Occurrence and antimicrobial susceptibility pattern of ESBL-producers among Gram-negative bacteria isolated from burn unit at the Al Shifa hospital in Gaza, Palestine: A short original article

Ghassan Tayh^{1,2}, Nahed Al Laham^{1*}, Abdelraouf Elmanama², Karim BEN SLAMA³

¹ Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al Azhar University-Gaza, Palestine

² Medical Laboratory Science Department, Islamic University-Gaza, Gaza Strip, Palestine

³ Institut Supérieur des Sciences Biologiques Appliquées de Tunis, Université de Tunis El Manar, 2092 Tunis, Tunisie

Abstract

Background: Bacterial infection can be an important cause of death in burn cases. The emergence of antimicrobial resistant pathogens associated with extended spectrum beta-lactamases (ESBLs) is leading to inappropriate/or treatment failure and increased morbidity and mortality.

Objective: The purpose of this study was to determine the antimicrobial susceptibility pattern with the identification of ESB -producers among pathogens isolated from burn patients at the Al-Shifa burn unit in Gaza, Palestine.

Methods: A total of 40 gram-negative bacterial isolates were recovered from burn wound patients between August 2012 and December 2012. Antibiotic susceptibility profiles of the identified isolates were determined by the standard disc diffusion method according to CLSI guidelines. The ESBL detection was screened by using double disc diffusion method.

Results: *Pseudomonas aeruginosa* was the predominant isolate 15 (37.5%) followed by *Klebsiella pneumoniae* 10 (25%), *Escherichia coli* 9 (22.5%), *Enterobacter cloacae* 4 (10%) and *Acinetobacter baumannii* 2 (5%). ESBL was detected in 37.5% of isolates. Most of ESBL-producing

Corresponding author:

Dr. Nahed Al Laham

✉ n.lahamm@alazhar.edu.ps,
dr.allaham@hotmail.com

isolates were susceptible to meropenem and imipenem (80% and 73%, respectively). However, ESBL producing isolates showed 100% resistance against cefotaxime and ampicillin. The resistance rate towards sulfamethoxazole/trimethoprim, tetracycline, cefepime, tobramycin and aztreonam was 76.9%, 69.2%, 66.7%, 60% and 60%, respectively. *A. baumannii* isolates were resistance to all tested antibiotics.

Conclusion: *P. aeruginosa* is the most common bacteria isolated from burn wound of patients at the Al-Shifa burn unit. The frequency of ESBL-producers among gram-negative bacteria was high, and most of these isolates except *A. baumannii* were still highly susceptible to carbapenems including imipenem and meropenem.

Key words: Burns infection, Antimicrobial resistance, ESBL, Gaza hospitals, Palestine

Introduction

Burns infection remains the major leading cause of morbidity and mortality among burn patients in developing countries [1]. It has been estimated that 75% of the mortality following burn injuries are related to bacterial infection [2].

Majority of bacteria that cause burn wound infection in hospitals are resistant to at least one of the most commonly antimicrobial drugs used for treatment [3]. The emergence of antimicrobial resistance worldwide among burn wound pathogens, limits the available therapeutic options for effective treatment of burn wound infections [4]. Extended Spectrum β -Lactamases (ESBLs) producing organisms pose a major problem for treating burn infected victims [3]. ESBL-producer organisms have been emerging as serious challenges in hospitalized patients. These organisms can be transmitted easily from one patient to another [5]. Inappropriate treatment of severe infections caused by ESBL

producers has been associated with increased mortality [6]. Pattern of antimicrobial agents and bacterial resistance should be known and considered when prescribing antimicrobial agents for treatment [7]. Therefore, it is necessary to carry out periodic monitoring of patterns of isolation and susceptibility profiles of microorganisms in burn wounds in order to modify the preventive and therapeutic strategies. Colonization rates in burn patients and the antimicrobial drug resistance of burn infections pathogens from Gaza strip have been rarely reported.

