

Research Paper

Three-year Review of Bacteriological Profile and Anti-biogram of Burn Wound Isolates in Van, Turkey

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Abstract

The risk of infection in burns is well-known. In recent decades, the antimicrobial resistance of bacteria isolated from burn patients has increased. For this reason, a retrospective study was conducted at Van Training and Research Hospital to analyze the bacterial isolates from the wounds of patients admitted to the Burn Unit and to determine the susceptibility patterns of the commonly cultured organisms over a 3-year period, January 2009 to December 2011.

A total of 250 microorganisms were isolated from burn wounds of 179 patients. Our results revealed that the most frequent isolate was *Acinetobacter baumannii* (23.6%), *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* (11.2%), *Escherichia coli* (10%) respectively. Multidrug-resistance has emerged as an important concern in our burn unit. Tigecycline, and colistin were found to be the most active drugs against *Acinetobacter baumannii*. Carbapenems and amikacin, were found to be the most active drugs against other gram negative bacteria. Vancomycin and linezolid were active against gram positive bacteria.

Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.

Key words: antibiotic, burns, resistance, wounds.

Introduction

Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world [1]. The burn patients have unique predisposition to different infections which are linked to impaired resistance from disruption of the skin's mechanical integrity and generalized immune suppression. The skin barrier is replaced by a protein rich, avascular environment that provides a favourable niche for microbial colonization and proliferation. Additionally migration of immune cells is hampered, which contributes to septic process [2-6].

In spite of considerable advances in the last 60 years in antimicrobial treatment, infection still continues to pose the greatest danger to burn patients. It was shown that approximately 73 per cent of all death

within the first five days post-burn has been caused by sepsis [7-9]. Also the worldwide emergence of antimicrobial resistance among bacterial pathogens, limits the available therapeutic options for effective treatment of infections [10, 11].

Thus, the aim of the current study was to determine the microorganisms and their susceptibility patterns which were isolated from burn wounds of patients at Van Training and Research Hospital in Van, Turkey.

Material and Methods

Data Collection

This study was conducted retrospectively at a 10-bed paediatric and adult burn unit located in a

400-bed tertiary referral hospital. The burn unit is the reference burn center in Van province (with one million inhabitants), Turkey. Consequently patients hospitalized in this unit come from the emergency of the hospital as well as from transfers from other hospitals.

Wound swaps were obtained twice weekly to monitor colonisation and when infection was suspected in the burn unit. All wound specimens were collected by sterile swabs from registered patients. Positive wound swap culture results during a 3-year period (from January 2009 to December 2011) were reviewed and two hundred fifty non-duplicate bacterial species isolated from one hundred seventy-nine patients' wound swabs, were included in the present study.

Assessment and management of burns patients

Early burn excision and skin grafting is practiced in our burn unit. No routine systemic and topical antimicrobial (e.g. mafenide, silver sulfadiazine) used in our burn unit. Fucidic acid is used as topical agent only during deep excisions.

Bacterial identification and antimicrobial susceptibility

All samples were inoculated on 5% sheep blood agar and Eosin methylene blue overnight at 37°C. Identification of isolates was done by conventional biochemical methods according to Standard microbiological techniques [12]. After determining mainly morphologic criteria of bacteria, panels of automated identification device Phoenix Automated Microbiology System (Becton, Dickinson-USA) was used in order to determine the certain identification and anti-microbial susceptibility rates. Duplicate isolates defined as repeated isolation of the same bacterial species for the same patient with the same profile of antibiotic susceptibility were excluded. *Pseudomonas aeruginosa* and *Acinetobacter* spp were accepted as multidrug-resistant if the microorganism was resistant against at least three antimicrobials groups among antipseudomonal cephalosporins, β -lactam- β -lactamase inhibitor combination, antipseudomonal fluoroquinolones, antipseudomonal carbapenems or aminoglycosides. The antimicrobial susceptibilities were determined according to the Clinical and Laboratory Standards Institute (CLSI). Information on all bacterial isolates including their antibiotic susceptibility pattern were extracted and processed by SPSS 17.0 software package.

Results

A total of 250 bacterial isolates were obtained

from 179 patients' wound swap over a 3-year period. The most predominant bacterial isolate was *Acinetobacter baumannii* (*A. baumannii*) (23.6%) followed by coagulase negative *Staphylococci* (13.6%), *Pseudomonas aeruginosa* (*P. aeruginosa*) (12%), *Staphylococcus aureus* (*S. aureus*) (11.2%), and *Escherichia coli* (*E. coli*) (10%) as shown in Table 1.

