FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ISOLATED FROM BURN WOUNDS: A THREE-YEAR ANALYSIS AT BURNS AND PLASTIC SURGERY CENTER, PESHAWAR

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ABSTRACT

Objectives: To determine the frequency of bacteria isolated from burn wounds, and their percent susceptibilities against a panel of antibiotics by analyzing burn wound culture, and susceptibility test results data of the patients managed in our burn center over a period of three years.

Methodology: In this descriptive, observational, cross-sectional, study a summary of antibiotic susceptibilities of isolated bacteria to tested antibiotics in percentages was prepared to assess the susceptibility pattern of bacteria. The prevalence of each of the isolated specie and genus of bacteria was determined. For susceptibility testing and antibiogram construction, the guide lines of Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Supplement M100, and Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—5th Edition: M39-2022 were followed. Excel and SPSS 24 were used for data entry and calculation of percentages and frequencies.

Results: Gram-negative bacteria were more commonly isolated as compared to gram-positive bacteria. Out of total 5166 isolated bacteria, 4334 (83.89 %) of isolates were gram-negative, whereas 832 (16 .11%) were gram-positive. The most commonly isolated bacteria were Klebsiella pneumoniae (K. pneumoniae) with 1095 (21.20%), followed by Pseudomonas aeruginosa (P. aeruginosa) with 989 (19.16%) occurrences and gram-positive cocci, respectively.

Conclusions: It is clear from our results that Gram-negative bacteria were more commonly involved in burn wound infection at our center. In addition, the isolated organisms had shown resistance to co-amoxiclav and cephalosporins and several other tested antibiotics.

Keywords: Antibiotic Susceptibility; Bacteria; Burn Wound; Infection

INTRODUCTION

A severe burn injury is an unfortunate incident that places an otherwise healthy individual at a substantial risk of morbidity and even mortality, depending on the characteristics of sustained burn insult and socio-dynamic conditions of the patient[1] in developed countries, most patients, and in developing countries, many patients manage to receive successful initial resuscitation and management.[2] However, the peculiar characteristics of burn injuries, including extended hospital stays due to prolonged healing time, the need for multiple interventions, the environment of the burn wound which is suitable for microbial proliferation, and the weakened immune state of burn patients, burn wounds commonly get infected inpatients admitted to burn units. Additionally, many patients may already have developed infection of the wound or are suspected of having one when they are received in hospitals. Burn wound infection and its complications are one of the leading causes of morbidity and mortality among these patients worldwide.[3,4]

In addition to the fact that burn wound is more susceptible to infection, the organisms involved are usually more resistant to antibiotics.[5] The increasing antibiotic resistance in bacteria is a phenomenon all over the world. The origin of resistance is a natural genetic evolutionary phenomenon in microorganisms, serving as a self-defense mechanism. However, the inappropriate use of antibiotics exerts selective pressure on microorganisms, to enhance and accelerate this process. As a result, resistance is now commonly observed with second, third-line, and even last-resort antibiotics and gets

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on increasing day by day.[6] The bacteria commonly involved in burn wound infections are already known, but the frequency with which one specific bacterium is involved can vary in different geographical areas. Additionally, their antibiotic susceptibility profiles may differ from region to region, and even among different institutes and facilities, where compliance with infection control measures and antibiotic prescription practices also play important role.[7,8]

In cases of burn infections, it is highly desirable that the antibiotics are used rationally and only according to the culture and sensitivity test reports. However, the culture and sensitivity / susceptibility test results take some time to become available to the clinicians. In resource limited setups, and due to a greater influx of patients in government hospitals there could be even more unwanted delay in identifying the specific organisms responsible and their antibiotic susceptibility profile through culture and sensitivity testing.[9] This is the time when need for appropriately selected empirical antibiotic arises. The knowledge of the commonly prevalent microorganism and antibiotics that are most appropriate for empirical therapy is of great importance at this stage and can be advantageous if known at facility and even unit's level.[10]

