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Cross Sectional Study of Burn Infections and Antibiotic Susceptibility Pattern for the Improvement of Treatment Policy

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ABSTRACT

Introduction: Burn is a devastating form of trauma, and based on its condition, it could run the risk of infections. Infection of wound is a major cause of morbidity and mortality in burn cases. The current study investigates the prevalence of infectious agent in three years and antibiotic resistant pattern to improve and predispose a good policy of treatment in our environment.

Materials and Methods: In the present study, a total number of 3330 samples from 713 patients were evaluated for detection of the most prevalent infections and for finding out the antibiotic susceptibility pattern with routine microbiology procedures.

Results: Based on the results, 598 samples were reported positive. According to the results Pseudomonas aeruginosa, Coagulase Negative Staphylococcus and Acinetobacter sp. were the three most prevalent bacteria with a prevalence rate of 42.1%, 22.1% and 18.4% respectively. Furthermore, based on the results of distribution and diversity of bacterial infections, wound samples were the most infected samples with 73.6% of total infections. Finally, during these three years, there were no significant changes in the resistance pattern of Gram positive and Gram negative infectious agents.

Conclusion: By evaluating the infectious agents during the period of the study, it was found that due to the focus on treatment of Gram negative bacteria, Gram positive bacteria especially Staphylococcus aureus and Coagulase Negative Staphylococcus have at least doubled. This increase in two important nosocomial infections is a next threat of infection and septicemia for burn victims.

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Introduction

Burn injuries are one of the most common and distressing forms of trauma (1). Burn injury is a global public health problem and infection in this group of patients is still considered as the most important cause of mortality in all ages in both developed and developing countries (2, 3). Data from the National burden of disease in Iran, indicate that burn injury in population is the 13th cause of mortality (4). Beside some complications like dehydration in burn victims, infections remain one of the most important and potentially serious complications that occur in the acute period following the injury (1, 5-7). Nosocomial infection or Health Care-Associated Infections (HAIs) is an infection developed about 2 to 3 days after the patient is admitted in the health care setting and is an agent with a lack of evidence that the infection was present or incubate at the time of admission at the health care setting (8). Despite the considerable advances in the management of burn injuries, the humidity and nourishing environment of wounds still makes it a good media for infectious agents like bacteria (3, 9). Recent studies show that more than 70 percent of all post-burn deaths within the first five days directly or indirectly are caused by septic processes (10). Epidemiological studies on the prevalent infectious agents in burns showed that the most common pathogens are Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus),

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Klebsiella spp., various coliform bacilli and some rare Gram negative bacteria like Acinetobacter sp., and Aeromonas spp (9-12). Multi drug resistant (MDR) bacteria are a significant and growing source of nosocomial infections, which limit the treatment options and are especially problematic for immunocompromised patients such as burn patients. (13) Besides that, increase in the release of MDR in hospital effluents has an important role in the global spread of antimicrobial resistance and is therefore an important public health concern. Different reports from burn units show that MDR bacteria are the most common results of nosocomial outbreaks of infection and are colonizers of the wounds of burn patients(14, 15).

One of the most important reasons to determine the algorithm of infection and its antibiotic sensitivity pattern is unavailability of an appropriate alternative antibiotic for resistant nosocomial infections (3, 9).

Given that infections are the major life threatening elements in burn patients, and considering the fact that they have not yet been diagnosed and treated properly, still determining the prevalence of bacterial agents in burn units and sensitivity of the isolates to the antibiotics used in these centers may help clinicians to control the infection and use appropriate antimicrobial agents. Therefore, the present study was conducted to determine the microbial profile during three years of burn infections, the antimicrobial susceptibility patterns of the cultured bacteria and the frequency of infections with respect to the duration of wound, in Ghotbeddin Shirazi Burn Centre affiliated with Shiraz University of Medical Sciences. The results of current study were used in order to improve the policy of antibiotic treatment and infectious control at the target centre.

Materials and Methods

This cross sectional and experimental study was carried out at Ghotbeddin Shirazi Burn Centre, Shiraz University of Medical Sciences (SUMS), Shiraz, Iran, over a period of three years (from January 2013 to December 2015). This study included patients of both genders from all ages, who had sustained deep burns (degree 2 and more).