A recent study in 2013 showed that 45.8% of specimens collected from burn patients in Gaza strip were infected with different bacteria species with predominance of *Pseudomonas aeruginosa* (50%) and *Enterobacter cloacae* (28.3%) as the commonest isolated pathogens [8]. Here in this study, we attempt to determine the incidence of facultative anaerobic bacterial isolates from burn wound specimens, their antimicrobial susceptibility

patterns and the occurrence of ESBL-producers among those pathogens isolated from Al-Shifa burn unit in Gaza strip, Palestine.

Materials and methods

Study setting

Gaza Strip in Palestine is a narrow part of land of 365 km². The Gaza strip is one of the most overpopulated areas in the world, with a population of 1.7 million inhabitants and a population density of 4,073 per km² [9, 10]. There are two burn units in the two main governmental hospitals in Gaza strip –Al Shifa (503 beds) and Nasser (277 beds) – that serve this large population where most of them are middle and low socio-economic classes. Al Shifa's burn unit has ten beds while Nasser's has only five beds. An average of 289 and 30 patients per month are seen at Al Shifa and Nasser burn units respectively; most are treated as out-patients [11]. The samples included in this study were collected from burn patients admitted to Al Shifa burn unit.

Patients and Setting

This study was conducted for a period of 5 months (August 2012 to December 2012) at Al Shifa's burn unit in Gaza strip. A total of 40 isolates of Gram-Negative were obtained from wound samples during the study period from patients who admitted to burn unit (Gram positive isolates were not included). Only one specimen and one prevalent isolate per patient was processed. The study design was approved by local Ethical Helsinki Committee in Gaza, and an informed consent was obtained from participating patients who are freely accepted to participate in this study.

Sample collection and Microbiological investigation

A sterile cotton swab was used for sampling from all burn in-patients after three days of admittance.

The specimens were collected by the attending physicians using a sterile swab moistened with sterile normal saline. Swabs were collected from burn wound surface after the removal of dressings and topical antibacterial agents and cleansing of the wound surface with 70% alcohol [4]. The swabs were dipped in Stuart's transport medium and transported to the laboratory for bacteriological isolation and identification. The swabs were plated on blood agar and MacConkey agar. The isolates were identified using conventional identification techniques after incubation for 18–48 hrs at 37 °C [12]. Positive cultures were subcultured on MacConkey agar, as per routine bacteriologic guidelines. Conventional biochemical tests and API 20E system (BioMerieux, France) were used to identify the prevalent isolated gram-negative bacteria to the species level [13].

Antimicrobial susceptibility test

The antimicrobial susceptibility testing of all isolates was performed on Mueller-Hinton agar plates with commercially available discs by the Kirby-Bauer disc diffusion method. All the Gram negative isolated bacteria were tested for their sensitivity profile to the most common used antibiotics in Al Shifa burn unit: Amoxycillin–clavulanic acid, amikacin, Ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, sulfamethoxazole/trimethoprim, tetracycline and tobramycin (Biorad, California, USA). The experiments and results were performed, recorded and interpreted as per CLSI recommendations [14].

Detection of extended spectrum β -lactamase (ESBL) by the double disc diffusion method

For ESBL screening, the isolates in this study were inoculated onto Mueller-Hinton agar medium (Biolife, Milano, Italy). Amoxicillin/ clavulanic acid impregnated disc was then placed at the center

Figure 1. Distribution of pathogens isolated from burn patients

of the Mueller-Hinton plate while ceftazidime 30 µg and cefotaxime 30 µg discs were placed peripherally away from the amoxicillin/ clavulanic acid former two discs are applied 25-30mm apart. Band formation between amoxicillin/ clavulanic acid and any other disc was considered as ESBL positive [15, 16].

