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Bacterial Isolation and Antibiotic Susceptibility Test Results from Burn Wound Infection in the Southeastern Anatolia Region of Turkey

Türkiye'nin Güneydoğu Anadolu Bölgesinde Yanık Yarası Enfeksiyonundan Bakteri İzolasyonu ve Antibiyotik Duyarlılık Testi Sonuçları

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ABSTRACT

This study was conducted to find the bacterial profile isolated from the wound areas of the patients hospitalized in our burn center, which is the only one in our region and to find the sensitive antibiotics to be used in the treatment.
In this study, the culture reports of 395 patients, whose wound culture results were (+), obtained from 1415 patients hospitalized in Gazi Yaşargil Training and Research Hospital burn center between January 2010 and January 2020, were included.
Of the 394 patients, 217 (55%) were male and 117 (45%) were female. The average age of the patients is 12,86±17,34 years. The average burn degree is 2,67. In the wound culture results, 70,55% of gram (+) and 28,68% of gram (-) bacteria were found. Candida albicans were found in wound culture growth results with a rate of 0,07%. The most common gram (+) pathogen is Staphylococcus aureus with 23,09% (n: 91). In our study, the resistance rate of Staphylococcus aureus to methicillin was 20,31%. The most common gram (-) pathogen was E. coli with 9,13% (n: 36) and Pseudomonas aeruginosa with 9,13% (n: 36). Gram (+) organisms were mostly isolated with a rate of 78,95% in the culture results of patients who needed intensive care clinically due to infection. Staphylococcus aureus is the most common bacteria with 21,05%.
As a result of our study, it was found that the most common cause of burn infection in our region was S. aureus, P. aeruginosa, and E. coli. We hope that the use of broad-spectrum antibiotics that can be effective against these bacteria will contribute to clinical treatment until culture results are available.

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ÖZET

Bu çalışma, bölgemizde tek olan yanık merkezimizde yatan hastaların yara bölgelerinden izole edilen bakteri profilini ve tedavide kullanılacak uygun duyarlı antibiyotikleri bulmak amacıyla yapılmıştır.
Bu çalışmada Ocak 2010-Ocak 2020 tarihleri arasında Gazi Yaşargil Eğitim ve Araştırma Hastanesi yanık merkezinde yatan 1415 hastanın (+) yara kültürü sonucu alınan 395 hastanın kültür raporları dahil edildi.
394 hastanın 217'si (%55) erkek, 117'si (%45) kadındı. Hastaların ortalama yaşı 12,86 ± 17,34'tür. Ortalama yanma derecesi 2,67'dir. Yara kültürü sonuçlarında %70,55 gram (+) ve %28,68 gram (-) bakteri bulundu. Yara kültürü üreme sonuçlarında %0,07 oranında Candida albicans saptanmıştır. En sık görülen gram (+) patojen %23,09 (n: 91) ile Staphylococcus aureus'tur. Çalışmamızda Staphylococcus aureus'un metisiline direnç oranı %20,31 olarak bulundu. En sık görülen gram (-) patojen %9,13 (n: 36) ile E. coli ve %9,13 (n: 36) ile Pseudomonas aeruginosa idi. Enfeksiyon nedeniyle klinik olarak yoğun bakıma ihtiyaç duyan hastaların kültür sonuçlarında en çok %78,95 oranında gram (+) organizma izole edildi. Staphylococcus aureus %21,05 ile en yaygın bakteri idi.
Çalışmamız sonucunda bölgemizde en sık yanık enfeksiyonu nedeninin S. aureus, P. aeruginosa ve E. coli olduğu saptanmıştır. Kültür sonuçları çıkana kadar bu bakterilere karşı etkili olabilecek geniş spektrumlu antibiyotik kullanımının klinik tedaviye katkı sağlayacağını umuyoruz.

1. Introduction

Burn injuries to the skin and other tissues occur as a result of contact with heat, electricity, friction, radiation, or chemicals. Approximately 180.000 deaths occur annually from burns, and most of these deaths occur in low- or middle-income developing countries. Children and women are the two main groups most affected by burn injuries. In Bangladesh, Colombia, Egypt, and Pakistan, 17% of children with burns have a temporary disability

and 18% have a permanent disability. Non-fatal burn injuries result in significant morbidity, prolonged hospital stay, cosmetic impairment, and often stigmatization and rejection (1). In 2015, 67 million people were affected by burn injuries (2).