More than 2000 patients were admitted during the study period. Patients were evaluated for any sign and symptoms of infection within the first 72 hrs after the admission based on National Nosocomial Infection Surveillance System (NNIS) criteria (16).

Patients with any sign and symptoms of Urinary Tract Infection (UTI), Blood Stream Infection (BSI), and Wound Infection (WI) in the first 48h were excluded. Totally 3330 samples were taken from Blood (310), Urine (544), Stool (44) and Wound biopsy (2432) of patients on days 3, 5, 7 and 10 after admission and were cultured on blood Agar and EMB Agar. From the obtained samples, 598 were informative and were included for the next analysis.

Growth bacteria were classified in two Gram negative and Gram positive groups and were identified according to the standard microbial procedures (17, 18). The number of detected bacterial causative infectious agents in this step is presented by sample kind and year in Table 1 in number and percent.

Antibacterial sensitivity test for isolates was performed with disk diffusion method recommended by Clinical and Laboratory Standard Institute (CLSI, 2012) (19).

In this step, isolates were prepared in a suspension with turbidity equal to 0.5 McFarland standards and then plated onto Muller-Hinton agar (Difcos) plates.

Antibiotic disks (Oxoid) were applied to each plate (Tables 2 and 3). Plates were incubated at 35°C for 24 hrs. Inhibition zone diameter of each disc were measured and reported in millimeters for each isolate.

Isolates were reported in two resistant and sensitive types to each antibiotic.

In antibiogram test Escherichia coli (E. coli), Klebsiella, Proteus and Enterobacter isolates were grouped in Enterobacteriaceae and their results are reported in Table 5.

Included reference strains were ATCC 25923 (S. aureus), ATCC 35218 (E. coli), ATCC 27853 (P. aeruginosa) and ATCC 43816 (Klebsiella pneumoniae) obtained from Shiraz University of Medical Sciences, Department of Microbiology, Burn and wound healing research center, Shiraz, Iran.

The data analysis was performed with the SPSS 21 (SPSS inc, Chicago, IL). This study was approved by the Ethical Committee code: 94-01-01-9898 from Shiraz University of Medical Sciences.

Results

Since this study was conducted over a period of 3 years, the results in each year were first evaluated and compared with each other.

According to the study criteria, a total number of 713 patients, 510 (71.5%) male and 203 (28.5%) female with any grade of burn were selected and evaluated.

Among the selected patients, 304 (42.6%) patients were evaluated with second and more degree of burn. 200 (65.8%) were male and 104 (34.2%) were female.

The range age of patients was from 1 to 91 years old with the mean age of 32.03±12 years.

Patients' gender and their related ward of hospitalization according to years of study are summarized in Table 1.

According to the sex ratio of patients in different years of study it has been shown that the ratio of male to female in year 2014 had a higher proportion in comparison with the two other years of study.

Comparing the results in pediatric ward during the time of study showed that the number of patients has been declining by 31 percent and the deterioration of patients is significantly decreased as well.

Table1: Gender distribution and ward section of patients by year of study

Year of the Study	2013		2014		2015	
Sex / ward of hospitalization	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
Male ward	102 (58.3)	0 (0.0)	118 (61.5)	0 (0.0)	92 (64.3)	0 (0.0)
Female ward	0 (0.0)	39 (52.0)	0 (0.0)	41 (65.1)	0 (0.0)	38 (58.5)
Pediatric ward	55 (31.4)	25 (33.3)	53 (27.6)	14 (22.2)	37 (25.9)	18 (27.7)
ICU	18 (10.3)	11 (14.7)	21 (10.9)	8 (12.7)	14 (9.8)	9 (13.8)
Total	175	75	192	63	143	65
Ratio of male to female	2.33		3.05		2.2	

In this study from a total number of 3330 samples, 598 (18%) of them were positive for aerobic bacteria.