Results

Out of 40 specimens, 23(57.5%) were from males and 17 (42.5%) were from females. The most

common cause of burns was scalding, followed by open fire. Pediatric patients (<15 years) accounted for 55% (22 cases) and adult patients (≥15 years) were 45% (18 cases). Third and second degree burns accounted for 65% and 35%, respectively.

A total number of 40 prevalent positive Gram-negative bacilli were recovered. *Pseudomonas aeruginosa* was the predominant isolate 15 (37.5%), followed by *Klebsiella pneumoniae* 10 (25%), *Escherichia coli* 9 (22.5%), *Enterobacter cloacae* 4 (10%) and *Acinetobacter baumannii* 2 (5%) (**Fig. 1**).

All 40 gram-negative bacilli were screened for ESBL production. Fifteen (37.5%) of them were ESBL-producers. Out of these 40, putative ESBL-production was observed in five (55.6%) of the nine *E. coli* isolates, 2/4 (50%) of the *E. cloacae* isolates, 4/10 (40%) of the ten *K. pneumoniae* isolates, and only 2/15 (13.3%) of *P. aeruginosa* isolates. Additionally, two isolates (100%) of *A. baumannii* were ESBL-producer (**Table 1**).

As shown in Table 2, meropenem and imipenem were found to be the most resistant antibiotics against ESBL producing isolates, with efficacy rate reached 80% and 73%, respectively. Meanwhile, amikacin and ciprofloxacin were the next best resistant drug against ESBL producing isolates with efficacy rate that reached 60% (**Table 2**).

Table 1. ESBLs producer among the Gram negative bacteria isolated from Burn patients

Type of Bacteria	ESBL Production		Total (%)
	No. (%)Positive	No. (%)Negative	
<i>P. aeruginosa</i>	2 (13.3)	13 (86.7)	15 (100)
<i>K. pneumoniae</i>	4 (40)	6 (60)	10 (100)
<i>E. coli</i>	5 (55.6)	4 (44.4)	9 (100)
<i>E. cloacae</i>	2 (50)	2 (50)	4 (100)
<i>A. baumannii</i>	2 (100)	-	2 (100)
Total	15 (37.5)	25 (62.5)	40 (100)

Table 2. Antimicrobial resistance pattern of ESBL-producing pathogens

Antimicrobial agents (concentration/ μ g)	<i>E. coli</i> (N 5;%)	<i>K. pneumoniae</i> (N 4;%)	<i>E. cloacae</i> (N 2;%)	<i>Paeruginosa</i> (N 2; %)	<i>A.baumannii</i> (N 2;%)	Total (%)
Amoxycillin–clavulanic acid (20/10)	1(20)	2(50)	2(100)	2(100)	2(100)	9(60)
Amikacin (30)	1(20)	2(50)	1(50)	1(50)	1(50)	6(40)
Ampicillin (10)	5(100)	4(100)	2(100)	2(100)	2(100)	15(100)
Aztreonam (30)	2(40)	2(50)	1(50)	2(100)	2(100)	9(60)
Cefepime (30)	3(60)	2(50)	1(50)	2(100)	2(100)	10(66)
Cefotaxime (30)	5(100)	4(100)	2(100)	–	2(100)	13(100)
Ceftazidime (30)	1(20)	2(50)	1(50)	1(50)	2(100)	7(46)
Ciprofloxacin (5)	2(40)	0	1(50)	1(50)	2(100)	6(40)
Gentamicin (10)	1(20)	3(75)	2(100)	0	2(100)	8(53)
Imipenem (10)	0	0	2(100)	0	2(100)	4(26)
Meropenem (10)	0	0	1(50)	0	2(100)	3(20)
Piperacillin-tazobactam (100/10)	2(40)	2(50)	1(50)	2(100)	1(50)	8(53)
Sulfamethoxazole/ trimethoprim (1.25+23.75)	4(80)	2(50)	2(100)	–	2(100)	10(77)
Tetracycline (10)	4(80)	2(50)	1(50)	–	2(100)	9(69)
Tobramycin (10)	1(20)	3(75)	2(100)	1(50)	2(100)	9(60)