Local antimicrobial resistance pattern is reflected in the antibiogram of an institution or health care facility which is the most important tool and apart of antibiotic stewardship programs, aiding in the selection of appropriate empiric antibiotics in any institution.[11] Appropriate selection of empirical antibiotics can save the life of burn patient and reduce the risk of morbidity.[10,12] Overall, appropriate use of antibiotics helps in preventing the emergence of resistant strains of microorganisms,[13,14] protect the patient from the effects of unnecessary systemic antibiotics, save the resources, and guide the hospital formulary in the selection of antibiotics for purchase.[15]

The current study was designed to find out the frequency of isolation of different aerobic bacteria from burn wounds of patients, as well as, determined their susceptibility profile against antibiotics. This study aims to provide valuable insights into local epidemiology, by bacteria development of tailored antibiogram and antibiotic stewardship programs. The findings from this study will have a significant role in combating antimicrobial resistance and infection control measures at burn units.

METHODOLOGY

This descriptive, observational, cross-sectional study was conducted at a tertiary care facility for burn cases, Burns and Plastic Surgery Center (B&PSC) Hayatabad, Peshawar. The sampling was by non-probability, convenience, sampling technique. The study period ranged from, 1st October 2019 to 31st march 2021, and 1st October 2021 to 31st March 2023 that was a three year time in total. Permission was obtained from the concerned authorities to use the laboratory data for research purposes. Ethical approval was obtained from IREB Post Graduate Medical institute Hayatabad Peshawar, Reference No.10138. Dy. REG. PGMI, dated 5-10-23. It was an observational study with no intervention, and the identity of the patients was not disclosed, taking into consideration the Helsinki Declaration1975, last revised in 2013.

In this study, an analysis of three years' records of burn wound culture and sensitivity test results of burn patients was performed. The culture and susceptibility tests were performed at microbiology laboratory of B & PSC. The isolates were from the clinical specimens including pus, swabs, tissue and foreign bodies from the wounds of burn patients. All the reports with positive bacterial growth were included in the analysis. If there were multiple reports from the same patient, subsequent reports were not included. Where more than one bacterial isolates were obtained from a single wound specimen, they were considered separately. For some isolated bacteria, the count was less than 30, which is the minimum count required by, antibiogram construction guidelines for antibiotic susceptibilities to be statistically representative (CLSI. Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fifth Edition: M39-2022). Therefore, their susceptibility results were not included in the study results [16]. In all tests, samples were incubated to obtain pure cultures of bacteria. Gram staining was performed on the isolates and appropriate biochemical tests were applied to identify the bacteria. Resistance and susceptibility were measured using Kirby Bauer, disk diffusion method on Mueller Hintonagar. The guidelines laid down by the Clinical and Laboratory Standards Institute (CSLI), were followed (CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28thed. CLSI supplement M100).

Gram-positive isolates were tested against an antibiotic panel selected from Penicillin, Ampicillin, Co-amoxiclav, Piperacillin/Tazobactam, Cefoxitin (surrogate for oxacillin), Ceftriaxone, Cefoperazone/Sulbactam, Imipenem, Tetracycline, Tigecycline, Gentamycin, Amikacin, Linezolid, Vancomycin, Erythromycin, Ciprofloxacin, Trimethoprim/ Sulfamethoxazole. Gram-negative isolates were tested against an antibiotic panel selected from Ampicillin, Co-amoxiclav, Piperacillin /Tazobactam, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime, Cefoperazone /Sulbactam, Imipenem, Meropenem, Doripenem, Gentamycin, Amikacin, Tobramycin, Ciprofloxacin, Levofloxacin, Tetracycline, Tigecycline, Colistin / Polymyxin B. In panel for Pseudomonas species Ticarcillin and Cefepime were also included. Cumulative antimicrobial susceptibility testing (AST) results from microbiology laboratory records were generated using manual data collection approach. The frequency of different aerobic bacteria which were isolated and their antibiotic susceptibilities against the tested antibiotics were noted individually and plotted in tabular forms on Excel sheet. A summary of antibiotic susceptibilities of isolated bacteria to tested antibiotics, in form of percentages was prepared to observe the susceptibility pattern of bacteria. The prevalence of isolated species and genera of bacteria was determined after counting their total numbers for each, in percentages. Data was analyzed using IBM SPSS version 24 statistical package for social sciences) and Microsoft excel. Descriptive statistics were used to calculate frequencies and percentages for categorical variables. The results were summarized in form of tables.

