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Bacterial infection pattern in ventilated burn patients in the southwest of Iran

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ABSTRACT

Burn is one of the most traumatic injuries and life-threatening states which is a global public health problem, accounting for an estimated 180 000 deaths annually. In burn trauma patients, intubation and ventilator-associated pneumonia (VAP) is a major threat. Added to other complications this may be responsible for the higher morbimortality of burn patients. In this one-year cross-sectional study (September 2018 to September 2019), a total of 70 patients hospitalized in the burn ICU ward were sampled according to the study criteria. Cases were hospitalized in Amir-al-Momenin burn hospital, affiliated with Shiraz University of Medical Sciences. Tracheal samples were evaluated for bacterial infection with standard microbiological techniques. In the following process, an antibacterial susceptibility test was performed for confirmed isolates with the Kirby-Bauer method and recommended antibiotics by the Clinical and Laboratory Standards Institute. According to the results of the study, a total of 21 (30%) positive growth samples were detected. The age range of patients was estimated at 1–60 years old with the mean age 29.61±21.56 years. The lowest resistance rate was seen in *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates against gentamycin. Based on the results, it was seen that all *Escherichia coli* isolates were resistant against all of the examined antibiotics. Appropriate infection control policies and knowing the antimicrobial pattern in burn patients especially in intensive care units may help to provide the best treatment for burn patients and save their life.

1. Introduction

Burn is a devastating form of trauma that causes these patients to be one of the most complex groups in hospital care units. Despite burn injuries is preventable, but this is still a global public health issue with an estimation of 180,000 deaths annually in the world (WHO 2018 March). According to the lifestyle and some high-risk jobs such as petrochemical units and gas fuels in Iran, this country is in a maximum burn threat, and burn injuries are still significant (Karimi et al., 2014; Javanmardi et al., 2019). Infection, sepsis, organ failure, and mortality are the most complications that result from skin damage and hypermetabolism in burn victims (Dolp et al., 2018). Intensive care management is a preventive measure in burn patients who are at high risk of organ failure (Ansermino and Hemsley, 2004). One of the threats in burn victims especially intensive care unit (ICU) hospitalized patients, is ventilator associate pneumonia (VAP). VAP is usually developed after 48 hours of mechanical ventilation and remains an important cause of morbidity and mortality in burn ICU patients

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(Huzar and Cross, 2011). Based on different reports, 20-50% of healthcare-associated infections (HAI) are developed in ICU, which may cause many adverse health effects on hospitalized patients. This group of infections is caused by prolonged hospitalization, increasing mortality, and significantly increasing the costs for treatment (Rutkowska et al., 2013). Due to HAI complications in ICU patients, as a result of device utilization threat, VAP remains an important cause of morbidity and mortality in burn ICU victims. Accordingly, this study was aimed to analyze the epidemiology of ventilator-associated pneumonia (VAP) in BICU wards of Amir-Al-Momenin burn hospital as the main center for burns at the south of IRAN.

2. Materials and Methods

2.1. Case selection

This one-year cross-sectional study was performed from September 2018 to September 2019 in Amir-al-Momenin burn hospital, affiliated with Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. This study was approved by the Local Ethical Committee with the Code: IR.SUMS.MED.REC.1398.176. During the study time, a total of 70 cases were hospitalized in burn intensive care units (BICU). Sputum samples were taken from all BICU patients after >48h stay in BICU. Patients who referred from other centers and who were treated with antibiotics before, referring after 24 h of a burn accident, and patients with immune system disorders (such as leukopenia or immunosuppressed patients) were excluded. Included patients' who developed new or progressive infiltration on chest radiograph, leukocytosis $>12\times10^9$/ml, and purulent tracheobronchial secretions were selected for tracheal sampling. Besides these criteria, the results of some other non-specific signs such as fever of > 38°C and tachycardia were either recorded in their questionnaire. Demographic information of patients such as age, sex, and total burn surface area (TBSA) was recorded completely. According to the non-specificity of radiography this criteria was not considered. According to some previous reports, pulmonary infiltrates consistent with VAP can be caused by numerous noninfectious disorders, such as chemical pneumonitis, asymmetric cardiac pulmonary edema, pulmonary embolism, pulmonary contusion, pulmonary hemorrhage, drug reaction, and asymmetric acute respiratory distress syndrome (ARDS) (Koenig and Truwit 2006).