All the ESBL Gram-negative bacterial isolates were resistant to cefotaxime and ampicillin (100%). Resistance to sulfamethoxazole/trimethoprim, tetracycline, cefepime, tobramycin and aztreonam among ESBL producing isolates was 77 %, 69%, 67 %, 60% and 60%, respectively. The two isolates of *A. baumannii* were resistance to all tested antibiotics. All ESBL isolates were found to be multidrug resistant (MDR) and *A. baumannii* isolates even were extremely drug resistant (XDR) (**Table 2**).

Comparing the antibiotics resistance profile of ESBL producers and non-ESBL producers; we can notice that both were more resistant to ampicillin. Resistant profile to aminoglycosides, cefepime, cefotaxime, sulfamethoxazole/trimethoprim and piperacillin-tazobactam was greater for ESBL

producers, than non-ESBL producers, whereas imipenem resistance was higher for non-ESBL producers. There was no obvious difference in resistance to amoxycillin–clavulanic acid, meropenem, ciprofloxacin, ceftazidime, aztreonam, tetracycline or ampicillin (**Table 3**).

Discussion

The present study isolated and characterized the prevalent etiological bacterial pathogens of burn patients in Al-Shifa burn unit in Gaza strip. Also, the study determined the antimicrobial susceptibility profile and the occurrence of the ESBL -producers among gram-negative isolates.

Table 3. Antimicrobial resistance pattern of the ESBL Negative Bacteria

Antimicrobial	<i>E. coli</i> (N4;%)	<i>K.pneumoniae</i> (N6;%)	<i>E.ter cloacae</i> (N2;%)	<i>P. aeruginosa</i> (N13;%)	Total (N25;%)
Amoxycillin–clavulanic acid	1(25)	4(67)	1(50)	10(77)	16(64)
Amikacin	1(25)	4(66 .7)	0	1(7.7)	6(24)
Ampicillin	3(75)	6(100)	1(50)	12(92)	22(88)
Aztreonam	2(50)	3(50)	0	8(62)	13(52)
Cefepime	2(50)	3(50)	0	6(46)	11(44)
Cefotaxime	2 (50)	4(67)	0	–	6(50)
Ceftazidime	1(25)	4(67)	0	5(39)	10(40)
Ciprofloxacin	3(75)	3(50)	0	5(39)	11(44)
Gentamicin	1(25)	4(67)	0	5(39)	10(40)
Imipenem	1(25)	4(67)	0	6(46)	11(44)
Meropenem	0	1(17)	1(50)	5(39)	7(28)
Piperacillin-tazobactam	1(25)	1(17)	0	3(23)	5(20)
Sulfamethoxazole/trimethoprim	4(100)	3(50)	0	–	7(58)
Tetracycline	3(75)	4(67)	1(50)	–	8(67)
Tobramycin	2 (50)	3(50)	0	6(46)	11(44)

The present study found that the most frequently isolated pathogens from burn wounds were *P. aeruginosa* (37.5%), followed by *K. pneumoniae* (25%), *E. coli* (22.5%), *E. cloacae* (10%) and *A. baumannii* (5%). This result is in agreement with the findings of other studies conducted over the world, especially in developing countries. Most studies showed that the most common bacteria isolated were *P. aeruginosa* and *K. pneumoniae* [5, 17- 19]. A previous study conducted in Gaza strip burn units found that *P. aeruginosa* accounted for 50% of isolates, and it was the highest isolated bacteria from burn patients, followed by *E. cloacae* (27.8%), coagulase negative staphylococci (CoNS) (9.3%) and *E. coli* (5.6%) [20]. The high occurrence of *P. aeruginosa* and *K. pneumoniae* infections among burn patients could be due to the fact that

these organisms are frequently found in hospital environments, and burn wounds are an ideal body site for their survival. These are mostly MDR or even XDR to the most routinely used antibiotics in burn units.