Antibiogram: The cumulative antibiogram is a profile that shows the periodic antibiotic susceptibility patterns of various organisms isolated from patients within an institution; it may help to choose the best empirical antimicrobial therapy for that institution.[17]

Empirical therapy: Before the data from the standardized susceptibility tests are known, physicians base their anti-infective strategies on the cumulative percent susceptibility data for the most commonly used antimicrobial agents against key bacterial pathogens. This data is developed from the pooled results of all patient specimens collected over a defined time period.[18]

RESULTS

A total of 6255 culture test reports were studied, out of which 4454 (71.20%) had a positive growth of bacteria, while 1821 (29.11 %) yielded no growth of any type or a non-bacterial growth, if any. Out of 4454 cultures with growth, 3741 (83.99 %) showed growth of a single bacterium (Table 1), while 713 (16.01%) reports showed growth of more than one bacterium (poly-microbial) (Table 2). The total number of bacterial isolates that were analyzed was 5166.

Out of 5166, 4334 (83.89 %) of bacterial isolates were gram-negative, while 832 (16 .11%) were gram-positive. The most frequent bacterial isolate was K. pneumoniae with 1095 (21.20%) occurrences, followed by P. aeruginosa with 989 (19.16%) occurrences. In third place were gram-positive cocci [(Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis)] with 831 (16.08%) occurrences, followed by Acinetobacter spp. 689 (13.33%), Enterobacter 522 (10.10%), Citrobacter366 (07.08%), Proteus spp. 364 (07.04%), and Escherichia coli (E. coli) with 310 (06.01 %) occurrences (Table 1). The antibiotic susceptibility profile of gram-negative bacteria isolated from burn wound samples is given in Table 3. The antibiotic susceptibility profile of gram- positive bacteria isolated from burn wounds is given in Table 4. Out of gram-positive cocci, 398 (48 %) were methicillin sensitive, while 434 (52%) were methicillin resistant.

DISCUSSION

The burn wound gets colonized by different microorganisms within a few hours of the burn injury, even though the wound is sterile at the beginning.[19] Many patients develop an infection of burn wound over time, which may lead to sepsis and septicemia.[20,21] Whenever an infection is suspected clinically, the recommended step is to detect the specific organisms responsible and determine their antibiotic susceptibility through a culture and sensitivity test which in turn guides in selection of appropriate antibiotic therapy for the patient.[22] While waiting for the culture and sensitivity test report for individual patients, it may be necessary to start some antibiotics treatment.[16] It has been observed in various studies that the organisms causing infection in burn wounds vary across different geographical regions, healthcare facilities, and even within different units of one hospital, such as the ICU and general wards.[23-25] Additionally, the antibiotic susceptibility pattern of isolated organisms also varies. Therefore for every burn facility having knowledge of the most frequent organisms involved in infections and their antibiotic susceptibility profile helps in selection of most appropriate empiric antibiotics,[16] which, in turn, helps in reducing the emergence of resistant strains due to selective pressure as well and saves the patient from the harms of unnecessary systemic antibiotics and their side effects.[16,27,28] In our study 71.20 % of cultures showed positive growth of organisms, while 29 .11% were growth negative. In other local studies, the range of positive culture frequencies varied from 49% to 92.5 %.[29,30] Our frequency of positive culture was closest to the one found by Chaudhary et al. In 83.99% of our cultures, one organism was isolated, while in 16.01%, two or more organisms were detected. In other Pakistani studies, poly-microbial growth was found in 19% to 34.65% of wound cultures.[9,29] The frequency of poly-microbial growth varied widely in local studies. In worldwide studies,

Table 1: Frequencies of Different Bacteria Isolated

Organism	Number n=5166)	Percent (%)
K. pneumoniae	1095	21.20
P. aeruginosa	989	19.16
Gram positive cocci	831	16.08
Acinetobacter	689	13.33
Enterobacter	522	10.10
Citobacter	366	07.08
Proteus spp.	364	07.04