The results for detection of bacteria are separately listed in Table 2 based on the sample type. According to the results P. aeruginosa (42.1%), Coagulase Negative Staphylococcus (CoNS) (22.1%) and Acinetobacter sp. (18.4%) were the three most prevalent bacteria respectively, while Streptococcus (0.33%), Proteus (0.50%) and Aeromonas spp. (0.67%) as the less common infectious agents were isolated.

According to the results of distribution and diversity of bacterial infections, it has been deduced that the wounds were the most infected samples with 73.6 percent of total samples, while the variation of infectious agents in wound and urine samples were higher compared with other samples. These results are demonstrated in details in Table 2.

Table2: Detected bacteria in wound, Urine, Blood and Stool samples by year of study

Pathogen	Wound (by year/percent)			Urine (by year/percent)			Blood (by year/percent)			Stool (by year/percent)			Total
	2013 (%)	2014 (%)	2015 (%)	2013 (%)	2014 (%)	2015 (%)	2013 (%)	2014 (%)	2015 (%)	2013 (%)	2014 (%)	2015 (%)	
Pseudomonas aeruginosa	101 (40.1)	98 (38.9)	33 (13.1)	1 (0.4)	4 (1.6)	3 (1.2)	0 (0.0)	3 (1.2)	1 (0.4)	1 (0.4)	4 (1.6)	3 (1.2)	252
Staphylococcus aureus	8 (17.0)	6 (12.8)	14 (29.7)	2 (4.3)	1 (2.1)	8 (17.0)	3 (6.4)	3 (6.4)	2 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	47
CoN* Staphylococcus	21 (15.9)	35 (26.5)	47 (35.6)	5 (3.8)	3 (2.3)	3 (2.3)	0 (0.0)	8 (6.1)	5 (3.8)	1 (0.7)	1 (0.7)	3 (2.3)	132
Acinetobacter sp.	13 (11.8)	18 (16.4)	25 (22.7)	4 (3.6)	6 (5.4)	2 (1.8)	4 (3.6)	6 (5.4)	4 (3.6)	6 (5.4)	13 (11.8)	9 (8.2)	110
Streptococcus	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2
Enterobacter	0 (0.0)	0 (0.0)	3 (50.0)	2 (33.3)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6
Escherichia coli	0 (0.0)	8 (26.6)	4 (13.3)	4 (13.3)	5 (16.7)	5 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.7)	2 (6.7)	30
Klebsiella spp	0 (0.0)	1 (8.3)	0 (0.0)	1 (8.3)	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	3 (25.0)	4 (33.4)	12
Proteus spp.	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
Aeromonas spp	0 (0.0)	0 (0.0)	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4
Total	143	166	131	21	21	24	7	20	12	9	23	21	598

According to the comparative results of infections in the four selected days of hospitalization, it was found that, an increase in the duration of hospitalization leads to the increase of the frequency of infection.

According to these results, the maximum number of infections is gained at day 5 and 7, with the highest number at day 5. Furthermore, the peak of diversity of infectious agents is observed at day 5 and 7. These comparing results are presented in details in Table 3. In this part of study, it has been shown that the frequency and diversity of infections is significantly reduced on day 10 (P value < 0.05). Overall based on the comparison of the results among all patients, 241 (33.8%) suffered at least from one type of nosocomial infections and the remaining patients, suffered from two or more infectious agents.

Table3: The progress of the bacterial infections number in four selected days

Bacteria	Day 3	Day 5	Day 7	Day 10
Pseudomonas aeruginosa	31	105	98	19
Staphylococcus aureus	22	18	5	2
CoN* Staphylococcus	45	35	40	12
Acinetobacter sp.	5	13	6	2
Streptococcus	40	33	19	18
Enterobacter	1	1	0	0
Escherichia coli	2	3	1	0
Klebsiella spp.	1	4	3	0
Proteus spp.	2	5	3	1
Aeromonas spp.	0	0	3	0
Total	149	217	178	54

* Coagulase Negative

Table 4: Percentage of resistant Gram +ve and -ve isolates according to year of study and the investigated antibiotics