Table 1. Distribution of microorganisms isolated from burn wounds.

Microorganism	n	%
<i>Acinetobacter baumannii</i>	59	23.6
Coagulase negative <i>Staphylococci</i>	34	13.6
<i>Pseudomonas aeruginosa</i>	30	12.0
<i>Staphylococcus aureus</i>	28	11.2
<i>Escherichia coli</i>	25	10.0
<i>Enterococcus</i> spp.	22	8.8
<i>Klebsiella pneumoniae</i>	18	7.2
Other <i>Enterobacteriaceae</i> spp.*	26	10.4
Others**	8	3.2
Total	250	100.0

*: 14 *Enterobacter* spp., 4 *Klebsiella oxytoca*, 3 *Proteus* spp., 1 *Morganella morgani*, 1 *Pantoea agglomerans*, 1 *Proteus mirabilis*, 1 *Providencia rettgeri*, 1 *Serratia fonticola*. **: 5 *Streptococcus* spp., 2 *Stenotrophomonas maltophilia*, 1 *Achromobacter* spp..

The susceptibility of the organisms to different antibiotics varied depending on the isolate. Although all tested *Acinetobacter* spp. isolates were sensitive to tigecycline (n=37) and colistin (n=43), fifty-five (93%) of isolates were multidrug-resistant. Thirteen (43%) of *P. aeruginosa* isolates were multidrug-resistant. Meropenem, amikacin, ciprofloxacin and cefepime were found to be most active antimicrobial agents against *P. aeruginosa*. All tested *E. coli* isolates were susceptible to amikacin (n=17), imipenem (n=19) and meropenem (n=21). Besides these three antimicrobial agents, gentamicin was also found to be in vitro active against *Klebsiella pneumoniae* (*K. pneumoniae*) and other *Enterobacteriaceae* spp. The antibiotic resistance patterns of gram negative isolates were as shown in Table 2. Extended-spectrum beta-lactamases (ESBL) were found to be 13/25 (52%) and 7/18 (39%) among *E. coli* and *K. pneumoniae* isolates respectively.

Among the *S. aureus* isolated from patients within the burn center, the incidence of methicillin-resistant *S. aureus* (MRSA) was 19% and the most active antimicrobial agents were found to be vancomycin and linezolid against *S. aureus* isolates respectively. None of the *Enterococcus* spp. was found to be resistant to vancomycin. Antimicrobial susceptibilities of gram positive isolates were presented in Table 3.

Table 2. Susceptibilities of gram negative isolates to various antimicrobials.

Antimicrobials	<i>A. baumannii</i> n=59		<i>P. aeruginosa</i> n=30		<i>E. coli</i> n=25		<i>K. pneumonia</i> n=18		Other Gram negative microorganisms n=26	
	n*	R (%)	n*	R (%)	n*	R (%)	n*	R (%)	n*	R (%)
Ceftazidime	58	54 (93)	28	9 (32)	22	12 (55)	18	7 (39)	25	5 (20)
Piperacillin/ tazobactam	57	51 (90)	29	9 (31)	24	10 (42)	17	8 (47)	24	3 (13)
Imipenem	58	50 (86)	28	13 (46)	19	0 (0)	18	0 (0)	19	1 (5)
Meropenem	56	43 (77)	27	5 (19)	21	0 (0)	16	0 (0)	22	1 (5)
Gentamicin	56	48 (86)	28	10 (36)	22	5 (23)	13	0 (0)	25	1 (4)
Cefepime	55	47 (86)	24	6 (25)	22	13 (59)	15	3 (20)	25	2 (8)
Ciprofloxacin	59	51 (86)	28	7 (25)	25	8 (32)	18	3 (17)	26	2 (8)
Tigecycline	37	0 (0)	NT	NT	NT	NT	NT	NT	NT	NT
Amikacin	57	30 (53)	29	6 (21)	17	0 (0)	14	0 (0)	24	2 (8)
Colistin	43	0 (0)	20	0 (0)	NT	NT	NT	NT	NT	NT

*: number of isolates which were tested, R: resistant, NT: not tested.

Table 3. Susceptibilities of gram positive isolates to various antimicrobials.

Antimicrobials	<i>S. aureus</i> n=28		<i>Enterococcus spp.</i> n=22	
	n	R (%)	n	R (%)
Penicilin	26	25 (96)	9	1 (11)
Trimetoprim-Sulfametoksazol	27	2 (7)	NT	NT
Klindamisin	23	1 (4)	NT	NT
Vankomisin	22	0 (0)	22	0 (0)
Linezolid	17	0 (0)	22	0 (0)
Daptomisin	11	0 (0)	6	1 (17)
Ampisilin	NT	NT	22	0 (0)

R: resistant, NT: not tested.

