), *Klebsiella* (2.22%), and *Acinetobacter* (1.4%). *E. coli* exhibited high resistance to ciprofloxacin (73.6%) and amoxicillin-clavulanate (68.4%), while remaining sensitive to cefepime (84.2%) and amikacin (84.2%).

Conclusion: This study highlights the increasing prevalence of multidrug-resistant Gram-negative bacteria in wound infections. Judicious antibiotic use and routine microbial surveillance are essential to curb the spread of resistant strains in hospital settings.

Keywords: Amikacin, Anti-Bacterial Agents, Drug Resistance, Escherichia coli, Gram-Negative Bacteria, Pseudomonas aeruginosa, Wound Infection.

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INTRODUCTION

The skin serves as the body's first line of defense, offering a physical and immunological barrier against the invasion of pathogenic microorganisms. This protective function is supported by the skin's normal flora, which inhibits colonization by external pathogens. However, when the integrity of the skin is compromised due to trauma, the underlying tissues become exposed, creating an ideal environment—characterized by warmth and moisture—for microbial growth and potential infection (1). Literature consistently supports the notion that wounds, especially in clinical settings, are inherently vulnerable to microbial contamination, and if not managed appropriately, are at constant risk of progressing to sepsis (2). Wound infections are clinically significant due to their association with delayed healing, prolonged hospital stays, increased treatment costs, and elevated morbidity and mortality rates (3). Hallmarks of infected wounds typically include purulent discharge, localized inflammation, pain, and systemic signs such as fever (4). However, it is crucial to recognize that the presence of exudate alone is not definitive evidence of infection, necessitating a more nuanced approach to diagnosis. Differentiating between wound contamination, colonization, and true infection is essential in clinical decision-making. While initial contamination by pathogens is often minimal, sepsis arises when multiple microorganisms overwhelm host defenses, leading to active tissue destruction and systemic involvement (5,6).

Colonization refers to the presence and replication of low-virulence organisms or normal flora within the wound without invoking a pathological response, and its clinical implications differ significantly from infection (4). The progression from colonization to infection is largely influenced by both microbial virulence and the host's immune status (7). As such, assessing the immune competence of a patient becomes critical in predicting susceptibility to infection and interpreting the microbiological profile of wounds. Among the various microorganisms implicated in wound infections, both aerobic and anaerobic pyogenic bacteria are frequently identified. These organisms are responsible for the formation of pus and are particularly associated with post-operative complications. Surgical site infections remain among the most common nosocomial complications, contributing to increased morbidity and healthcare burdens (6–8). *Staphylococcus aureus* has been repeatedly identified as the leading cause of wound infections, accounting for approximately 20% to 40% of cases, followed by *Pseudomonas aeruginosa*, which is especially prevalent in burn wounds and constitutes around 5% to 15% of nosocomial infections (8-10). Members of the *Enterobacteriaceae* family and *Enterococci* species are also frequently isolated, particularly in post-abdominal surgeries and immunocompromised patients (11,12).

In recent years, the emergence of Metallo Beta-Lactamase (MBL)-producing Gram-negative bacteria has posed a critical threat to infection management. These enzymes confer resistance to nearly all beta-lactam antibiotics, including carbapenems—agents often reserved as the last line of defense in severe infections—while sparing only monobactams such as aztreonam. The genes encoding MBLs are often located on mobile genetic elements like plasmids and transposons, which facilitate the rapid and widespread transmission of resistance among hospitalized patients. This alarming trend is further exacerbated by the scarcity of region-specific data on antimicrobial resistance patterns in wound infections. Given the growing burden of antimicrobial resistance and the lack of comprehensive local data, this study was designed to isolate Gram-negative bacterial pathogens from infected wounds in hospitalized patients and to determine the antibiotic susceptibility profiles of these isolates. The objective is to inform targeted therapeutic strategies and contribute to antimicrobial stewardship efforts in clinical settings.

METHODS

Present study held at Microbiology section, Department of pathology, Combined Military Hospital Multan. Sample size calculated turned out to be 170. Written informed consent was taken at the time of enrollment. Study was approved by ethical committee. Inclusion samples include gram negative rods while anaerobic bacterias were excluded. Aseptically, wound swabs were with sterile cotton wool swab in Amies transport media. Present study used mediums like Blood agar, MacConkey agar, Muller Hinton agar, Triple sugar iron agar, Simmon citrate agar and Urease agar for pathogen growth. Mediums were prepared according to the guidelines in 500 ml bottle and sterilized by autoclaving at 121°C for 15 minutes at 15 pound pressure. Reagents which were used for biochemical tests included Oxidase reagent, Indole reagent, Methylred, Voges proskauer reagents, sulphide indole motility media (SIM)and reagents for gram

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staining. The following control strains were used to check the performance of the method and were arranged from National Institute of health.