Burn wounds are a sensitive area for the growth of endogenous and exogenous opportunistic organisms (3). Infection in a burn patient is an important cause of morbidity and mortality and poses a challenge for the burn team (4). The risk of infection, which can lead to sepsis, is increased due to the change in immunity (5). After the first care, complications due to infections in burn patients constitute 50% - 75% of the mortality (6-8). Many factors cause infection in burn patients, such as body surface, immunocompromised state, invasive procedures performed in healthcare facilities, and prolonged hospital stays. Factors related to the patient, such as age, total body surface area (TBSA), and the depth of the burn wound, and factors related to microbiological organisms such as the type and number of infections, enzyme/toxin production, and the motility of organisms, both determine the patient's mortality and morbidity. Superficial bacterial contamination may cause sepsis in burn patients and both are directly related to each other (8-10). There are three characteristic areas of a burn wound. The first is the coagulation site closest to the heat source which forms a scab. Adjacent to this zone is the stasis zone with an increased risk of ischemia due to the loss of perfusion. The outermost zone consists of relatively normal skin with a zone of hyperemia, increased blood flow and vasodilation, and minimal cellular damage. Most importantly, it is the most protein-rich eschar-containing area that supports microbial growth due to its avascular structure and prevents the delivery of immune cells and antibiotics (6). The burn wound remains sterile immediately after thermal injury (3), but then becomes rich in organisms transported by the hands of healthcare personnel and other staff (11).

The gastrointestinal system is an important source of organisms in burn patients, and these endogenous organisms can contaminate the surface with fecal contamination of wounds (11).

Increasing patient load in public hospitals causes a delay of several days between taking patient cultures and receiving the results of culture reports. At such a critical time, empirical antibiotic therapy can be given to patients to control infection. However, antibiotic resistance increases due to inappropriate antibiotic prescription (12) and overuse (12-14). For this reason, this study was carried out to find the profile of bacteria isolated from the wound sites of the patients hospitalized in our burn center, which is the only one in our region, and to find the appropriate sensitive antibiotics for use in the treatment.

2. Materials and Methods

In this study, patients hospitalized in the Gazi Yaşargil Training and Research Hospital burn center between January 2010 and January 2020 were retrospectively screened using the clinical registry and computer records. We included male and female patients of all ages who applied to our hospital's burn center. Patients who were re-admitted for follow-up or outpatient clinic control after one time were excluded from the study. Culture reports of 395 (+) from 1415 patients were included. Patients with prolonged hospital stays had more than one culture result report, while those with mild burn injuries and short hospital stays had only one culture report at the time of admission. Wound cultures were taken from all patients at admission. Samples were collected from the burn wounds with culture swabs under aseptic techniques. They were then sent to the microbiology laboratory, where the samples were inoculated on agar plates. Wound cultures were made on blood and McConkey agar. Cultures were incubated at 37 °C for 24 - 48 hours. Microbes were identified under a microscope by observing morphological features and applying biochemical tests. Antibiotic susceptibility pattern was performed on Muller Hinton agar using the Kirby Bauer disk diffusion method. Age, gender, admission time, place of application, burn degree, burn total body surface area (TBSA), burn type, and length of hospital stay were recorded from the patient's records.

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS), v23.0 (IBM SPSS Statistics, Armonk, NY). Descriptive statistics were applied to find frequencies, percentages, means, and standard deviations. Quantitative variables such as age and TBSA were expressed as means and standard deviations. Qualitative variables such as bacterial type and burn type were expressed in frequency and percentage. The normality of quantitative variables was checked by applying Kolmogorov-Smirnov and Shapiro-Wilk tests. Mann-Whitney U test and Kruskal Wallis test were used to find a significant difference between the means of nonparametric variables with two and more than two categories, respectively. Chi-square tests were applied to compare the percentages of the two categorical variables. The level of significance was set at $p<0.05$.

3. Results

The culture-positive report of 394 patients from 1415 patients was included. 194 (49,2%) of the patients were hospitalized in the emergency room and 200(50,8%) of them were from the polyclinic. 201 (51,0%) patients from rural, 193 (49,0%) patients from the city center applied to our hospital. 284 (72,1%) patients arrived on the day of the burn injury, and 110 (27,9%) patients applied one day or more after the burn injury.

