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Evaluation of Antimicrobial Pattern of Difficult-To-Treat Resistant Gram-negative Bacteria from Pus Samples in a Tertiary Care Hospital

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ABSTRACT

Objective: To assess the antimicrobial profile of difficult-to-treat resistant Gram-negative bacteria, isolated from pus specimens at a tertiary care hospital.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Pak Emirates Military Hospital and Army Medical College (NUMS) Rawalpindi, Pakistan, from Jun to Aug 2024.

Methodology: A total of 1,250 pus specimens were collected. Pus was processed according to standard procedure of microbiology and antibiotic susceptibility testing was done following Clinical and Laboratory Standards Institute 2023 protocols. Analysis of data was done using Statistical Package for Social Sciences version (SPSS) version 26. Descriptive factors including frequencies and percentage were calculated. Chi square test was applied to check for association. A *p*-value of less than or equal to 0.05 was assumed significant.

Results: Out of 1,250 pus specimens received during one year, 416(33.2%) were Gram-negative rods and 356(28.4%) were Gram-positive cocci. The most frequently isolated organism was Escherichia coli, seen in 156(30.3%) samples, followed by Klebsiella pneumoniae and Pseudomonas aeruginosa. Acinetobacter baumannii appeared as the most resistant pathogen. It was sensitive to only colistin and tigecycline.

Conclusion: By determining various types of Gram-negative rods present in pus specimens, as well as their sensitivity for different antibiotics an institutional antibiogram can be constructed. It will guide clinicians in making valuable treatment decisions and indirectly help preventing antimicrobial resistance and promote optimal patient care.

Keywords: Antibiotic Resistance, Antibiotic Pattern, Bacterial Infection, Gram-negative Bacteria, Pyogenic.

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INTRODUCTION

Skin and its related structure infections are among of the most commonly occurring infectious syndromes reported in healthcare facilities all around the globe. The disease spectrum ranges from mild superficial abrasions to complicated dirty infections, which can cause grave repercussions leading to loss of limb or even life.[1] The highest number of new cases and deaths from skin and soft tissue infections were reported from South Asia posing an economic burden to the government.[2]

Classically, Gram-positive bacteria notably Staphylococci and Streptoccocci spp were in lead in causing such infections. However, in recent years Gram-negative bacteria either alone or mixed with other pathogens including yeasts have emerged as formidable adversaries.[3] This legion of Gram-negative bacteria has equipped itself with resistance mechanisms such as extended-spectrum β-lactamases (ESBLs), carbapenemases, and efflux pumps that baffle routinely utilized antimicrobial drugs. This surge in antibiotic resistance has significantly complicated the management of wound infections.[4] Multidrug resistant, extensively, and even pan drug resistant Gram-negative rods are increasingly being isolated from pus and tissue specimens, posing a threat to the efficacy of available therapeutic choices.[5] Recently, these superbugs come under the umbrella of a newly coined term difficult-to-treat resistance (DTR) in which beta-lactams, particularly carbapenems and fluoroquinolones are rendered inefficient.[6] Therefore, there is a dire need to know the local antibiogram of bacterial isolates yielded from pus, tissue and other specimens helping physicians in making an appropriate choice for selection of an antibiotic that hits the bacteria hard and fast ultimately leading to optimal therapeutic outcome.

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Skin and skin structure infections complications can indeed have a significant financial burden on individuals, healthcare systems and society as a whole.[7] The purpose of our study was to identify Gram-negative pathogens causing wound infections and their susceptibility pattern. We aimed to assess the antimicrobial profile of difficult-to-treat resistant Gram-negative bacteria, isolated from pus specimens at a tertiary care hospital.

METHODOLOGY

This cross-sectional study carried out at the Microbiology Laboratory of Pak Emirates Military Hospital, Army Medical College, National University of Medical Sciences Rawalpindi, Pakistan, from June to August 2024. Ethics approval was obtained from the Ethical review Committee of Army Medical College (ERC no. ERC/ID/372 dated 11th June 2024).

Inclusion Criteria: All the pure pus samples yielding growth of Gram-negative bacteria, and ultra sound guided pus samples were included.

Exclusion Criteria: To ensure accurate representation, repeat specimens from the same patient received within 24 to 48 hours, were excluded.

Sample size was calculated using formula WHO calculator, taking previous prevalence as 70.59%.[8] The minimum sample size came to 319, however we collected all available samples during the study period, which was 1250. Pus swabs and samples were collected without any identification of patient. Non-probability convenience sampling technique was used to collect data. Consent was taken from each patient before the specimen was collected.

Information was gathered from the hospital management system, tailored to store detailed patient laboratory data, including specimen information. Each patient was assigned a laboratory identification number, ensuring that data used in the study was anonymized to protect participant identities. Their demographic details were also accessible, along with the submission time and date of the specimen, as well as the issuance time of the report.