Inoculation of Culture Media:
All collected wound samples were inoculated on Blood agar and MacConkey agar and incubated at 37°C for 24 hours. After incubation period on MacConkey agar, gram negative rods produced two types of colonies, pink and colourless colonies. Lactose fermenters (*Escherichia coli, Enterobacter and Klebsiella*) produced pink colonies while non-fermenter species (*Proteus, Pseudomonas*) produced colourless colonies. Gram positive were not processed further while gram negative rods were further evaluated with the help of different biochemical reactions.

STATISTICAL ANALYSIS:

The data was entered and analyzed in SPSS 25. Mean ± SD were given for numeric data i.e., age. Frequency and percentage were calculated for categorical data i.e., gram staining, culture report, drug sensitivity. Chi square was applied. A p-value ≤ 0.05 was considered significant.

RESULTS

Standard results of different gram-negative bacteria including *Pseudomonas aeruginosa* on Culture media, Microscopy and Motility test were shown in table-1.

Table-1: Gram negative rods on culture, Microscopy and Motility Test

Species	Blood agar	MacConkey	Microscopy	Motility
Pseudomonas aeruginosa	Large flat spreading colonies, majority haemolytic, greenish blue pigment	Pale or colourless non-lactose fermenter colonies	Gram negative rods	Motile
Escherichia coli	1 to 4 mm colonies	Smooth, pink colonies	Gram negative rods	Motile
Enterobacter	Large non mucoid colonies	Pink lactose fermenter colonies	Gram negative rods	Motile
Proteus	Colonies produce swarming, fishy odour	Colourless colonies (non-lactose fermenter)	Gram negative rods	Motile
Klebsiella	Large, grey, white, mucoid colonies	Mucoid pink colonies	Gran negative rods	Non motile
Citroabacter	Circular flat colonies	Late or non-lactose fermenter	Gram negative rods	Motile
Acinetobacter	White smooth, raised, opaque colonies	Colourless colonies	Short, stout, gram negative cocobacilli	Non motile

Swabs taken were directly sent to microbiology laboratory CMH Multan and applied on appropriate media for culture report after 24 hrs. Results showed that 135(79.4%) were culture positive after 24 hours of incubation while 35(20.5%) samples were culture negative (table-3) while among 135 culture positive organisms, 25 (18.5%) samples were Gram positive cocci and 110 (81.4%) were Gram negative rods as shown in table-2.

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Table-2: Culture Report of Samples After 24 hours of incubation		
Culture report	**Number N=170**	**%age**
Culture positive	135	79.4%
Culture negative	35	20.5%
Gram Staining Analysis		
Gram staining	**Number N=135**	**%age**
Gram negative rods	110	81.4
Gram positive cocci	25	18.5

Table-3 showed that out 110 culture positive gram negative rods, 45 (33.3%) were *Pseudomonas aeruginosa*, *Escherichia coli* 19(14.07%), *Proteus* 18(13.3%), *Enterobacter* 16 (11.8%), *Citrobacter* 7(5.18%), *Klebsiella* 3 (2.22%) and *Acinetobacter* 2 (1.4%) and remaining 25(18.5%) were gram positive cocci.

Table-3: Bacterial Isolates from Culture Positive Samples of Wound Swabs	
Isolates	**No of Isolates N=135(110+25)**
Pseudomonas aeruginosa	45 (33.3%)
Escherichia coli	19 (14.07%)
Proteus	18 (13.3%)
Enterobacter	16 (11.8%)
Citrobacter	7 (5.18%)
Klebsiella	3 (2.2%)
Acinetobacter	2 (1.4%)
Gram positive cocci	25 (18.5%)
Total	135 (100%)

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Figure-1 showed that out of 170 samples 95 (55.8%) were taken from abdomen, 43 (25.2%) from legs, 12 (7.05%) from foot, 3 (1.76%) from hands, 9 (5.29%) from arms and 8 (4.705%) were from chest. Highest number of samples were taken from abdominal wounds and lowest were from hand wounds.