Of all patients, 27 (6,85%) on December, 41 (10,40%) on January, 37 (9,39%) on February 30 (7,61%) on March, 24 (6,09%) on April, 33 (8,37%) on May, 32 (8,12%) on June, 32 (8,12%) on July, 20 (5,07%) on August, 42 (10,65%) on September, 39 (9,90%) on October and 37 (9,39%) on November applied to our hospital.

224 (56,8%) patients were in the 0-4 ages range, 39 (9,9%) patients were in the 5-9 ages range, 14 (3,5%) patients were in the 10-14 ages range, 37 (9,4%) patients were in the 15-24 ages range, 30 (7,6%) patients were in the 25-34 ages range, 17 (4,3%) patients were in the 35-44 ages range, 23 (5,8%) patients were in the 45-64 ages range, and 10 (2,5%) the patients were over 65 years old.

317 (80,45%) of the patients were scalded, 45 (11,42%) were flame burns, 22 (5,58%) were electrical burns, 3 (0,76%) were embers, 2 (0,05%) patients were frostbite, 1 patient (0,02%) each applied to us because of burns due to tandoori, grape leaves and green walnuts.

Burn injury had seen in the head and neck region 76 (19,28%) of patients, in the right upper extremity 133 (33,75%) of patients, in the left upper extremity 132 (33,5%) of patients, in the anterior chest and abdomen 112 (28,42%) of patients, in the lumbar region and back of the chest 35 (8,88%) of patients, in the perineum 29 (7,36%) of patients, in the right lower extremity 209 (53,04%) of patients and the left lower extremity 194 (49,23%) of patients (Table 1).

The average burn degree is 2,67 (min: 2-max: 4). Burn percentage average is 14,18% (min: 2-max: 50). The average of our laboratory results was WBC (White Blood Cell): 13,6 ± 7,13 and CRP (C-Reaktif Protein): 21,25 ± 29,49.

Table 1. General information about the patients

Patients		Number	Percent	All patients %	p-value
Gender	man	217	55%	56,40%	
	women	177	45%	43,60%	
Age group	0-4 ages	224	56,80%	53,20%	0,012**
	5-9 ages	39	9,90%	12,50%	
	10-14 ages	14	3,50%	5,00%	
	15-24 ages	37	9,40%	8,90%	
	25-34 ages	30	7,60%	7,70%	
	35-44 ages	17	4,30%	4,80%	
	45-64ages	23	5,80%	5,50%	
	65+	10	2,50%	2,40%	
Application Place	emergency	194	49,20%	49,75%	
	policlinic	200	50,80%	50,25%	
Application time	1 or more day delay	110	27,90%	23,10%	
	Within 24 ours	284	62,10%	66,90%	
Place of residence	rural	201	51,00%	47,85%	0,065**
	urban	193	49,00%	52,15%	
Season*	winter	105	26,64%	24,02%	
	spring	87	22,08%	25,72%	
	summer	84	21,32%	24,17%	
	autumn	118	29,95%	26,09%	
Cause of burns	scalding burns	317	80,45%	76,60%	
	flame burns	45	11,42%	9,18%	
	electrical burns	22	5,58%	3,67%	
	embers burns	3	0,76%		
	asphalt burns	2	0,05%		
	frostbite	2	0,05%	0,03%	
	tandoori	1	0,02%		
	grape leaf burn	1	0,02%		
	green walnut	1	0,02%		
Location of burns	head-neck	76	19,28%	21,41%	
	upper right extremity	133	33,75%	32,08%	
	upper left extremity	132	33,50%	32,79%	
	front chest + abdomen	112	28,42%	27,85%	
	posterior chest + lumbar region	35	8,88%	7,63%	
	Perineum	29	7,36%	5,30%	
	Lower right extremity	209	53,04%	42,54%	
	lower left extremity	194	49,23%	40,56%	
Burn degree			2,67 (min:2-max:4)	2,64 (min:2-max:4)	0,04**
% TBSA* burned**			14,18% (min:2-max:50	9,22% (min:1-max:50)	
Length of stay in the hospital			12,88 (min:1-max:39)	6,0 gün (min:1-max:39)	

*Winter: December, January, February. Spring: March, April, May. Summer: June, July, August. Autumn: September, October, November. **p<0,05 ***TBSA: Total Body Surface Area

In the wound culture results, 70,55% of a gram (+) and 28,68% of gram (-) bacteria were found. Candida albicans was found in wound culture growth results with a rate of 0,07%. Staphylococcus aureus was the most common gram (+) bacteria with 23,09% (n: 91). The most common gram (-) bacteria were E. coli with 9,13% (n: 36) and Pseudomonas aeruginosa with 9,13% (n: 36) (Table 2).