ESBL are capable of hydrolyzing broad spectrum cephalosporins and monobactams. In the present study, 37.5% of gram-negative bacteria were ESBL-producers. No previous study has investigated the prevalence of extended spectrum beta-lactamases resistance in burn infection in Gaza strip, Palestine. The incidence of ESBL in gram-negative isolates was 37.5% (15 out of 40 isolates) which is similar to many studies, particularly from India, which showed a high prevalence of ESBL-producers that reached 39.8% and 35.16% in burn patients, respectively [3, 21] and lower than reported from

in Iraq (61.5%) among gram-negative bacteria [22]. In Iran, 21% of *A. baumannii* isolates from burn patients were found to be ESBL-producers [23]. A recent study from Egypt, showed that ESBL were detected in 58.5% of Gram-negative bacteria isolates from patients with burn infection [24]. A second study from Egypt, reported that 57.1 % *P. aeruginosa* (12 /21) isolates from burn wounds were positive for ESBLs production [25]. The high prevalence of the ESBL-producing isolates may be indicative of wide spread of bacteria species acquiring resistance to many useful antimicrobial drugs.

The ESBL-producing isolates exhibit co-resistance to many other classes of commonly used antibiotics resulting in limitation of their therapeutic options. The high level of resistance (>50 %) seen against most tested antibiotics could be due to overuse or misuse of these antimicrobials agents in our country, easy availability of these antibiotics, purchase without doctor prescription and lack of any antibiotic policy in Palestine. A similar study that was previously done in burn units of the main two hospitals in Gaza strip (Al Shifa and Nasser burn units), has reported that bacteria isolates from clinical and environmental samples were also resistant to most of tested antimicrobials [8]. The major risk factors for acquiring infection with ESBL-producing organisms in burn patients are long-term antibiotic exposure and prolonged hospital stay.

The prolonged hospital stays, hospitalization cost, and the mortality rate due to infections caused by ESBL-producing gram-negative isolates is significantly higher than that caused by ESBL non-producers gram-negative isolates [26-28]. Prevalence of ESBL-producers varies in different countries, and has been reported to be 21.4%, 28%, 58.7%, and 61.5% in gram-negative bacteria from the burn units of Bangladesh, India, Pakistan and Iraq, respectively [22, 29-31].

The significant findings of this study is that MDR gram-negative bacilli were still susceptible

to imipenem (73%) and Meropenem (80%). Therefore, imipenem and meropenem remain effective treatment options against gram-negative producing ESBL. This result is in agreement with the findings of a previous study that conducted in 2013 in Palestinian burn units and which found imipenem is the most effective antimicrobials agent [8]. Also, the same findings were reported by a recent study conducted in Indian burn unit [32]. Carbapenems drugs are considered as the last option against ESBL-producing bacteria, and therefore, they should not be extensively used in our burn units or other hospital wards to minimize the resistance rate against these drugs. This study shows that *A. baumannii* and *E. cloacae* isolates were 100% and 50% resistant to carbapenems, respectively. This result is alarming and will complicate treatment of burn patients if they were infected with pan-drug resistant bacteria [33].

The most important limitation of this study is that we have only investigated the facultative anaerobic bacterial pathogens, and were not able to investigate the occurrence of anaerobic and/or fastidiously growing bacterial pathogens as well as mold and yeast which could be involved in burn cases. Moreover, it would be important to investigate the occurrence of potential ESBL genes in bacterial isolates from hospitalized patients by molecular techniques.

Conclusion

P. aeruginosa was the most common bacteria isolated from burn wound patients. The frequency of ESBL-producers among gram-negative bacteria isolates was high, and most of these isolates except *A. baumannii* were susceptible to mipenem and meropenem. Continuous monitoring of antimicrobial susceptibility of isolates from burn patients is the best preventive and therapeutic strategies.

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