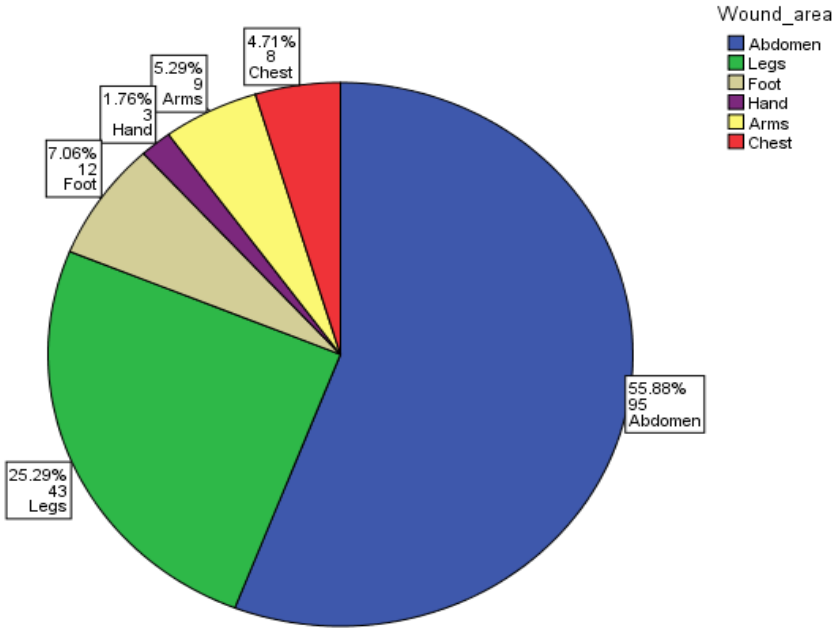

Figure 1: Source of wound swabs from different wound sites

Table-4 reflected the antibiotic susceptibility pattern of gram-negative bacteria other than *Pseudomonas aeruginosa*. The susceptibility pattern of gram-negative bacteria (N=65) isolated from wound infection against 06 selected antibiotics. Most of the bacteria showed mild to moderate rate of resistance against most of these antibiotics except *Klebsiella* and *Acinetobacter* which showed high rate of resistance against these drugs. *E. coli* showed 10.5% resistance against Cefipime, 21.05% against Ceftazidime, 73.6% against Ciprofloxacin, 10.5% against Amikacin, 31.5% against Ceftriaxone, 68.4% against Amoxicillin/Clavulanic.

Table-4: Antibiotic Susceptibility Pattern of Gram-Negative Bacteria

Antibiotics (µg)	**E.coli N=19(%)**			**Enterobacter N=16(%)**			**Proteus N=18(%)**			**Citrobacter N=7(%)**			**Klebsiella N=3(%)**			**Acinetobacter N=2(%)**			**Total N=65(%)**		
	S	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**
Cefipime (30)	16 84.2	1 5.2	2 10.5	12 75	2 12.5	2 12.5	14 77.7	0	4 22.2	5 71.1	0	2 28.5	1 33.3	0	2 66.7	0	0	2 100	48 73.8	3 4.6	14 21.5

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Table-4: Antibiotic Susceptibility Pattern of Gram-Negative Bacteria

Antibiotics (µg)	E.coli N=19(%)			Enterobacter N=16(%)		Proteus N=18(%)				Citrobacter N=7(%)		Klebsiella N=3(%)		Acinetobacter N=2(%)					Total N=65(%)		
	S	IS	R	S	IS	R	S	IS	R	S	IS	R	S	IS	R	S	IS	R	S	IS	R
Ceftazidime (30)	15 78.9	0	4 21.5	10 62.5	2 12.5	4 25	10 55.5	1 5.5	7 38.8	4 57.1	0	3 42.8	1 33.3	0	2 66.7	0 0	0	2 100	40 61.5	3 4.6	22 33.8
Ciprofloxacin (5)	2 10.5	3 15.7	14 73.6	1 6.25	3 18.7	12 75	0 0	8 44.4	10 55.5	0 0	1 14.2	6 85.7	0 0	0	3 100	0 0	0	21 00	3 4.6	15 23.0	47 72.3
Amikacin (30)	16 84.2	1 5.2	2 10.5	12 75	3 18.7	1 6.25	16 88.8	1 5.5	1 5.5	6 85.7	0	1 14.2	2 66.6	0	1 33.3	0 0	0	2 100	52 80	5 7.6	8 12.3
Pipracillin/ Tazobactam (100/10)	19 100	0 0	0 0	16 100	0	0 0	17 94.4	1 5.5	0 0	4 57.1	0	3 42.8	1 33.3	2	0 0	1 50	1 50	0 0	58 89.2	4 6.1	3 4.61
Ceftriaxone (30)	10 52.6	3 15.7	6 31.5	2 12.5	2 12.5	12 75	4 22.2	0	14 77.7	1 14.2	1 14.2	5 71.4	0 0	0	3 100	0 0	0	2 100	17 26.1	6 9.2	42 64.6
Amoxicillin/Clavulanicacid(20/10)	2 10.5	4 21.0	13 68.4	0 0	0	16 100	0 0	0	18 100	0 0	0	7 100	0 0	0	3 100	0 0	0	21 00	2 3.07	4 6.1	59 90.7