Table 2: Frequency of Poly-microbial Infections

Organisms	Number N=713	Percent of each combination
P. aerugenosa+S.aureus	146	20.47
P. aerugenosa+ CONS	122	17.11
P. aerugenosa+Acinetobacter spp.	116	16.26
P. aerugenosa+K. pneumoniae	101	14.16
P. aerugenosa+ Proteus spp.	81	11.36
P. aeruginosa + E. coli	09	1.26
K. pneumoniae+ S. aureus	67	9.39
K. pneumoniae+ S. aureus + Acinetobacter spp.	01	0.14
Acinetobacter spp. + Proteus spp.	46	06.45
Acinetobacter spp.+ E. coli	07	0.98
Acinetobacter spp.+ CONS	03	0.42
Acinetobacter spp.+ S. aureus	03	0.42
Acinetobacter spp. +Entreobacter spp.	03	0.42
Acinetobacter spp. +Ctrobacter spp.	01	0.14
Proteus spp.+S.aureus	03	0.42
Proteus spp.+E.coli	02	0.28
E.coli+ CONS	02	0.28

Table 3: Sensitivity pattern of gram - negative bacterial isolates from burn wounds

Antibiotic tested	K.pneumoniae n=1095	P.aerugenosa n=989	Acinitobactern n=689	Enterobactern n=522	Citrobactern n=366	Proteus n n=364	E.coli n=310
Ampicillin	00	IR	IR	IR	IR	IR	0.5
Co-amoxiclav	05	IR	IR	IR	IR	05	32
Piperacillin/Tazobactam	57	57	55	59	50	69	69
Imipenem	54	55	51	52	53	63	77
Meropenem	65	57	66	57	73	79	79
Doripenem	76	62	68	74	79	83	79
Cefotaxime/Ceftriaxone	19	NT	09	05	08	09	13
Cefoperazone/ Sulbactam	49	31	27	19	39	39	59
Ceftazedime	21	32	20	12	20	18	21
Trimethoprim/Sulfameth-oxa-zole	06	NT	09	06	11	11	07
Tetracycline	18	IR	16	12	12	IR	19
Tigecycline	72	IR	66	51	59	IR	54
Gentamycin	32	31	29	19	30	19	48
Amikacin	52	39	43	33	39	35	74
Tobramycin	31	35	30	19	27	19	43
Ciprofloxacin	27	37	27	22	23	18	17
Levofloxacin	NT	20	NT	NT	NT	NT	NT
Colistin	84	70	54	55	38	IR	84
Ticarcillin	NT	07	NT	NT	NT	NT	NT
Cefipime	NT	NT	NT	NT	NT	NT	NT

N=Number, n=number, IR=Inherently Resistant, NT=Not Tested

Table 4: Antibiotic Sensitivity Pattern of Gram-Positive Bacterial Isolates from Burn Wounds

Antibiotics tested	Methicillin Sensitive S. aureus (MSSA) n= 335 40.26%	Methicillin Resistant S.aureus (MRSA) n=416 50%	Methicillin Sensitive S. epidermidis (MSSE) n=63 07.60%	Methicillin Resistant S. epidermidis (MRSE) n=18 02.14%
Penicillin	00	IR	00	IR
Ampicillin	00	IR	00	IR
Co-amoxiclav	11 %	IR	10	IR
Cefoxitin (surrogate for oxacillin)	100%	IR	100	IR
Piperacillin/tazobactum	86	IR	88	IR
Imipenem	100	IR	100	IR
Gentamycin	70	15	86	50
Amikacin	81	27	86	80
Tetracycline	30	13	86	100
Tigycycline	95	91	100	100
Vancomycin	99	98	100	100
Linezolid	89	97	86	99
Ceftriaxone	16	IR	13	IR
Cefoperazone/sulbactam	41	IR	44	IR
Ciprofloxacin	25	01	20	08
Erythromycin	24	07	18	04
Trimetoprim /Sulfamethoxazole	24	02	44	02

n=number, N=Number, IR=Inherently Resistant

there was wide variation in the frequency of positive growth cultures,[31,32] which was also related to the timing of the culture test being taken with respect to burn injury sustained. Similarly, the frequency of poly-microbial growth was also widely different in different studies.[31]

We found that gram-negative bacteria were more commonly isolated from burn wounds than gram- positive bacteria, which was in agreement with local studies by Chaudhary et al. and Khattak et al. However, in some local studies, gram-positive bacterium S. aureus, was most commonly isolated from burn wounds.[33,34] In our study 52% of gram-positive cocci were methicillin-resistant. In other local studies, the frequency of methicillin-resistant Staphylococci ranged from 40 to 71%.[9,29]

