

PREVALENCE OF BETA LACTAMASE PRODUCING SPECIES OF PSEUDOMONAS AND ACINETOBACTER IN PEDIATRIC BURN PATIENTS

PREVALENCE DE PSEUDOMONAS ET ACINETOBACTER SECRETEURS DE BLSE ET METALLO-BETA LACTAMASES CHEZ LES BRULES PEDIATRIQUES

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SUMMARY. Burn wound infection is a major cause of morbidity and mortality in burn victims. *Pseudomonas* and *Acinetobacter* species are among the most common organisms complicating burn wounds. Presence of extended spectrum β -lactamase (ESBL) and metallo- β -lactamase (MBL) genes plays an important role in spreading β -lactam resistant strains of these organisms and is a serious condition in the treatment of the affected patients. As a result, we aimed to determine the prevalence of SHV, TEM, PER and VIM β -lactamases in *Pseudomonas* and *Acinetobacter* species isolates from burn wound swabs of children with burn injury. In this descriptive observational study, 107 *Pseudomonas* and *Acinetobacter* isolates collected from burn patients were subjected to PCR assay. Using PCR method and DNA sequencing, the existence of SHV-, TEM-, PER- and VIM-type β -lactamase encoding genes were determined. Out of the 107 *Pseudomonas* and *Acinetobacter* isolates, 66 (77.6%) were ESBL positive, 26.2% were positive for SHV gene, 37.4% were positive for TEM gene, 14% were positive for PER gene and 15.9% of them harbored VIM gene. More than half of the *Pseudomonas* and *Acinetobacter* strains in our pediatric burn unit harbor β -lactamase encoding genes that make them resistant to a wide range of β -lactam antibiotics. Consequently, it is suggested to choose an appropriate antibiotic regimen based on the antibiogram pattern of the strains.

Keywords: Acinetobacter species, burn wound, extended-spectrum β -lactamases, metallo- β -lactamase, Pseudomonas species

RÉSUMÉ. Les infections cutanées sont une cause majeure de morbidité et de mortalité chez les brûlés. *Pseudomonas* et *Acinetobacter* sont parmi les micro-organismes les plus communs chez les brûlés. La présence des gènes codant les β -lactamases à spectre étendu (BLSE) et métallo- β -lactamases (MBL) joue un rôle important dans la dissémination des souches résistantes et obère le traitement des patients infectés. Nous avons étudié la prévalence des gènes encodant pour des enzymes des groupes SHV, TEM, PER et VIM dans des isolats de *Pseudomonas* et *Acinetobacter* chez les brûlés pédiatriques, grâce à des techniques de PCR. Dans cette étude observationnelle descriptive, 107 isolats de *Pseudomonas* et *Acinetobacter*, obtenus chez des patients brûlés ont été étudiés. Plus des 3/4 des souches de *Pseudomonas* et *Acinetobacter* expriment une BLSE (26.2% SHV; 37.4% TEM; 14% PER; 15.9% VIM), ce qui les rend résistants à de nombreuses β -lactamines. Il est donc suggéré de choisir un traitement antibiotique approprié, basé sur l'antibiogramme des souches infectantes.

Mots-clés: Acinetobacter, Pseudomonas, brûlure, bêta-lactamases à spectre étendu, métallo- β -lactamase

Introduction

Despite improvements in the medical and surgical care of burn patients, no significant improvement has been doc-

umented on the mortality of burn patients in developing countries. Infection is a major complication of burn injury. Disruption of the normal skin barrier and compromised immune responses increase the vulnerability of burn patients to seri-

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ous infections. Furthermore, burn wounds contain a large amount of necrotic tissue and protein-rich exudates which provide a rich growth medium for colonized organisms.^{1,2} Burn wound infection is responsible for 50 to 75% of in-hospital deaths. Moreover, infection of burn wounds leads to graft failure, increased tissue necrosis, and scarring.³⁻⁵

Serious infections caused by *Pseudomonas aeruginosa* and *Acinetobacter spp.* are common complications in burn pediatric patients leading to substantial morbidity and mortality. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are two important nosocomial pathogens that are highly amenable to multidrug resistance. Moreover, Gram-negative bacteria including *Pseudomonas* and *Acinetobacter* species (*spp.*) are usually the predominant etiologies of bloodstream and wound infections in the pediatric population.^{6,7}