Table 2. Wound site culture results

Pathogen	Subgroup	Number	Percent
Gram (-)		113	28,68%
Escherichia	coli	36	9,13%
Enterobacter	aerogenes	4	1,01%
	cloacae	6	1,52%
Pseudomonas	aeruginosa	36	9,13%
	flourescens	1	0,02%
	putida	2	0,50%
Acinetobacter	Baumannii	8	2,03%
Proteus	mirabilis	6	1,52%
Klebsiella	pneumoniae	5	1,26%
Pantoea	agglomerans	3	0,07%
Serratia	marcescens	2	0,50%
Sphingomonas	paucimobilis	2	0,50%
Aeromonas	Hydrophila	1	0,02%
Burkholderia	cepacia	1	0,02%
Gram (+)		278	70,55%
Staphylococccus	aureus	91	23,09%
	capitis	3	0,07%
	epidermidis	76	19,28%
	haemolyticus	27	6,85%
	hominis	33	8,37%
	lugdunensis	1	0,02%
	pseudintermedius	1	0,02%
	saprophyticus	2	0,50%
	simulans	1	0,02%
	warneri	2	0,50%
	xylosuse	5	1,26%
Streptococcus	spp	4	1,01%
	pyogenes	3	0,07%
	muthis	3	0,07%
	agalactiae	1	0,02%
Enterococcus	avium	1	0,02%
	faecalis	18	4,56%
Kocuria	kristinae	3	0,07%
	rosea	1	0,02%
Lactococcus	garvieae	1	0,02%
Micrococcus	lylae	1	0,02%
YEAST		3	0,07%
Candida	albicans	3	0,07%
TOTAL		394	100

In the wound culture results of the patients who arrived late for a day or more, the most gram (+) result was obtained at 65,14%. The most common of these was Staphylococcus aureus with 20,18% (Table 3).

19 patients had to be followed up in the intensive care unit because of the infection clinic. The average length of stay in intensive care was 11 days (min: 2- max: 36). Gram (+) organisms were isolated with the highest rate of 78,95% in the culture results of patients in need of intensive care. Staphylococcus aureus was the most common organism with 21,05% (Table 4).

Table 3. Cultural results in patients arriving late for 1 day or more

Pathogen	Subgroup	Number	Percent
Gram (-)		38	34,86%
Acinetobacter	baumannii	2	1,83%
Escherichia	coli	10	9,17%
	faecalis	7	6,42%
Klebsiella	pneumoniae	2	1,83%
Pantoea	agglomerans	1	0,91%
Proteus	mirabilis	3	2,75%
pseudomonas	aeruginosa	10	9,17%
	putida	2	1,83%
serratia	marcescens	1	0,91%
Gram (+)		71	65,14%
enterococcus	avium	1	0,91%
kocuria	kristinae	1	0,91%
lactococcus	garvieae	1	0,91%
staphylococccus	aureus	22	20,18%
	epidermidis	17	15,59%
	haemolyticus	9	8,25%
	hominis	12	11,00%
	lugdunensis	1	0,91%
	xylosus	2	1,83%
streptococcus	Spp.	2	1,83%
	agalactiae	1	0,91%
	mutis	1	0,91%
	pyogenes	1	0,91%
Total		109	100%

Table 4. Pathogens insulated in cultural results in patients in intensive care

PATHOGEN	SUBGROUP	NUMBER	PERCENT
Gram (-)		4	21,05%
Acinetobacter	baumannii	1	5,26%
Escherichia	coli	2	10,52%
Proteus	mirabilis	1	5,26%
Gram (+)		15	78,95%
Enterococcus	feacilis	1	5,26%
Staphylococcus	aerous	4	21,05%
	epidermidis	8	42,10%
	heamolyticus	1	5,26%
	hominis	1	5,26%
Total		19	100%

In the antibiogram of Staphylococcus, which was the most common bacterium in culture results, the antibiotics to which it is most resistant are; cefazoline, tobramycin, and cefoxitin. Antibiotics to which he is most sensitive; are moxifloxacin, teicoplanin, imipenem, and colistin (Figure 1).