Pus specimens received were processed in accordance with standard microbiological protocols. Initial processing included Gram staining, followed by inoculation on 5% sheep blood agar, MacConkey agar, and chocolate agar. The inoculated media were then incubated at 35 to 37°C for a minimum of 48 hours for open wound cultures. For specimens from deep sites, anaerobic incubation was also performed.

After incubation, culture plates were observed for bacterial growth. Isolates were identified by Gram stain and colony morphology. For Gram-negative bacilli, oxidase test and motility were performed and further identification was done by standard biochemical reactions on Analytical Profile Index for Enterobacterales (API 20E) bioMerieux and for non Enterobacterales (API 20NE) bioMerieux.

Antibiotic susceptibility was carried following CLSI 2023 guidelines. Using modified Kirby Bauer Disc Diffusion method on Mueller Hinton Agar (Oxoid UK) for all antimicrobials except for colistin for which agar dilution method was done, and in case of Acinetobacter baumannii colistin susceptibility was checked using MIC method using VITEK2 GNR card all isolates with MIC ≤2µg/mL were taken as intermediate and ≥4µg/mL as resistant according to CLSI. Results of antimicrobial susceptibility were interpreted according to CLSI 2023. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 were used as control strains performing identification and susceptibility testing of Gram-negative rods.

For the statistical analysis of data, Statistical Package for Social Sciences (SPSS) version 26 was used and *p*-value was calculated. Frequencies and percentages were use to present the descriptive data, whereas Chi-square test was applied to check for association. A *p*-value of less than or equal to 0.05 was taken as significant.

RESULTS

One thousand two hundred and fifty (1250) pus specimens were received within a year, 772 had growth, whereas 478 had no growth of any organism. Four hundred and sixteen (33.28%) pus specimens had Gram-negative rods, while 356(28.48%) pus specimens had Gram-positive cocci.

Further analysis revealed that among patients with Gram-negative rod infections, 312(75.00%) had single-organism pyogenic infections, whereas 104(25.00%) had polymicrobial infections involving two different Gram-negative bacilli.

According to our study, 78 out of 416 patients were from different outpatient departments. On the other hand, among hospitalized patients, surgical departments sent 202 out of 416 pus specimens from which Gram-negative bacillus were isolated. As mentioned in Table-I, male patients had a higher number of pyogenic infections 224(53.8%) as compared to females.

Total number of Gram-negative rods isolated from 416 pus samples were 514. The highest frequency of isolated organism was Escherichia coli 156/514(30.3%) followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Proteus mirabilis and other Gram-negative rods, as shown in Figure-1.

Table-I: Demographic Data of patient with Skin and Skin Structure Infections (n=416)

		Male	Female	Total
	Outpatient	40	38	78
Inpatient	Surgery	90	112	202
	Medicine	24	14	38
	Dermatology	20	12	32
	Infectious Diseases ward	18	6	24
	High Dependency Unit	22	4	26
	NICU/PICU	10	6	16
Total		224	192	416

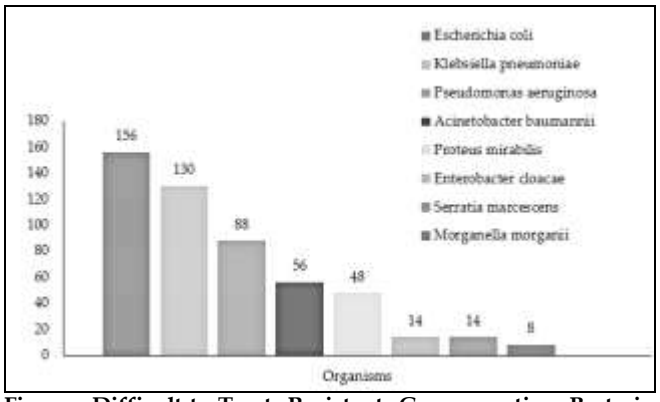

Figure. Difficult-to-Treat Resistant Gram-negative Bacteria Identified from Pus Specimens (n=514)

Among the 56 Acinetobacter baumannii specimens that were isolated, rising trends of antimicrobial resistance was noted throughout 12 months of our study. All the isolates were 100% resistant to amikacin, ciprofloxacin, ceftazidime and ceftriaxone. All isolated Acinetobacter baumannii were completely sensitive to colistin and tigecycline, as susceptibility of tigecycline was reported following EUCAST. In Table-II. antimicrobial resistance pattern of other isolated Gram-negative bacilli have been shown.

Association between variables was checked using Chi square test, and the *p*-value was found to be less than 0.05 for wards of patients, polymicrobial and monomicrobial pyogenic infections in our study. High statistical significance was noted among the isolates and the wards in which infected patients were admitted.

DISCUSSION

Any skin or skin structure injury can lead to inflammation, resulting in pyogenic infections.[9] Moreover, the rapid development of antimicrobial resistance is a common strategy employed by these pathogens to evade treatment.[10] Alarmingly, the prevalence of difficult-to-treat, resistant Gram-negative bacteria in pyogenic infections is on the rise, posing a significant challenge to effective management and treatment.