Pseudomonas aeruginosa is an opportunistic Gram-negative organism. Several mechanisms including beta-lactamase production, up regulation of efflux systems, and decreased outer membrane permeability result in beta-lactam resistance in *P. aeruginosa* and render the organism very difficult to treat or eradicate, consequently predisposing the burn wound and medical equipment and devices in burn units to *Pseudomonas* contamination and infection.^{8,9}

Recently, *Acinetobacter spp.* has emerged as a main cause of nosocomial infections associated with considerable morbidity and mortality.^{10,11} *Acinetobacter spp.* exhibit multidrug resistance through production of β -lactamases, alterations in outer membrane proteins (OMPs) and penicillin-binding proteins (PBPs), and increased activity of efflux pumps.^{11,12-15} It is believed that *Acinetobacter spp.* are resistant to almost all currently available antimicrobial agents, including the aminoglycosides, the quinolones, and broad-spectrum β -lactams.¹⁶ The broad spectrum of antibiotic resistance in these organisms in association with their unique survival capabilities represents a major challenge in the treatment of these types of infections in hospitals, especially burn units. Most strains of *Acinetobacter* are resistant to cephalosporins and resistance to carbapenems is increasingly common.^{17,18}

Spread of beta-lactamase producing mediated antibiotic resistance in *P. aeruginosa* and *Acinetobacter spp.* is a major concern in pediatric burn units. As a result, in the present investigation, we aimed to assess the presence of beta lactamase producing genes in *Pseudomonas* and *Acinetobacter* strains isolated from the burn wounds of pediatric burn patients.

Patients and methods

This cross-sectional study was conducted at the Pediatric ward of Motahari Burn center in Tehran, Iran from September 2012 to June 2013. Hospitalized patients aged 1 to 18 years old with burn injury were enrolled if their

burn wound culture was positive either for *Pseudomonas* or *Acinetobacter* strains.

The study was reviewed and approved by the Institutional Review Board at Iran University of Medical Sciences and a written informed consent was obtained from parents or guardians of all participants.

On admission, patients with burns greater than 20% of the Total Body Surface Area (TBSA) were resuscitated with intravenous fluid (20 mL/kg per hour) and a Foley catheter was placed and urine output was monitored hourly, the goal being 1.0 mL/kg per hour. Once the extent of the burn was estimated according to Lund-Browder chart, resuscitation continued using the Parkland formula (approximately 6 mL/kg per percent TBSA of burn). The burn depth was estimated clinically by an experienced trauma surgeon. A topical anti-microbial agent (preferentially silver sulphadiazine) was applied to burn wounds and cotton gauze dressings used for all patients were changed daily. Sampling from burn wounds was carried out using swabs from various parts of the wound before cleansing and application of the topical antimicrobial. Swabs were transferred into sterile tubes immediately following sampling and sent to a microbiology laboratory. Identification of isolates was performed according to the conventional bacteriologic methods or using commercial identification kits. In cases with *Pseudomonas spp.* or *Acinetobacter spp.* positive cultures, samples were subjected to PCR analysis to examine the β -lactamase producing genes including PER, SHV, TEM (extended-spectrum β -lactamase encoding genes) and VIM (a metallo- β -lactamase encoding gene, also known as carbapenemase). Patients received antibiotic therapy only if there was a clinical suspicion of infection (i.e. change in appearance or color of wound or positive blood or wound cultures) at the discretion of the treating surgeon.

Statistical analysis

All analyses were conducted by Statistical Package for Social Sciences (SPSS) software, version 19 (SPSS Inc., Chicago, IL, USA). Continuous data are presented as mean \pm SD. Unpaired t-test was used for comparison of age of patients between *Pseudomonas* and *Acinetobacter* positive cultures. Categorical data were given as frequencies and percentages. A *P* value of <0.05 was considered statistically significant.

Results

There were 107 patients including 84 boys (78.5%) and 23 (21.5%) girls. The mean age was 3.8 ± 2.5 years. The burn size in 43 patients (40.2%) was less than 30% TBSA, in 29 patients (27.1%) between 31-40% of TBSA, in 17 (15.9%) patients it was between 41-50% and in 18 patients (16.8%) the burn size was more than 50% TBSA.