Organism (G+)										
Antibiotic	Concentration Disc (µg)	S. aureus			CoNS*			Streptococcus		
		1	2	3	1	2	3	1	2	3
Gentamicin	10	50	65	83	63	65	65	ND	ND	ND
Cephalothin	30	78	78	80	70	70	70	ND	ND	ND
Ciprofloxacin	5	75	76	76	40	40	42	ND	ND	ND
Cephalexin	30	68	70	70	42	42	40	ND	0	0
Clindamycin	2	68	70	70	38	42	40	ND	100	100
Oxacillin	1	43	59	63	80	90	80	ND	0	0
Vancomycin	30	0	0	0	0	0	0	ND	ND	ND
Ampicillin	10	98	98	100	99	99	99	ND	100	100
Cotrimoxazol	1.25/23.75	88	92	92	90	92	92	ND	0	0
Ceftazidime	30	78	78	78	60	62	62	ND	0	0
Penicillin	10	ND	ND	ND	ND	ND	ND	ND	0	0
Teicoplanin	30	0	0	0	0	0	0	0	0	0
Organism (G-)										
Antibiotic	Concentration Disc (µg)	P. aeruginosa			Aeromonas spp.			Acinetobacter sp.		
		1	2	3	1	2	3	1	2	3
Gentamicin	10	98	99	100	ND	ND	100	100	100	100
Cephalothin	30	95	95	100	ND	ND	100	100	100	100
Ciprofloxacin	5	90	98	100	ND	ND	100	90	90	90
Cephalexin	30	98	99	100	ND	ND	100	100	100	100
Amikacin	30	95	95	100	ND	ND	100	100	100	100
Carbenicillin	100	90	98	100	ND	ND	100	90	90	90
Ceftazidime	30	90	98	100	ND	ND	100	90	90	90
Tobramycin	10	98	99	100	ND	ND	100	100	100	100
Imipenem	10	45	50	60	ND	ND	40	40	45	45
Meropenem	10	10	12	12	ND	ND	0	0	0	0
Colistin	10	0	0	0	ND	ND	0	0	0	0

CoNS: Coagulase Negative Staphylococcus **1**: 2013, **2**:2014. **3**: 2015, **ND**: not done

Furthermore, during hospitalization period, 24 (10.0 %) patients had urinary tract infection (UTI), 20 (8.3 %) had blood stream infection (BSI), and 166 (68.9 %) had wound infection (WI). The results of this step indicated that the wound infection was the most frequent (68.9 %), followed by urinary tract infection (10 %) and blood infection (8.3 %). By the evaluation of infection and the pattern of infection during these three years, 598 bacterial isolates were obtained. The most predominant bacterial isolate was P. aeruginosa (42.1%) followed by CoNS (22.1%), Acinetobacter sp. (18.4%) and Streptococcus, Proteus and Aeromonas with a frequency of about 0.5 percent were the least common bacteria in the study samples. During the study period, it has been observed that there is a decrease in P. aeruginosa infection as the main nosocomial infectious agent of the center in the last year of study while there was an increase in other Gram negative bacteria such as Acinetobacter sp., Enterobacter spp. and Aeromonas spp.

Evaluating the infectious agents during the years of study it has been found that due to the focus on treatment of Gram negative bacteria, Gram positive bacteria especially S. aureus and CoNS have at least doubled. This increase in two important nosocomial infections is a next threat of infection and septicemia for burn victims. An epidemiological study of infectious agents in terms of bacteria-caused disease showed that the diversity of bacteria in the first year (2013) was in the lowest number and type and just four prevalent bacteria (P. aeruginosa, S. aureus, CoNS and Acinetobacter) were detected. In the next years of

study, the diversity is increased and reaches to 8 bacterial genera in 2015.

In this study bacterial isolates antibiogram results were evaluated in two Gram positive and Gram negative groups based on the years of study. Based on the antibiogram results it has been shown that during these three years there wasn't a significant change in resistance pattern of Gram positive isolates (Table4).

It is worth noting that about 43-63 percent of S. aureus and about 8-9 percent of CoNS strains were resistant to oxacilin. However, these isolates were susceptible to vancomycin and teicoplanin Disks. In evaluation of Antibiogram pattern of Gram negative bacteria it has been found that there isn't a significant change in resistance pattern of isolates especially in P. aeruginosa as the main nosocomial infection and other Gram negative bacteria such as Aeromonas spp., Acinetobacter sp. and Enterobacteriaceae. It has also been found that the antibacterial guideline in the study center is responsible for the treatment of hospitalized patients. (Table4).