Table-II: Antimicrobial Resistance observed in Isolated Gram-negative Bacilli from Pus Specimens

Antibiotics	Organisms resistant to antimicrobials				
	Escherichia coli n=156	Klebsiella pneumoniae n=130	Pseudomonas aeruginosa n=88	Acinetobacter baumannii n=56	Proteus mirabilis n=48
Ampicillin	152 (97.4%)	IR	IR	IR	0%
Amoxicillin-clavulanate	154 (96.2%)	120 (92.30%)	IR	IR	0%
Amikacin	84 (53.84%)	90 (69.20%)	64 (72.70%)	56 (100.00%)	0%
Ciprofloxacin	134 (85.89%)	100 (76.90%)	66 (75.00%)	56 (100.00%)	0%
Ceftazidime	-	-	22 (25.00%)	56 (100.00%)	0%
Ceftriaxone	142 (91.02%)	104 (80.00%)	IR	56 (100.00%)	0%
Gentamicin	98 (62.80%)	96 (73.80%)	64 (72.70%)	54 (96.40%)	0%
Meropenem	62 (39.70%)	56 (43.00%)	60 (68.10%)	40 (71.40%)	0%
Minocycline	42 (26.9%)	20 (15.30%)	-	4 (7.140%)	0%
Colistin	0%	0%	0%	0%	IR
Piperacillin tazocbactam	146 (93.50%)	96 (73.80%)	58 (65.90%)	52 (92.80%)	0%
Tigecycline	0%	0%	-	0%	IR
Trimethoprim-sulfamethoxazole	150 (96.10%)	124 (95.30%)	IR	50 (89.20%)	42(87.50%)

**IR: Intrinsic Resistance*

The main cause of skin and skin structure infections are Gram-positive rods, as evident from a study conducted in central India, where the proportion of these bacteria was 40.11%.[11] In our study infections caused by Gram-negative bacteria was 33.2% and it goes parallel to study conducted in Nepal where frequency was 73.15%.[12]

In our study 75% of wound infections were mono microbial which goes parallel to the study conducted in Haryana, India, where 97.4% specimen showed mono microbial growth.5 In comparison, a study conducted on refugees showed 84% poly microbial pyogenic infections which were due to poor hygiene and non-compliance with topical as well as oral antimicrobials.[13] Escherichia coli was the most frequently isolated pathogen followed by Klebsiella pneumoniae and Pseudomonas aeruginosa respectively. Our results are similar to the retrospective study on pediatric population with diagnosed pyogenic musculoskeletal infections.[14] However, studies conducted in India as well as Uganda, show that in their data, Klebsiella pneumoniae and Acinetobacter baumannii predominated.[8,15]

In our study, Acinetobacter baumannii emerged as the most resistant organism. It demonstrated

resistance to all antimicrobials except colistin and tigecycline. Notably, tigecycline has shown promising outcomes keeping in view detailed focus on antibiogram of hospital and according to literature it has adequate skin/soft tissue penetration.16 Considering the EUCAST guidelines, tigecycline was chosen for sensitivity testing. The susceptibility pattern of Acinetobacter baumannii underscores its persistent role as a highly resistant pathogen in causing pyogenic infections, which is similar to the findings of another regional study.[17]

Pseudomonas aeruginosa, the second most resistant pathogen in our study, exhibited a substantial 68.1% resistance to meropenem. For these patients, colistin remained the drug of last resort. According to one study, we are on a verge of losing this last resort also due to non-adherence to antimicrobial stewardship.[18]

Given that 81.2% of our study samples were received from inpatient departments, surgical site infections were a significant concern. Multiple risk factors, including prolonged hospital stays, exploratory surgeries, dirty wounds, and the presence of multiple pus pockets, contribute to the burden on the healthcare system and patient well-being, which is consistent with data from other studies.[19]

Poor infection control practices and policies which are not coherent to antimicrobial stewardship programs are setting grounds for high prevalence of complicated skin and skin structure infections. To overcome this crisis with limited options of treatment, the need of hour is that the clinicians and microbiologist should combine their efforts. Conducting studies of this nature and establishing an antibiogram derived from similar research is essential to aid clinicians in initiating empirical therapy to combat infections caused by difficult-to-treat resistant Gram-negative bacteria.

LIMITATIONS OF STUDY

This was a single centered study which limits the scope of study and limited data was used in this study, more data would have cleared the vision about difficult to treat resistant Gram-negative bacteria.

CONCLUSION

The antimicrobial profile observed in difficult-to-treat resistant Gram-negative bacteria detected from pus specimens serves as evidence indicating our restricted treatment optionsTop of Form. Resistance has developed and will continue to spread aggressively against antimicrobials among nosocomial pathogens until infection control practices are not strictly followed. Based on this study, we can develop an institutional antibiogram that will assist clinicians in making better treatment choices.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

WF & FH: Data acquisition, data analysis, critical review, approval of the final version to be published.

JU & RI: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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