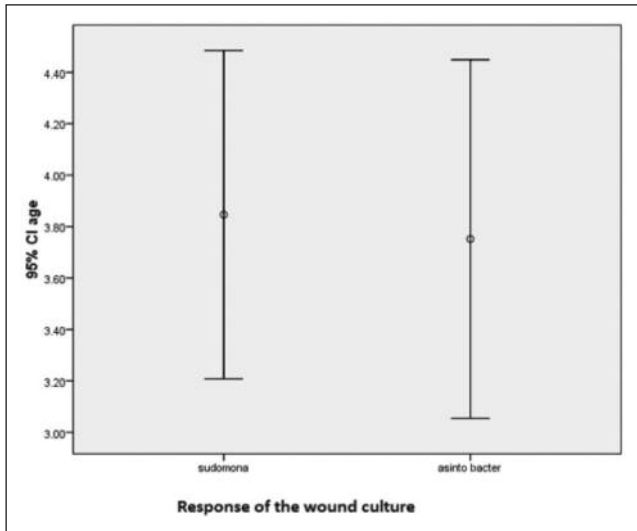


Fig. 1 - No significant difference was seen in the result of burn wound swab cultures and age of the participants ($p=0.62$).

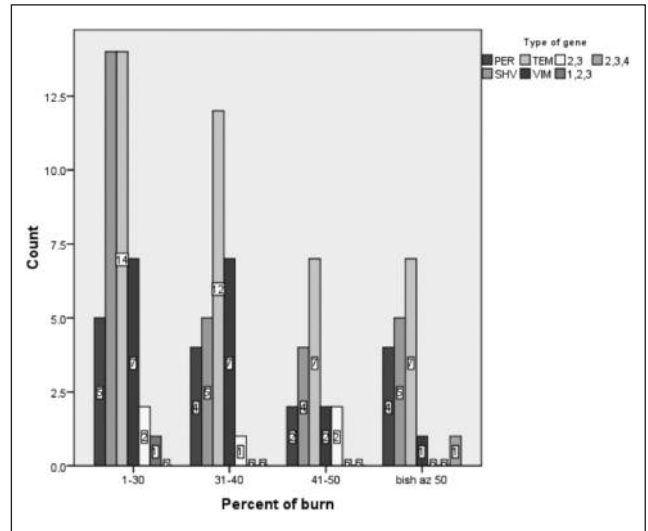


Fig. 3 - The distribution of β -lactamase producing genes in the isolated strains of *Pseudomonas* and *Acinetobacter* is shown based on the extent of burn as % of Total Body Surface Area (TBSA).

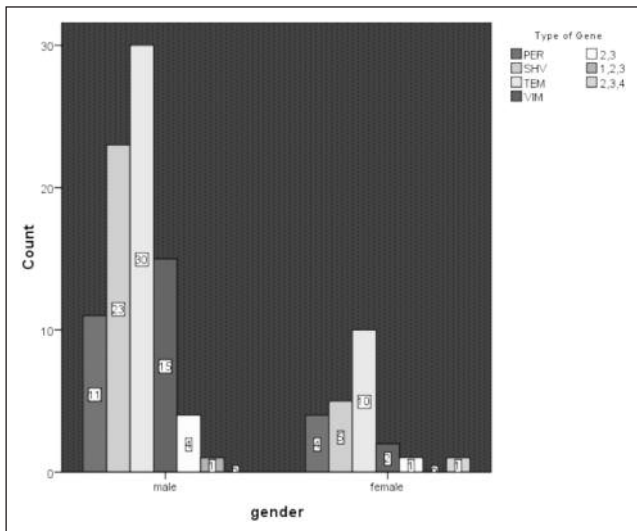


Fig. 2 - The distribution of β -lactamase producing genes in the isolated strains of *Pseudomonas* and *Acinetobacter* is shown based on the gender of participants.