2.2. Microbiological diagnosis:

Tracheal samples, were injected in collection tubes contain sterile normal saline (3 ml) and transferred to the burn and wound healing research center microbiology laboratory. Collected samples were first evaluated for bacterial infection with a standard biochemical microbiology tests. In brief, a loop full of samples were inoculated on standard bacteriology media (Blood agar and Eosin Methylene Blue), and growth colonies after the incubation time (24 hours/37°C) were isolated and a pure culture from each colony were prepared. For detection of isolates, standard techniques such as gram staining and biochemical tests such as catalase, oxidase, sugar fermentation pattern in Triple Sugar Iron agar (TSI), indole production, MR-VP, citrate utilization, were used. In following, confirmation of some isolates (*Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Klebsiella pneumonia*) was performed by the Polymerase Chain Reaction (PCR) with a specific introduced primers as one of the steps of the study (Table1). Other isolates (*E. coli*, *Staphylococcus aureus* and *Bacillus* spp.) were confirmed according to the specific microbiological/biochemical tests. After the confirmation of the isolates in the following process, an antibacterial susceptibility test was performed. For detection of antibacterial susceptibility pattern of isolates, strains were tested with a standard antibiogram protocol and recommended antibacterial agents by the Clinical and Laboratory Standards Institute ((CLSI) 2018). Kirby-Bauer technique was selected for antibiogram and recommended antibacterial disks were placed on Muller-Hinton agar plates which were inoculated with pure selected of strains. After the incubation time, the inhibition zone around the disks was measured in diameter. Selected antibacterial disks which was used in the current stsudy consist of: Tobramycin, Ampicillin, Tetracycline, Nitrofurantoin, Cephalothin, Gentamycin, Cloxacillin, Trimethoprim- Sulfamethoxazole, Chloramphenicol, Amoxicillin.

2.3. Statistical analysis

Due to the aim of study, quantities and qualitative variables were presented with mean ± standard deviation and frequency and parentage respectively. Chi-square test was used for evaluating the distribution of sex between infected non-infected patients. All the statistical analysis were done by SPSS 18. P-value less than 5% was considered as statistically significant.

Table1. Primer seq. target genes, product size and PCR protocol.

Microorganism	Primer	Sequence	Annealing temperature	Product length (bp)	Reference
Streptococcus pneumonia	16s-F 16s-R	AAGGTGCACTTGCATCACTAC GACGCATTTCACCGCTACA	66	499	(Emami et al., 2019)
Pseudomonas aeruginosa	F R	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	58	956	(Emami et al., 2019)
Klebsiella pneumonia	F R	GGC TGT ACT ACA ACG ATG AC TTG AGC AGG TAA TCC ACT TTG	62	931	(Jeong et al., 2013)

3. Results

During the study, a total of 70 ventilator-dependent patients was included with the mentioned criteria wards (male, female, and pediatrics, one sample one patient). From these 21 (30%) samples were positive growth. The age range of patients was estimated at 1–60 years old and the mean was estimated 29.61±21.56 years. All of the included patients had sustained deep burns (second-degree and more) with the average percentage of the Total Body Surface Area (%TBSA) 37.05± 11.74 (range: 10 – 60). All of the selected patients were in the normal weight and no one detected obese or low weight at the study time. Infected patients consist of 7 (33.33%) female, and 14 (66.66%) males, while significant differences were seen between male and female participants (P = 0.048). Based on the main goal of the study, the antibiogram resistance of the isolates was evaluated and the results are provided in table2. The lowest resistance rate was seen in *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates against gentamycin. Based on the results, it was seen that all *Escherichia coli* isolates were resistant against all of the examined antimicrobial agents.

4. Discussion

Infection complications are a major hidden threat for burn patients during hospitalizations and it is a risk factor for mortality and morbidity in these groups of victims. One of the most common nosocomial infections among hospitalized patients in intensive care units is ventilator-associated pneumonia (VAP) (Roquilly, Feuillet, Seguin et al. 2016). Frequent mechanical ventilation and intubation increase the risk of developing VAP in burn cases. In a study by Kollef et al, it was found that patients who admitted to ICU are more at risk of VAP (Kollef 2003). The present study indicated that Bacillus spp. and *Pseudomonas aeruginosa* were the most prevalent causative pathogens of VAP, this is while Streptococcus and Enterobacter were the predominant isolates in Wendy's research (Wahl, Taddonio, Arbabi et al. 2009). Due to the high resistance of isolates to multiple antibacterial agents, it is a major challenge for clinicians to choose appropriate antimicrobial agents for hospitalized patients. Due to the long time need for performing microbiological procedures, physicians have to treat infected patients with primary empirical therapy. An important point in empirical treatment is choosing a proper antimicrobial agents, which if not will increase mortality or at least cause to form resistant strains (Albrecht, Griffith, Murray et al. 2006). Based on these it is an urgent issue to know the predominant pathogenic agents especially VAP-related infections and their susceptible patterns. Various risk factors are known to threaten burn patients are included; age, inhalation burns, and TBSA. These risk factors are more dangerous for BICU patients. Based on previous researches, patients with

larger TBSA, present VAP more significantly. Patients with higher ages and weakness in their lungs have more inhalation injury which causes them to be more at risk of VAP (Mosier and Pham 2009).

