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Prevalence of Multidrug-Resistant (MDR) Gram-Negative Bacilli in Burn Infections among Patients at Al-Mouwasat Hospital, Damascus, Syria

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ABSTRACT

Burn infections pose a significant contribution to morbidity and mortality globally, particularly due to the emergence of multi-antibiotic-resistant strains, especially in developing nations. This study aims to ascertain the prevalence of Multidrug-Resistant (MDR) strains of gram-negative bacilli among patients with burn infections at the Burns and Plastic Surgery section in Al-Mouwasat Hospital. A total of 49 swabs were collected from patients between November 2023 and February 2024, at Al-Mouwasat Hospital. The isolation and identification of species was done by conventional microbiologic techniques. Antibiotic sensitivity was determined using Kirby-Bauer disk diffusion method. MDR strains were identified according to established international standards. All 49 samples contained Gram-negative bacteria. 10 samples (20.4%) exhibited the presence of two bacilli species, while the remaining (79.6%) contained a single one. Male samples were predominant (67.3%). *Pseudomonas aeruginosa* was the most frequently isolated organism, accounting for (39%) of the samples, followed by *Klebsiella* (34%), *Enterobacter* (22%), and *E. coli* (5%). Notably, all isolated strains were MDR. All isolates were resistant to Aztreonam and Erythromycin. Two *P. aeruginosa* and three *Klebsiella* isolates exhibited resistance to all tested antibiotics. Our study highlights the prevalence of Multidrug-Resistant Gram-negative bacilli at the burn and plastic surgery unit of Al-Mouwasat Hospital. Therefore, emphasis should be placed on prevention and control strategies, as well as the judicious selection of antibiotics to mitigate the spread of MDR strains causing infections.

1. Introduction

Burn injuries are associated with elevated rates of morbidity and mortality [1]. In burn patients, infections caused by multidrug-resistant strains (MDRs) often contribute significantly to mortality [2].

The compromised integrity of the skin barrier in burned areas facilitates the proliferation of pathogens,

rendering burn victims more vulnerable to hospital-acquired infections [3], [4]. The indiscriminate and improper use of antibiotics exacerbates the emergence of MDR strains, particularly prevalent in developing countries, complicating treatment, prolonging hospital stays, and heightening the risk of complications such as sepsis, potentially leading to fatal outcomes [5], [6].

Researches indicate that burn patients are at a heightened risk of developing MDR infections compared to other hospitalized even at early stages of hospitalization [7]. Among burn patients, Gram-negative bacilli, notably *Pseudomonas aeruginosa*, *Klebsiella*, *Enterobacter*, and *E. coli*, are frequently isolated pathogens, correlating with mortality rates [2], [8].

Variations in bacterial species distribution and their antibiotic sensitivity patterns can impact treatment strategies and available medications [9]. This study aimed to ascertain the prevalence of MDR Gram-negative bacilli among burn patients at Al-Mouwasat Hospital and characterize their antibiotic sensitivity profiles. The objective is to enhance treatment options, mitigate the rise of MDR strains, and reduce mortality associated with these infections.

2. Materials and Method

This study was designed as a cross-sectional study.

Samples and Collection:

Forty-nine samples were collected from the injury sites of patients, following informed consent, between November 2023, and February 2024. Samples were procured from all patients admitted to the Burns and Plastic Surgery section at Al-Mouwasat Hospital, displaying signs of infection at the injury site. Collection commenced from the fifth day of the patient's admission to the department. Each sample was properly labeled denoting the patient's gender. Swabs were directly immersed in a sterile preservation medium to maintain bacterial viability until transfer to the laboratory [10]. Subsequently, the swabs were cultured, streaked, and incubated at 37°C for 24 hours.

Isolation and Identification of Bacteria:

Bacterial identification was conducted through Gram staining and culture on blood agar, EMB, Cetrimide, and TSI agar media, in addition to motility and biochemical tests, according to standard guidelines [11]. All media were sourced from Himedia Laboratories, India. Media were prepared following the manufacturer’s instructions.

Antibiotic Susceptibility Test:

To assess the emergence of multidrug resistance in bacteria, antibiotic susceptibility testing was conducted for each isolate using Kirby Bauer disc diffusion method. This testing was performed on Mueller Hinton agar medium following the standards outlined by the Clinical and Laboratory Standards Institute (CLSI), as specified in the CLSI M100S 26th edition guidelines [12]. Discs impregnated with specific concentrations of antibiotics were utilized for this purpose.

A small inoculum of each pure bacterial isolate was emulsified in sterile normal saline. The bacterial suspension was prepared to match the turbidity equivalent to a 0.5 McFarland standard. A cotton swab was immersed in the bacterial suspension, and the swab content was evenly spread across the entire agar surface of Mueller Hinton medium in a Petri dish. Subsequently, the medium was left to air-dry near a flame for 3 - 5 minutes.