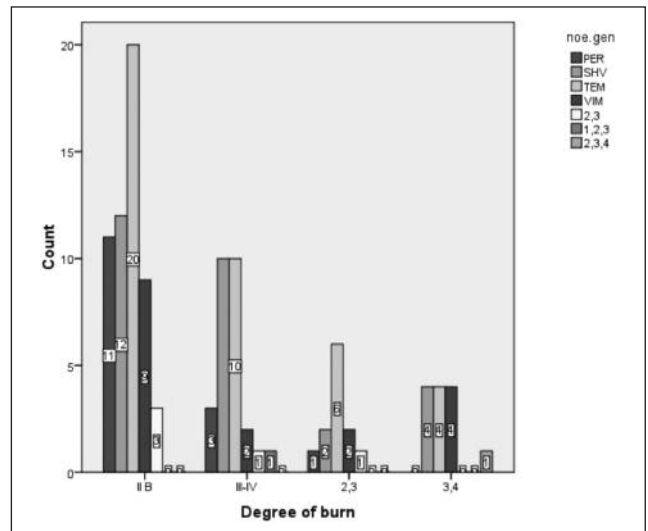


Fig. 4 - The distribution of β -lactamase producing genes in the isolated strains of *Pseudomonas* and *Acinetobacter* is shown based on the degree of burn.

In 55 patients (51.5%), depth and severity of burn were at stage IIb, in 27 (25.2%) patients at stages III to IV, in 12 (11.2%) at a combination of II and III, and in the remaining 13 (12.1) patients at a combination of stage III and IV. The isolated strains included *Pseudomonas* spp. in 76 (71%) patients and *Acinetobacter* spp. in 31 (29%) patients. No significant difference was seen between the isolated strains from burn wounds with respect to the patients' age ($p=0.622$, Fig. 1).

Table I shows the frequency of each of the beta-lactamase producing genes (PER, SHV, TEM and VIM) in the isolated strains of *Pseudomonas* and *Acinetobacter*.

The distribution of β -lactamase producing genes in the isolated strains of *Pseudomonas* and *Acinetobacter* based on gender is shown in Fig. 2. Figs. 3 and 4 also demonstrate the distribution of β -lactamase producing genes in the isolated strains of *Pseudomonas* and *Acinetobacter* based on the size and degree of the burn, respectively.

Table I - Distribution of β -lactamase producing genes

Type of gene	Frequency	Percent
PER	15	14.0
SHV	28	26.2
TEM	40	37.4
VIM	17	15.9
SHV, TEM	5	4.7
PER, SHV, TEM	1	.9
SHV, TEM, VIM	1	.9

Discussion

Burn wounds represent the most extensive surface wounds seen in surgery. Though the treatment of burn wounds has progressed over time, their management still provides considerable challenges. To date, there is still a remarkable mortality rate in burn victims in developing countries.

With improved resuscitation of the burn patients, the major etiologies of mortality in this population include inhalation injury, adult respiratory distress syndrome (ARDS) and sepsis ranging from systemic inflammatory response syndrome (SIRS) to frank septic shock.¹⁹ Wound infection, especially by nosocomial and opportunistic infections, are the main cause of sepsis in burned children. As a result, a growing cause of concern in burn victims is colonization of burn wounds by multidrug resistant organisms.

In a survey based on the data from 176 burn care centers in North America, *Pseudomonas* species was found to be the most important cause of life threatening infections in patients with thermal injuries.²⁰ Similarly, McManus et al. in a 25-year review of *Pseudomonas* bacteremia in burn patients demonstrated that bacteremia due to *P. aeruginosa* caused an overall burn mortality of 77% which was 28% higher than predicted.²¹ Revathi et al. also reported their experience in 600 infected burn patients.²² They showed that *Pseudomonas* spp. and *Staphylococcus aureus* caused the most common and severe infections followed by other Gram-negative organisms. In a study by Gastmeier on pediatric burn patients with hospital acquired infections, the most frequently isolated pathogens were *Staphylococcus aureus* (20.7%) and *Pseudomonas* spp. (14.6%), followed by *Enterococcus* spp. and *Escherichia coli* (both 12.2%).²³

In a study by Ganesamoni et al. conducted on burn patients, *Pseudomonas aeruginosa* was the most common organism identified followed by *Acinetobacter* species. They also showed that these strains were resistant to the majority of the commonly used antibiotics.²⁴ According to their results, amikacin and ceftriaxone had only less than 20% in vivo activity and only around one third of isolates of *P. aeruginosa* and a similar percentage of *Acinetobacter* spp. were sensitive to meropenem. This study empha-

sized the increasing prevalence of *Pseudomonas* colonization in burn wounds compared to the previously reported series. Furthermore, prevalence of *Acinetobacter* has also been reported to have increased in western studies.^{22, 25-30} However, *Acinetobacter* infection has not been demonstrated to affect mortality independently.³¹

In this study, 15.9% of *P. aeruginosa* and *Acinetobacter* isolates were found to produce carbapenemase i.e. VIM (a metallo-beta-lactamase, MBL). Also, 63% of *P. aeruginosa* and *Acinetobacter* isolates harbored SHV and TEM-typed ESBL while only 14% of isolates harbored PER, as an ESBL. Approximately 5% of isolates had both "class A" ESBLs, (i.e. SHV and TEM).