Discussion

Despite major advances in the treatment of burn patients, nosocomial infectious complications continue to play a major role in morbidity and mortality of these patients. (20) With regards to the importance of infection control and identification of the main cause of infection in burn centers especially the pattern of infectious agents in different wards, such studies may help improve the burn victims condition and decrease their time of hospitalization (21). These findings may help in implementation of appropriate policies to control infection and find the origin of infection and the transmission pattern (22). In burn injuries, there is a proper growth medium for microbes, due to the loss of skin as the body's largest defense system and the burning of eschar environment through the existence of the moist (23, 24). According to the recent comparative study and consistent with other studies' results, it has been found that the risk of infection in higher grades will increase due to the burn wound depth (25).

According to the latest results of studies in Iran about 73 percent of burn mortality is due to the wound infection especially in the first five days of hospitalization(26). Results of the recent study showed that the peak of infection in the study center happened in days 5 and 7 of hospitalization. These results altogether recommend that control of infection in the first week of hospitalization is of prime importance. Furthermore, results show that the prevalence of infection and the separate investigation for the main result of infection in each center and in each section, may help to control infection and save burn victims' lives. Like other studies the incidence of wound infection in our hospitalized patients was more than other samples (21, 25, 27-29).

According to the aim of the recent study, i.e. the determination of the frequency of prevalent infectious agents, P. aeruginosa and Acinetobacter sp. were the most prevalent Gram negative bacteria while Staphylococcus spp. was the most prevalent Gram positive bacteria in the study center. About the prevalence of P. aeruginosa the results were consistent with other centers in Iran and other countries (21, 30, 31) which shows that this bacteria should be taken into serious consideration in burn and immunodeficiency in patients. The important point about other prevalent infections is that Acinetobacter and Coagulase Negative Staphylococcus are also important and are in the same proximity rate of first nosocomial infection. Neglecting these bacteria and focusing on the control of specific bacteria alone, may cause next level of infection problem. While in the center where this study was performed as well as in some others centers, Staphylococcus spp. and Acinetobacter are now in the high prevalence (32, 33) while in some centers these bacteria are placed in first level of nosocomial infection (2). According to the result of recent study it has been deduced that in the first days of hospitalization, Gram positive bacteria are more prevalent and with elongation of patients' recovery time the chance for growth of opportunistic Gram negative bacteria such as Pseudomonas sp. and Acinetobacter sp. increases.

This result shows that while the patient needs to be hospitalized for a long time we must adapt the two policies of treatment according to the infection pattern. The management of patients with appropriate policy will help improve their condition and decrease their time of hospitalization, subsequently reducing the health care costs.

One of the important points that must be considered in determining the policy of treatment is to have knowledge of infection pattern and antibiotic sensitive algorithm of the infectious. For this purpose, related studies need to be performed periodically to determine the prevalent infectious agents and their antibiotic susceptibility pattern.

As shown in this study periodic study during years 2013 and 2014 with the aim of determining the infections and their drug resistance could help reduce the most prevalent bacterial infection, P. aeruginosa. Moreover, in line with the present epidemiology study, it has been determined that some other bacteria which were in low levels of frequency in the first years of study increased in recent years and must be considered in new treatment policy.

Overall, according to the present results, we decide to have different policies for short and long term hospitalization of burn patients according to the hurt degree.

Another conclusion of this epidemiology study is that problem may arise according to the focus on treatment of specific bacteria and the use of a choice drug for a long term treatment in the study center.

This policy will give chance to other infections and beside that increase the drug resistance of most prevalent nosocomial infection at the center of target.

Conclusion

Given the importance of knowing antimicrobial pattern of resistance these results can be helpful in deciding the appropriate time and the suitable treatment for using new extended-spectrum antibiotic. The results of this study could help increase our epidemiological information about recent situation of infection in burn cases and prepare the ground for an optimized care for the group of burn patients in southwest of Iran.

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