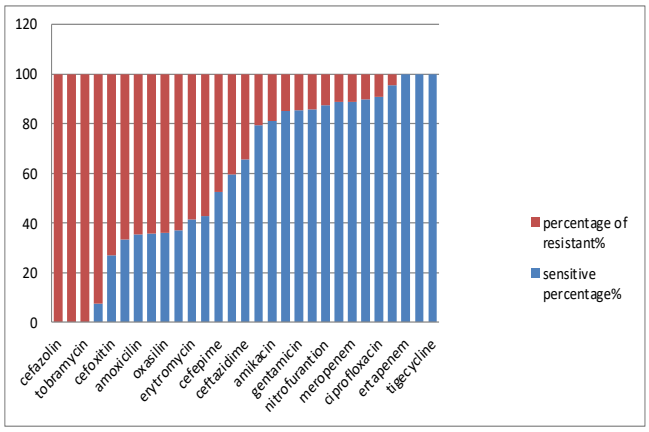


Figure 1. Staphylococcus antibiotic sensitivity

Antibiotics to which E. coli, one of the most common Gram (-) bacteria in culture results, was the most resistant in the antibiogram of; cefuroxime, amoxicillin, oxacillin, and ampicillin. Antibiotics to which it was most sensitive; ertapenem, meropenem, netilmicin, and teicoplanin (Figure 2).

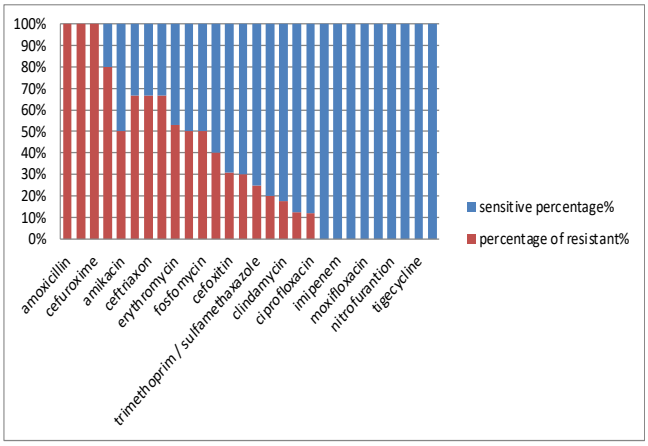
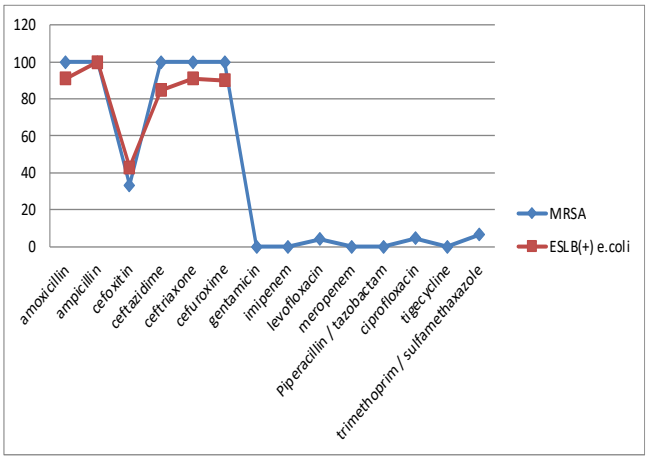


Figure 2. Escherichia coli antibiotic sensitivity

The resistance rate of all staphylococcus to methicillin was 24,80%, and the resistance rate of staphylococcus aureus to methicillin was 20,31%. Our rate of ESBL (+) E. coli is 36,11%. Percentages of resistance to MRSA and ESBL (+) E. coli microorganisms penicillin and cephalosporin group antibiotics were almost 100%. This group of bacteria was more sensitive to antibiotics such as piperacillin/tazobactam, and trimethoprim/sulfamethoxazole (Figure 3).