The present study revealed a high prevalence of β -lactamase producing genes in burn wounds colonized by *Pseudomonas* or *Acinetobacter* species. It seems that extended spectrum β -lactamase producing genes, including TEM-type ESBL encoding genes followed by SHV genes, are responsible for the majority of emerging resistance to β -lactams in the pediatric burn unit of our hospital. The roles of PER-type ESBL and VIM (a MBL) are less conspicuous.

Sepehriseresht et al. evaluated *vim1*, *vim2*, *ipm1* and *ipm2* metallo- β -lactamase encoding genes in imipenem-resistant and intermediate *P. aeruginosa* strains isolated from burn wounds.³² Among the 483 isolates evaluated in their study, 272 (56%) were imipenem-resistant with a growth zone of < 13mm, 63 (13%) isolates had intermediate pattern in their imipenem antibiogram with a growth zone between 13-15mm, and 148 (31%) isolates were susceptible to imipenem with a growth zone of > 15 mm. PCR assay for *vim1*, *vim2*, *ipm1* and *ipm2* genes were done for the isolates with resistant and intermediate patterns to imipenem. Results of DNA sequencing by PCR showed that 54 (16.1%), 7 (2.1%), 22 (6.6%), and 11 (3.3%) of these isolates had *vim1*, *vim2*, *ipm1* and *ipm2* genes, respectively. The incidences of *vim1*, *vim2*, *ipm1* and *ipm2* genes among the imipenem-resistant isolates were 15.4%, 1.8%, 6.6%, and 3.3%, respectively; while intermediate isolates, had frequencies of 4.8%, 3.1%, 6.3%, and 3.1%, respectively. However, in another study conducted in our hospital, Sadari et al. found that 38.28% of 128 clinical isolates of *P. aeruginosa* were resistant to imipenem.³³ Shahcheraghi et al. assessed 350 clinical isolates of *P. aeruginosa* from two general hospitals in Tehran, Iran and demonstrated that only 5% of the isolates were imipenem-resistant.³⁴

According to our study, and also in comparison with the study by Sepehriseresht et al., it seems that prevalence of VIM-typed MBL genes in the isolates of *P. Aeruginosa* has been in a steady state from 2008 to 2012.³² However, data regarding the time-dependent changes in the prevalence of ESBL genes in *P. aeruginosa* and *Acinetobacter* spp. and also of MBL encoding genes in *Acinetobacter* spp. are less available. However, Rasmussen and Bush be-

lieved that as carbapenems use goes up, an increase in MBL-producing organisms would be inevitable.³⁵ Consistently, a study by Lee et al. revealed that after nine years use of carbapenems in Korea, the imipenem-resistance rate of *P. aeruginosa* rapidly raised from 6% in 1996 to 19% in 2001.³⁶ Shibata et al. demonstrated that 35%, 0.5%, and 64.5% of 180 isolates of *P. aeruginosa* had *vim2*, *ipm1*, and *ipm2*-typed MBL genes, respectively.³⁷ Laupland et al. also found a 92% prevalence of *vim2* positive isolates of *P. aeruginosa* isolates in the Calgary Health Region in Canada between May 2002 and April 2004.³⁸ Lee et al. showed prevalences of 1.7% and 0% of *vim2* and *imp1* MBLs among 415 clinical isolates of *P. aeruginosa* in Korea, respectively.³⁹ These findings reinforce the fact that geographical conditions, hygienic states and chromosomal and plasmid differences of the isolated organism lead to different prevalence of MBLs all over the world.