Discussion

Burn injuries remain a huge public health issue in terms of morbidity and long-term disability throughout the world [13, 14]. Thermal injury impairs the skin its normal barrier function, thus allowing microbial colonization of the burn wounds. Severe dysfunction of the immune system, a large cutaneous colonization, the possibility of gastrointestinal translocation, a prolonged hospitalization and invasive diagnostic and therapeutic procedures, all contribute to infections [13, 15]. Patient factors such as age, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, and motility determine the likelihood of invasive burn wound infection. [16]. Although any organism is a potential pathogen in burned patients, coagulase-negative staphylococci and *S. aureus* and *Enterococcus spp.* were the most common gram positive pathogens and *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *Acinetobacter spp.*

were the most common gram negative microorganisms [6,17,18].

The most common pathogen isolated from burn wounds in our study was *Acinetobacter spp.* The high prevalence of *Acinetobacter baumannii* in our centre differs markedly from most other studies from Europe, the USA and South America [19-21]. Chim *et al.* have also found *Acinetobacter spp.* highly prevalent in Singapore and explained this situation by constant introduction of *Acinetobacter spp.* carried on human skin (endemic to tropical climate) with every patient admitted in their settings [22]. Other studies have supported the hypothesis that *Acinetobacter spp.* might be more prevalent in warm climates, with corresponding increase in colonization and nosocomial infection [23-25]. Although our center is placed in eastern part of Turkey where the climate is dry and cold, this hypothesis could be an explanation for our results also and should be further studied.

Our finding concerning the frequency of *P. aeruginosa* (12%) was much lower than many previous

reports where this organism was held responsible for the majority of invasive burn wound infections in burn-treatment facilities [26-29]. *S. aureus* was the third in the list of microbial isolates recovered in our study. This is contrary to many previous reports indicating a much higher frequency of isolation of this organism [30-31]. In our study, *E. coli* was the fourth most frequently recovered organism. This is higher than reported by other burn centers [29-32].

The pattern of bacterial resistance is important for epidemiological and clinical purposes. The results of the antimicrobial resistance pattern give serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antimicrobial agents in Turkey. *Acinetobacter baumannii* and *P. aeruginosa* were found to be multidrug-resistant. Despite the increased knowledge of the pathogenesis and antibiotic resistance mechanisms, multidrug-resistant isolates of *A. baumannii* and *P. aeruginosa* are special concerns in burn care units [33]. The incidence of *A. baumannii* resistant to imipenem (86%) was high, in contrast to other studies [34-36]. Presence of *Acinetobacter* spp. as normal skin flora, its easy transmissibility and ability to remain viable in a hospital environment due to its multidrug-resistant status and several other factors have been implicated in the increased incidence of nosocomial infections due to this organism. As reported in other studies [22-25] multidrug-resistant *Acinetobacter* spp. have emerged as a significant cause of wound infection in our burn unit. The incidence of *P. aeruginosa* resistant to ceftazidime (32%), Piperacillin/tazobactam (31%) and imipenem (46%) was much higher in our study in contrast to other studies [37, 38]. Also the incidence of methicillin-resistant *S. aureus* (MRSA) was 19%. This is consistent with those reported from other countries [37, 38] and supports the fact that there was increasing evidence that MRSA has become a significant problem. We found that vancomycin, linezolid, and ampicillin were still active drugs for the treatment of *Enterococcus fecalis*.

The emergence of ESBL producing strains among *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, *E. cloacae* and *P. mirabilis*) is a special concern [39]. Gugenheim *et al* have showed that imipenem and meropenem were the most active antimicrobial agents for ESBL producing strains [40]. Our results were consistent with aforementioned study.

The main limitations of our study are the retrospective design and use of only a single burn center's data. Culture isolates were unavailable for additional testing or molecular analysis to determine if isolates were acquired through nosocomial transmission. Additionally data obtained from electronic patient rec-

ords makes it difficult to distinguish infection from colonization. Although it is known that the widespread use of broad spectrum antimicrobials in burn units would provide a fertile ground acquisition of resistance and transformation to form new strains [22], detailed treatment regimens were unavailable in our study and it is unknown what impact antibiotic use had on culture data.

In conclusion, the growth of multidrug-resistant organisms should be considered as a serious risk in burn units. Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.

Conflict of interest

The authors have no conflict of interest to report.

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