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Antibiotic discs, impregnated with specified concentrations of antibiotics, were carefully placed on the Mueller Hinton medium using flame-sterilized metal forceps. Gentle pressure was applied to ensure the antibiotic disc adhered firmly to the medium. The Petri dishes were then incubated at 37°C for 24 hours.

Gram Negative bacteria were tested for following antibiotics: Amikacin (30 µg), Aztreonam (30 µg), Ampicillin/Sulbactam (20 µg), Cefepime (30 µg), Cefoperazone (75 µg), Ceftazidime (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Colistin (10 µg), Cotrimoxazole (25 µg), Doxycycline (30 µg), Erythromycin (15 µg), Gentamycin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Tigecycline (15 µg). Antibiotic discs obtained from Biomaxima, Poland.

After the incubation period, results were interpreted by measuring the diameter of the inhibition zones around each disc and comparing them with standard tables to determine bacterial resistance to various types of antibiotics. The isolates were considered as MDR when non-susceptibility to at least one agent in three or more antimicrobial categories [13].

3. Results

The majority of patients were male, accounting for (76.3%), while the remaining (23.7%) were female. Gram-negative bacilli growth was observed in all samples collected. Polymicrobial infections were determined in 10 samples, representing (20.4%) of the total. The remaining 39 samples (79.6%) contained a single bacterial species.

Isolation and identification results:

Among the samples, *Pseudomonas aeruginosa* was the most prevalent isolate, comprising 39% (23 out of 59) of the total samples. *Klebsiella* followed with a prevalence of 34% (20 out of 59), followed by *Enterobacter* at 22 % (13 out of 59), and *E. coli* at 5% (3 out of 59) as shown in (Figure 1).

Susceptibility test results revealed that all isolates were multidrug-resistant (MDR). Approximately 4% of *Pseudomonas aeruginosa* isolates and 6% of *Klebsiella* isolates exhibited resistance to all tested antibiotics. Furthermore, all isolates demonstrated resistance to Aztreonam and Erythromycin. Detailed susceptibility and resistance profiles of the isolated bacteria to different antibiotics are presented in (Table 1).

Klebsiella isolates exhibited complete resistance (100%) to the Ampicillin/Sulbactam combination, Aztreonam, Ceftazidime, Cotrimoxazole, and Erythromycin, as indicated in (Table 1).

Among *Pseudomonas aeruginosa* isolates, high resistance is observed against several antibiotics, such as Ampicillin/Sulbactam, Cefepime, and Cefoperazone, with resistance rates reaching 95.7% for these antibiotics. Conversely, *P. aeruginosa* shows relatively high susceptibility to antibiotics like Colistin and Tigecycline, with susceptibility rates ranging from 69.6% to 34.8% respectively.

Similarly, *Klebsiella* isolates also exhibit susceptibility to Colistin and Tigecycline, with rates ranging from 60% and 40% respectively.

Enterobacter isolates display varying levels of resistance to different antibiotics, ranging from full resistance observed against Aztreonam, and Erythromycin to high susceptibility to antibiotics like Colistin Tigecycline, Doxycycline and Gentamycin with susceptibility rates of 92.3%, 76.9%, and 61.5% for each of Doxycycline and Gentamycin.

E. coli isolates also exhibit high susceptibility to Imipenem and Tigecycline with a 100% susceptibility rate for both.

4. Discussion

This study examined the prevalence of multidrug-resistant Gram-negative strains among burn patients. The predominance of male patients in the samples can be attributed to occupational factors predisposing males to burn injuries, aligning with findings from studies by [1], [14].

The prevalence of *Pseudomonas aeruginosa* among the causative pathogens of infection was 39%, consistent with the findings reported by [1], [3], [15].

The susceptibility rate of *Pseudomonas aeruginosa* to Tigecycline was observed to be 34.8%, consistent with findings reported by (35.4%) [5].

The presence of strains resistant to all antibiotics underscores the emergence and progression of resistance, likely stemming from inappropriate and indiscriminate antibiotic use, as well as the use of experimental treatments without prior identification and susceptibility testing of the causative pathogen, particularly among burn patients. This assertion is supported by several previous studies, including those that were confirmed by [3], [4], [8].

Overall, the data underscores the importance of careful antibiotic selection in clinical settings, considering the varying resistance patterns observed among different bacterial species. It also highlights the emergence of multidrug-resistant strains and the need for ongoing surveillance and antimicrobial stewardship efforts to combat antibiotic resistance effectively.