Azimi et al. evaluated the prevalence of Carbapenemase (KPC) among isolated *Pseudomonas aeruginosa* and *Acinetobacter* spp. at a tertiary burn hospital in Tehran, Iran.⁴⁰ They isolated 64 strains from 20 patients with TBSA burns of 15-40% and an age range of 0-6 years. The isolated strains included 28 Gram-positive bacteria, 16 *Enterobacteriaceae*, 12 *Pseudomonas* and 7 *Acinetobacter baumannii*. Among 36 Gram-negative isolates, 10 isolates were resistant to all tested antibiotics except Colistin and 10 isolates were susceptible to at least one aminoglycoside. Moreover, 15 out of 36 isolates including 6 *Pseudomonas aeruginosa*, 6 *Acinetobacter baumannii* and 3 *Klebsiella pneumoniae* strains were found to be imipenem-resistant. Finally, 13 out of 15 imipenem-resistant strains were confirmed as KPC-producing organisms. In detail, four of six imipenem-resistant *Pseudomonas* strains, all six imipenem-resistant *Acinetobacter baumannii* strains and all three imipenem-resistant *Klebsiella pneumoniae* strains were producing KPC. Only six out of 36 isolated strains were ESBL producers; while four strains were both KPC and ESBL positive.

Similarly, Shakibaei et al. evaluated the presence of SHV, TEM and PER type extended-spectrum β -lactamase genes among clinical strains of *Pseudomonas aeruginosa* isolated from burnt patients at Shafa hospital, Kerman, Iran.⁴¹ Amongst 120 isolates of *P. aeruginosa* collected from 245 patients in the burn unit, 41 isolates (34%) were found to be ESBL producers; while none of the isolates produced MBL. The PCR assay of these ESBL producing isolates of *P. aeruginosa* demonstrated prevalences of 6.6%, 4.1% and 2.5% for SHV, PER and TEM type ESBL genes, respectively. Furthermore, Shojapour et al assessed the prevalence of TEM-1 type ESBL gene in *Pseudomonas aeruginosa* strains isolated from burn wounds in Shahrekord, Iran.⁴² They found 66 (37.7%) ESBL positive isolates among all 175 studied *Pseudomonas aeruginosa* isolates; and 15.15% of them were positive for TEM-1 gene. Sim-

ilarly, Ranjbar et al also evaluated the characteristics of *Pseudomonas aeruginosa* strains isolated from burned patients hospitalized in our hospital.⁴³ They studied a total of 70 *P. aeruginosa* isolates obtained from different clinical origins. They showed that 100% of *P. aeruginosa* strains were resistant to cefoxitin, 97% to cefotetan, 93% to ticarcillin, 89% to ticarcillin/clav, 76% to gentamicin and imipenem, 63% to piperacillin, 49% to tetracycline, and 20% to meropenem. However, they did not assess the prevalence of beta-lactamase producing genes in their *Pseudomonas aeruginosa* strains.

Although the antibiotic resistance of *Acinetobacter* spp. has been assessed in isolates recovered from general hospitals, especially ICUs, in Iran,^{44, 45} there is limited information regarding the antibiotic resistance of *Acinetobacter* spp. isolated from burn patients in Iran. It has been reported that most of the VIM-positive isolates (76%) of *Acinetobacter* spp. were multidrug-resistant. While it has recently been found that increased expression of chromosomal genes for efflux systems plays a major role in multidrug resistance, the role of beta-lactamase producing genes in the emerging resistance of *Acinetobacter* spp should also be seriously considered, as shown previously.^{46, 47}

Schlager et al. showed a significantly higher rate of burn wound infection in patients with >20% TBSA as compared to the patients with <20% TBSA burn injury.⁴⁸ Their results were in accordance with those reported by Weber et al. and Gastmeier et al., who also found a close association between the burn size and burn wound infection. The effects of TBSA on the development of burn wound infections could be explained by the fact that larger burn wounds increase the likelihood of wound contamination.^{23, 49-50}

Our study has several limitations which need to be discussed. First, we used a relatively small sample size. Second, we did not assess the antibiogram pattern of the strains to decide the in vitro consequences of the presence of extended-spectrum β -lactamase-producing and carbapenemase-producing genes. Furthermore, there is a need for assessment of the clinical outcome of the patients affected by these strains that would reflect an in vivo reflection of the effects of these genes in morbidity and mortality of burn victims.

Conclusion

Our findings show that more than half of the *Pseudomonas* and *Acinetobacter* strains in our pediatric burn unit harbor β -lactamase encoding genes which make them resistant to a wide range of antibiotics. This might independently affect the outcome of our patients. Increased morbidity, mortality and also high treatment costs are consequences of considerable burn wound infections which should be addressed in future studies with larger sample sizes.

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