*ESBL(+) E. coli: Extended Spectrum ß-Lactamase (ESBL) ra- tes in Escherichia coli

Figure 3. MRSA and ESBL(+)* E. coli resistance percentages

The antibiotics to which pseudomonas, the other gram (-) pathogen, which was the most prominent gram (-) pathogens, were most resistant in the antibiogram are penicillin G, erythromycin, and ampicillin. Antibiotics to which he was most sensitive; tigecycline, moxifloxacin, and levofloxacin (Figure 4).

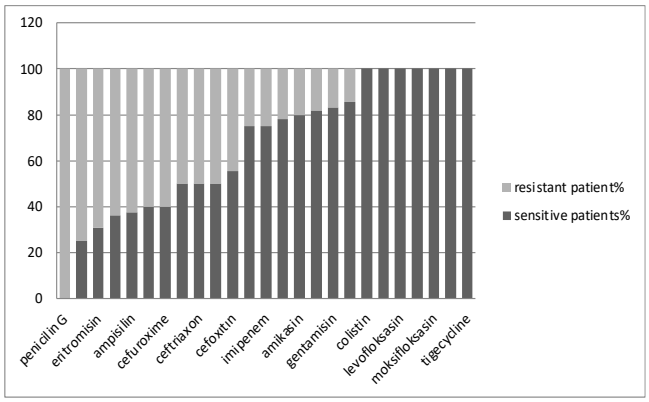


Figure 4. Pseudomonas antibiotic sensitivity

The average antibiotics sensitivity rate of Staphylococcus, which was the most common bacterium among gram (+) s, was 62.6% and the resistance rate to antibiotics was 37,4%. The average antibiotics sensitivity rate of escherichia and pseudomonas, which were among the most common bacteria in the culture of gram (-), was 64,9% and 68,1%, respectively, and the resistance rate to antibiotics was 35,1% and 31,9%, respectively (Figure 5).

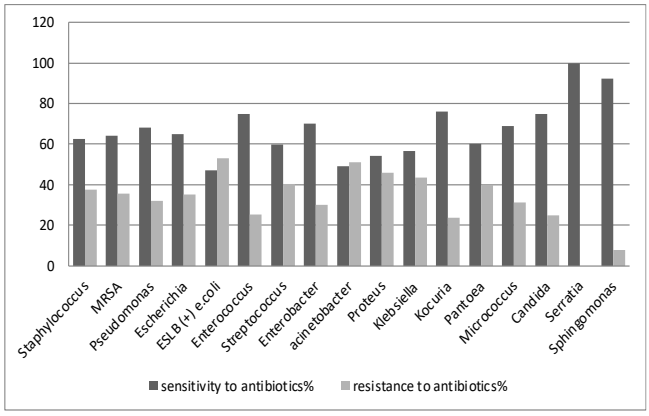


Figure 5. Susceptibility and resistance percentages to microorganisms

The average length of stay was 12,86 ± 17,34 days (1-39 days) and we had no mortality.

4. Discussion

The skin is a protective physical barrier against microorganisms that deteriorate in burn injuries. According to the American Burn Association, there are a number of infections in burn wounds, consisting of wound colonization, wound infection, invasive infection, and fasciitis (15). It is defined as the presence of a low concentration of bacteria on the surface without wound colonization, invasion, or systemic symptoms. When there are more than 105 bacteria in the wound, we call it a wound infection. When more than 105 bacteria in the burn wound cause pus formation and separation of the eschar, loss of graft with tissue

involvement, or the presence of systemic sepsis, this is called invasive infection. Cellulite is redness, hardening, warmth, and tenderness in the tissue surrounding the burn wound, and when invasive infection involves deeper structures under the skin, it becomes a necrotizing infection (8,15).

According to the study of Datta S. (16), Meroj A. et al. (17), more women were affected compared to men. In our study, more men (55%) were affected by burn injuries than women (45%). This result was consistent with the studies conducted by Saaiq M, Chaudhary N. Ave (18,19). The reason for this may be that men are more exposed to occupational hazards in order to earn living money, and therefore the risk of burns injuries was higher than women.

Our study involved patients of all ages. The most common cause of burn injuries varied by age groups. In our region, scalding burns and hot object contact burns were mostly seen in children, and electrical burns, flame burns, and chemical burns were mostly seen in the adult group. The age group with the most wound culture (+) was the 0-5 age group. Our results are consistent with the studies of Dhopte A. et al. (20). The reason for this is the most active years when the 0-5 age group did not know the danger. While TBSA was 2 - 50% in patients hospitalized in our study, Saaiq M. et al. It was between 5-40% (18) and it varied between 3-93% in the study of Chaudhary N. A. et al. (19).