Table 2. Resistance Pattern of isolates

Isolates *Antibacterials*	*P. aeruginosa* (n=6)	*S. aureus* (n=3)	*K. pneumonia* (n=3)	*E. coli* (n=1)	*S. pneumonia* (n=1)	*Bacillus* spp (n=7)
Tobramycin	4 (66.6%)	3 (100%)	3 (100%)	1 (100%)	0 (0%)	5 (71.4%)
Ampicillin	6 (100%)	3 (100%)	2 (66.6%)	1 (100%)	1 (100%)	7 (100%)
Tetracycline	2 (33.3%)	1 (33.3%)	3 (100%)	1 (100%)	0 (0%)	4 (57.1%)
Nitrofurantoin	4 (66.6%)	3 (100%)	3 (100%)	1 (100%)	0 (0%)	6 (85.7%)
Amikacin	3 (50%)	3 (100%)	3 (100%)	1 (100%)	0 (0%)	6 (85.7%)
Cephalothin	5 (83.3%)	3 (100%)	3 (100%)	1 (100%)	0 (0%)	5 (71.4%)
Gentamycin	2 (33.3%)	1 (33.3%)	3 (100%)	0 (0%)	1 (100%)	4 (57.1%)
Cloxacillin	5 (83.3%)	3 (100%)	2 (66.6%)	1 (100%)	1 (100%)	6 (85.7%)
Trimethoprim-Sulfamethoxazole	2 (33.3%)	2 (66.6%)	3 (100%)	0 (0%)	0 (0%)	6 (85.7%)
Chloramphenicol	3 (50%)	1 (33.3%)	2 (66.6%)	1 (100%)	1 (100%)	3 (42.8%)
Amoxicillin	6 (100%)	3 (100%)	2 (66.6%)	1 (100%)	0 (0%)	4 (57.1%)

According to a recent analysis, it was found that high resistance may be due to some mechanisms such as expressing multiple efflux pump systems and hydrolyzing enzyme activity. Multi-drug resistance is observed in clinical tracheal samples from burn patients, especially in Acinetobacter sp. and P. aeruginosa. According to the previous literature the rate of bacterial resistance ranged from 10% to 50% which is similar to our results (Santucci, Gobara, Santos et al. 2003). Production of hydrolyzing enzymes such as extended-spectrum beta-lactamases is a major reason which may cause gram-negative bacteria such as *Klebsiella*, *Enterobacter*, and *Serratia* to become resistant to various antibacterial agents. Moreover, the pattern of infections is differing widely in different groups of patients, this is while the difference may be more seen in patients with acute respiratory distress syndrome (ARDS) (Sepsis, Group, Greenhalgh et al. 2007). In ARDS patients polymicrobial infection is another important risk factor in their treatment. Dennesen et al have reported most of the clinical signs of pneumonia were resolved by 6 days of antibacterial therapy, and a recent multicenter randomized trial in France demonstrated no difference in mortality or recurrent infection in patients treated with 8 days course of antibacterial compared with those treated with a 15-day course. In addition, those patients treated with shorter antibacterial treatment course shows a lower incidence of multi-drug resistant especially in recurrent infections (Dennesen, van der VEN, Kessels et al. 2001, Chastre, Wolff, Fagon et al. 2003, Wahl, Taddonio, Arbabi et al. 2009). Although defining a suitable treatment about the kind and duration of antibacterial therapy is an important issue in ICU patients but due to limited performed researches in this regard in burn patients, there are different outlooks about the pattern of antibacterial therapy in BICU patients. Burn victims due to their immunocompromised and malnourished condition especially prior to burn injury are more susceptible to microbial infections(Sen, Johnston, Greenhalgh et al. 2016, Emami, Pirbonyeh, Keshavarzi et al. 2020). Although various researches have been conducted about VAP prevention more researches are needed in burn victim groups. It is urgent to determine the causative agents and provide the best treatment, diagnosis, and infection control strategies.

Conclusion

Appropriate infection control policies and knowing the antimicrobial pattern in burn patients especially in intensive care units will help to provide the best treatment for burn patients and save their life. Overall, in the current study, it was found that polymicrobial infections are present in our cases with the most prevalence of Bacillus spp. and *Pseudomonas aeruginosa*. Given that nosocomial infections are acquired in the hospital, knowing the infection and antibacterial-resistant pattern of each ward/center can be a significant help in choosing the appropriate antimicrobial for empirical treatment and infection control policies.

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Author contribution: A. E designed and supervised the study, N.BS and N.P did the laboratory tests. F.J did the statistical analysis.

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Refereces

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