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DISCUSSION

Infection remains one of the principal barriers to timely wound healing, often leading to complications that delay recovery. The clinical practice of wound culture serves as an essential diagnostic tool for identifying causative organisms and guiding appropriate antimicrobial therapy. Although not all wounds require culture, samples should be collected when there is clinical suspicion of infection to avoid unnecessary antibiotic exposure and development of resistance. In the present study, culture positivity was observed in 79.4% of wound swabs, with Gram-negative rods accounting for 81.4% of the isolates. This predominance of Gram-negative organisms is consistent with findings from similar studies conducted in comparable hospital environments (13-15). However, other investigations have reported higher isolation rates of Gram-positive cocci, reflecting the variability in pathogen distribution due to differing hospital practices, patient populations, and antimicrobial usage patterns (16,17). A key finding of this study was the higher frequency of Gram-negative rods isolated from abdominal wound sites. This aligns with existing evidence suggesting that Gram-negative bacteria are predominant pathogens in abdominal surgical infections (18). The site-specific collection bias in this study likely contributed to the increased isolation of Gram-negative organisms, underscoring the influence of wound location on microbial profiles. Among Gram-negative isolates, *Pseudomonas aeruginosa* was the most prevalent, followed by *Escherichia coli* (14.07%), *Proteus* spp. (13.3%), *Enterobacter* spp. (11.8%), *Citrobacter* spp. (5.18%), *Klebsiella* spp. (2.22%), and *Acinetobacter* spp. (1.4%). These distribution patterns closely resemble those reported in previous investigations, which demonstrated comparable prevalence rates of *E. coli*, *Enterobacter*, and *Proteus* from wound infections (19-21).

The antibiotic susceptibility profiles revealed notable multidrug resistance among several isolates. *E. coli* demonstrated high resistance to ciprofloxacin (73.6%), amoxicillin-clavulanic acid (68.4%), chloramphenicol (94.7%), and tetracycline (78.95%). Conversely, it remained highly sensitive to cefepime (84.2%), ceftazidime (78.9%), amikacin (84.2%), and imipenem (100%). These findings align with prior literature, where high resistance to older antibiotics and preserved susceptibility to carbapenems and aminoglycosides was reported (22). Similarly, *Enterobacter* exhibited high resistance to ciprofloxacin and ceftriaxone (75% each), and complete resistance to chloramphenicol and amoxicillin-clavulanic acid. Sensitivity to piperacillin-tazobactam (100%), imipenem (93.7%), and aztreonam (87.5%) was noted, reflecting patterns seen in earlier studies (22,23). The resistance patterns highlight an alarming trend of antimicrobial resistance, particularly among commonly prescribed oral agents. These observations emphasize the urgent need for antimicrobial stewardship programs and adherence to evidence-based prescribing guidelines in hospital settings. The study's findings also suggest a preference for reserving broad-spectrum and last-resort antibiotics, such as carbapenems, for culture-confirmed infections to avoid accelerating resistance development.

One of the key strengths of this study was its focus on wound-specific Gram-negative pathogens and the provision of a detailed susceptibility profile, which holds clinical relevance for empirical treatment decisions. Moreover, the use of standardized biochemical and culture-based diagnostic procedures enhances the reliability of the results. However, the study was limited by its single-center design and modest sample size. The lack of stratification by patient comorbidities, immune status, and previous antibiotic exposure may have influenced the observed microbial patterns and resistance rates. In addition, data on the susceptibility profile of *Pseudomonas aeruginosa*—despite its high frequency—was not reported, representing a critical gap given its clinical significance and known resistance challenges. Future studies should aim to include larger, multi-center cohorts with stratified patient data to allow for a more comprehensive analysis of wound microbiota and resistance dynamics across diverse clinical settings. Additionally, inclusion of molecular typing methods and resistance gene profiling may provide further insights into resistance mechanisms and transmission trends. In conclusion, this study reaffirms the predominance of Gram-negative rods, particularly *Pseudomonas aeruginosa* and *E. coli*, in infected wound sites and underscores the need for targeted antimicrobial strategies based on localized susceptibility data. The rising trend of multidrug resistance among these organisms necessitates continuous surveillance and judicious antibiotic use to preserve the effectiveness of available therapies.

CONCLUSION

This study concluded that the rise in multidrug-resistant Gram-negative bacteria from wound infections is closely linked to the irrational and widespread misuse of antibiotics, posing significant therapeutic challenges in clinical settings. Timely identification of these resistant pathogens through microbiological testing is essential for guiding effective treatment and limiting their spread within healthcare environments. The findings emphasize the urgent need for strengthened antibiotic stewardship programs and evidence-based prescribing

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practices. Formulating and enforcing clear national guidelines on antibiotic use can play a pivotal role in preserving antimicrobial efficacy and improving patient outcomes.

AUTHOR CONTRIBUTION

Author	Contribution
Mehreen Afzal	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Inam Ullah Khan	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Furqan Muhammad Iqbal	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Talha Laique*	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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