In our study, the most common cause of burns was scaling, followed by flame and electrical burns. Saaiq M., Chaudhary N.A. In the study of et al., flame burns were the most common cause of burns (18,19). The reason for the higher rate of scalding burns in our study was that families with many children have more unprotected contact with hot liquids in the kitchen. As a result, we think that scalding burns occurred.

According to the data of the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) study, S. aureus ranks 3rd among the most frequently isolated microorganisms from invasive samples in our country (21) In the 2017 CAESAR study, the rate of MRSA in our country was reported as 26% (21).

Staphylococcus aureus was the most common (23,09%) cause of wound culture result (+) in our study. Staphylococcus aureus was resistant to 20,31% methicillin. Our methicillin resistance rate was close to the CAESAR study. In the study of Mater M.E et al., it was found to be 36,36% (22). In our study, it was lower. We attribute the low level to the use of antibiotics depending on the culture results. According to the results of the antibiotic sensitivity test in our study, the antibiotics most sensitive to MRSA are ciprofloxacin, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole.

In our study, the most common microorganism isolated from the burn wound was Staphylococcus aureus. Then comes P. aeruginosa and E. coli from gram (-) pathogens, respectively. Many studies, including studies conducted by Mehta M. Al Laham NA et al. (23,24). was inconsistent with our results. According to studies conducted by Mehta M. et al. P. aeruginosa and E. coli rates were 51,5% and 10,0%, respectively. According to studies conducted by Al Laham et al., P. aeruginosa and E. coli rates were 50,0% and 5,6%, respectively. In our study, the rates of P. aeruginosa and E. coli were 9,13% and 9,13%, respectively. Studies carried out by Mehta M et al. P. aeruginosa resistance to gentamicin amikacin, and ciprofloxacin was high (40% to 75%). In our study, this rate was lower (16% to 19%). In the study conducted by Rahman M. et al., The rate of ESLB (+) E. coli found in the burn wound culture results was 45,5% (25). In our study, this rate was lower at 36,11%.

Various potential risk factors were investigated for their role in burn wound colonization in burn centers. If we compare the positive culture results of male patients to all patients (56,4% / 55,0%), the result was found in female patients (43,6% / 43,0%) on the contrary. We think that the male gender plays a (+) role in the outcome of culture, but it is not statistically significant (p:0,387).

The percentage of patients coming from rural and city centers in all our patients was 47,85% and 52,15%, respectively. This ratio is 51,0% and 49,9% in those who have (+) wound culture results. We think that residing in rural areas increases the rate of wound infection. But it isn't statistically significant (p:0,065).

We found that the upper and lower extremities of the body were more commonly contaminated areas than other areas. In particular, the percentage of perineal infection increased more than the general patient rate. (Perineal burn rate in all patients / perineal burn rate in infected patients: (5,3% / 7,36%). But it isn't statistically significant (p:0,36).

The risk of wound culture (+) increases as the burn percentage increases on average by 14,18% (min: 2-max: 50 and 9,22% (min: 1-max: 50) in all patients with culture results). But it isn't statistically significant (p: 0,083). Considering the length of stay in the hospital, the mean is 12,88 (min: 1-max: 39 days) in patients with wound culture (+), and 6,0 days (min: 1-max: 39) in all patients.

5. Conclusion

This study is the first large-scale study in our region to confuse the results of organisms that cause burn wound infection. Due to the recent resistance to antibiotics, patients' length of stay, morbidity, and mortality are increasing. As a result of our study, it was found that the most common cause of burn infection in our region was S. aureus, P. aeruginosa, and E. coli. We hope that the use of broad-spectrum antibiotics that can be effective against these bacteria will contribute to clinical treatment until culture results are available. In this way, we hope to reduce the rate of resistance to antibiotics by preventing the unnecessary use of antibiotics.

Conflict of Interest: There is no conflict of interest in this study.

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Ethics Committee Approval: Ethical approval was obtained from Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (Date: 2021, Decision No: 810) to conduct the study.

Authorship Contribution:

HA: Data collection and analysis, manuscript writing and final controls.

YDY: Research design, manuscript writing and final controls.

EY: Literature review, manuscript writing and final controls.

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