5. Conclusion

Our investigation uncovered a significant prevalence of multi-resistant Gram-negative bacilli infections among burn patients at Al-Mouwasat Hospital in Damascus. This heightened prevalence contributes to prolonged hospital stays and extended periods of hospitalization for these patients. Moreover, it poses a potential risk for the transmission of such infections among patients within the department. Additionally, our study identified the presence of resistant strains against all antibiotics utilized in current treatment protocols, amplifying the risk of mortality.

Hence, future studies should involve larger sample sizes to identify the widespread resistant species and continually assess resistance patterns. This approach is essential for refining treatment strategies by ensuring the appropriate selection of medications. Furthermore, the reinforcement of preventive measures is imperative to curb the transmission of resistant strains among patients in the department, thereby curtailing their spread and mitigating the escalation of resistance.

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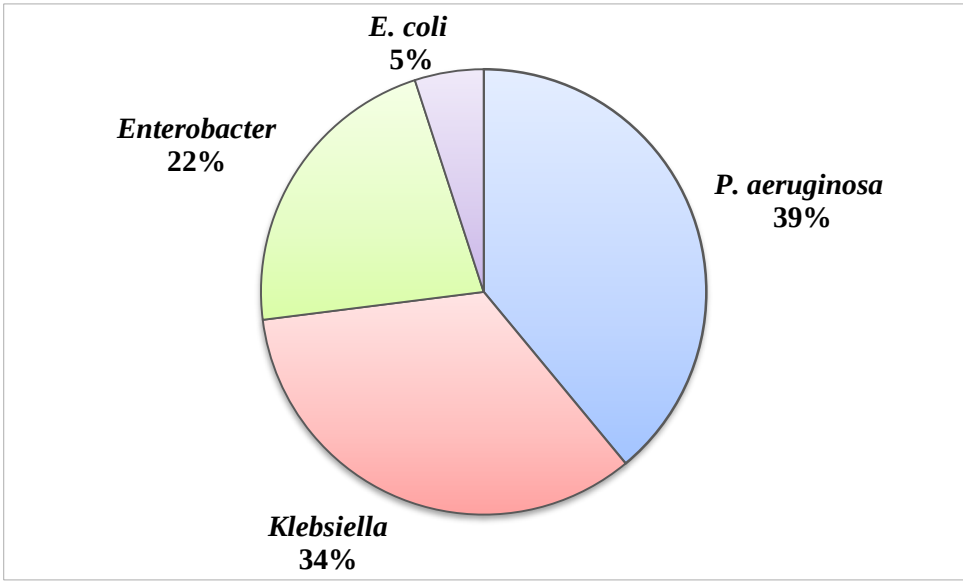

Figure 1. Distribution of Gram- negative bacilli species isolated from burn infections.

Table 1. Antimicrobial sensitivity and resistance profile of Gram-negative organisms isolated from burn infections.

Antibiotic	*P. aeruginosa* (N= 23)			*Klebsiella* (N= 20)			*Enterobacter* (N= 13)			*E. coli* (N= 3)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Amikacin	13	13	73.9	20	10	70	46.2	7.7	46.2	33.3	0	66.7
Ampicillin /Sulbactam	4.3	0	95.7	0	0	100	15.4	0	84.6	66.7	0	33.3
Aztreonam	0	0	100	0	0	100	0	0	100	0	0	100
Cefepime	4.3	0	95.7	5	0	95	7.7	0	92.3	0	33.3	66.7
Cefoperazone	4.3	0	95.7	0	0	100	7.7	0	92.3	0	33.3	66.7
Ceftazidime	4.3	4.3	91.3	0	0	100	7.7	0	92.3	0	33.3	66.7
Chloramphenicol	8.7	17.4	73.9	20	10	70	30.8	23.1	46.2	66.7	0	33.3
Ciprofloxacin	8.7	8.7	82.6	10	15	75	23.1	7.7	69.2	33.3	0	66.7
Colistin	69.6	21.7	8.7	60	25	15	92.3	0	7.7	33.3	0	66.7
Cotrimoxazole	8.7	0	91.3	0	0	100	7.7	0	92.3	0	33.3	66.7
Doxycycline	8.7	4.3	87	5	5	90	61.5	0	38.5	66.7	0.0	33.3
Erythromycin	0	0	100	0	0	100	0	0	100	0	0	100
Gentamycin	4.3	8.7	87	20	0	80	61.5	7.7	30.8	33.3	33.3	33.3
Imipenem	13.0	0	87	10	5	85	46.2	0	53.8	100	0	0
Meropenem	13.0	4.3	82.6	15	0	85	15.4	0	84.6	33.3	33.3	33.3
Tigecycline	34.8	8.7	56.5	40	5	55	76.9	15.4	7.7	100	0	0