Before the antibiotic era, Streptococcus pyogenes (S. pyogenes) was the most common bacterium involved in burn wound infections, but it was almost completely eradicated after the start of antibiotic era.[35] A single isolate of S. pyogenes was isolated in one Pakistani study.[30] In earlier global studies, S. aureus was considered to be the common organism.[36] However, presently, gram-negative bacteria are as commonly involved as gram-positive organisms, and are even commoner in some regions. That's why determining the frequency of different types of organisms involved in burn wound infections on a facility-by facility basis is beneficial, as this knowledge helps in deciding empirical antibiotics. In our study, gram-negative organisms were collectively more commonly isolated, accounting for 83.89% of all organisms. This finding is consistent with many local and global studies.[9,25,37]

In this study the most common organisms isolated from burn wound infection was K. pneumoniae followed by P. aeruginosa and than gram-positive cocci were in third place. These findings were not much different from other studies where these three types of bacteria were found to be most commonly involved in burn wound infections.[38-41] In this study the other gram-negative bacteria isolated in significant number were Acinetobacter, Enterobacter, Citobacter, Proteus spp., and E. coli. In many studies gram negative-bacterium P. aeruginosa [9,42-44] was the most commonly involved bacterium while in some studies gram-positive bacterium S. aureus was the most common one.[34] In one local study Proteus was the bacterium most commonly isolated.[45] In our study K. pneumoniae was the most commonly isolated bacterium which was not found to be the most common in most of other local and global studies.[9,42,43] These findings show that it is quite common that frequency with which bacteria are involved in burn wound infection might be different in different facilities; as well as their antibiotic susceptibility profiles, this variability should influence the choice of empirical antibiotic selection for each individual facility.

In our study gram-negative bacteria showed very high resistance to co-amoxiclav, second and third generation cephalosporins, quinolones as well as resistance to carbapenems which was an alarming finding and a finding similar to many other local and studies from developing countries,[9,29,38,41] while in developed countries these organisms are still relatively sensitive to first and second line antibiotics and carbapenems,[46] which points towards inappropriate use of antibiotics in developing countries and inadequate measures to prevent emergence of resistance in microorganisms. In our study gram-negative organisms showed higher level of sensitivity to colistin as compared to other antibiotics tested. Colistin need to be used strictly as reserved antibiotic and an antibiotic of last resort in view of increasing resistance to carbapenems and other antibiotics in gram-negative bacteria. Non judicial use and increased reliance on colistin is associated with increasing report of resistance in gram-negative bacteria globally against this antibiotic, colistin is also generally, associated with higher incidence of toxic side effects.[47] In our study gram positive-organism showed very high resistance to antibiotics against which they were tested, including penicillin, co-amoxiclav, and cephalosporins which is similar finding as in other local and studies from developing countries.

The strength of our study lies in the information obtained regarding the frequency of organisms involved in burn wound infections and their antibiotic susceptibility profiles. This data could be used to develop antibiotic prescribing guidelines for our burn center and help our formulary in selecting antibiotics.

The weaknesses of the study were that the data was collected retrospectively from manually maintained registers which was laborious work. The colonization of burn wounds from invasive infection could not be distinguished with surety.

CONCLUSIONS

It is clear from our results that Gram-negative bacteria were more commonly involved in burn wound infection at our center. In addition, the isolated organisms had shown very high resistance to all tested antibiotic.

RECOMMENDATIONS

On the basis of our results, we suggest that further studies on epidemiology and antibiotic susceptibility patterns should be performed at the other burn centers of our country to check the robustness of our data for any meaningful variation over time in frequency of organisms isolated from burn wounds and their antimicrobial resistance pattern. In addition, institution specific antibiotic policies and appropriate timely measures in form of strict infection control and antibiotic stewardship programs should also be promoted.

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Author’s Contribution

ZI conceived the idea, designed the study, participated in data collection and writing the manuscript. TU helped in designing the study, writing manuscript and revising it critically. RM helped in writing the manuscript and data collection. All author's made substantial intellectual contributions to the study.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.