

Plasmid Mediated Antibiotic Resistance in Isolated Bacteria From Burned Patients

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Background: Nowadays, the treatment of burned patients is difficult because of the high frequency of infection with antibiotic resistance bacteria.

Objectives: This study was conducted to evaluate the level of antibiotic resistance in Gram-negative bacteria and its relation with the existence of plasmid.

Materials and Methods: The samples were collected from two hundred twenty hospitalized burned patients in Isfahan burn hospital during a three-month period (March 2012 to June 2012). The samples were isolated and the Gram-negative bacteria were identified using phenotypic method and API 20E System. Antibiotic susceptibility and plasmid profile were determined by standard Agar disc diffusion and plasmid spin column extraction methods.

Results: Totally 117 Gram-negative bacteria were isolated, the most common were *Pseudomonas aeruginosa* (37.6%), *P. fluorescens* (25.6%), *Acinetobacter baumannii* (20/5%) and *Klebsiella pneumoniae* (7.6%), respectively. The isolates showed high frequency of antibiotic resistance against ceftazidime and co-amoxiclavate (100%) and low frequency of antibiotic resistance against amikacin with (70%). The results indicated that 60% of the isolates harboured plasmid. On the other hand, the patients infected with *A. baumannii* and *P. aeruginosa* were cured (with 60% frequency) whereas, those infected with *P. fluorescens* were not cured. Hence, probably antibiotic resistance markers of *A. baumannii* and *P. aeruginosa* are plasmid mediated; however, *P. fluorescens* is chromosomally mediated.

Conclusions: Based on our findings, *P. aeruginosa* is a major causative agent of wound infections and amikacin could be considered as a more effective antibiotic for treatment of the burned patients.

Keywords: Plasmid; Infection; Patients

1. Background

Bacterial infection in burned patients is a major problem for health care settings because of the increasing level of mortality and hospitals cost (1). Gram-negative bacteria including *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are the most frequent pathogens isolated from burned infected wounds (2-4). In 2008, the antibacterial resistance pattern of aerobic bacteria isolated from burn patients showed that ceftazidime and cefotaxime resistant *Pseudomonas* spp. is a common etiological agent of infections (5). In 2010, *A. baumannii* isolated from burned patients contained carbapenemase genes in their genome (6). Generally, many mechanisms are responsible for antibiotic resistance in Gram-negative bacteria. Relatively, mobile gene elements such as transposon, integrons and plasmids, play important roles in antibiotic resistance character (7-9).

2. Objectives

The present study was conducted to evaluate the antibiotic resistance patterns and its relation with the exis-

tence of plasmid in Gram-negative bacteria isolated from burned patients in Isfahan burned hospital, Iran.

3. Materials and Methods

3.1. Bacterial Strains

A total of 139 non-duplicate bacterial isolates were collected from two hundred twenty patients from March to June 2012. Samples were taken at the beginning of the first bondage during the hospital stay (10). The phenotypic features of isolates were identified by standard biochemical tests such as Gram staining, sugar fermentation and API 20E system (Biomerix Co, France).

3.2. Determination of Antibiotic Susceptibility of the Isolates by Disc Diffusion Method

Determination of antibiotic susceptibility patterns were carried out by the disc diffusion method according to CLSI guidelines (11). To perform the test, antago-

nistic bacteria viz., *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 cultured in nutrient broth and their turbidity were adjusted to 0.5 McFarland standard. The suspensions were streaked onto Mueller-Hinton agar plates and the following antibiotic disks (Hi-Media, India) amikacin (30 µg), cefotaxim (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (5 µg), co-trimoxazole (25 µg), piperacillin/tazobactam (25 µg), ceftriaxone (30 µg), amoxicillin-clavulanic acid (30 µg), ceftazidime (30 µg) were placed on the seeded media. The plates were incubated at 37°C for 18-24 hours and then the inhibition zone around each antibiotic disk was measured and recorded.

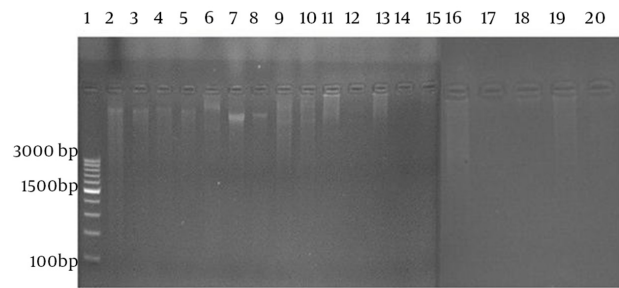
3.3. Plasmid Isolation

Plasmid was isolated using standard method (Vivantis nucleic acid extraction kit, GF-1 model, version 2.2, Germany) recommended by Birnboim et al. (12) and the purity of plasmid was evaluated at 260 nm by spectrophotometer (Eppendorf Biophotometer 6131, Germany). The plasmid DNA was observed by gel electrophoresis. To prepare the gel, Agarose gel powder (2%) was dissolved in TAE buffer (40 mM Tris-HCl, 50 mM Sodium acetate, 1 mM EDTA; pH 8). The extracted DNA was added to the gel and the Gel was run for two and half hours at 50 V, stained for 30 min with ethidium bromide (0.5 µg/mL). The plasmids were visualized under UV light in Alpha imager gel documentation system (Syngene, UK) (Table 1).

3.4. Plasmid Curing

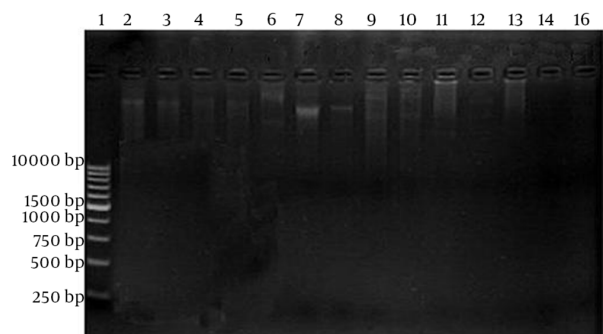
The isolates were showing resistance character (100%) subjected to plasmid curing. Ethidium bromide was serially diluted in Muller Hinton broth. The curing agent was tested at 500, 250, 125, 62.5, 31.2 µg/mL concentrations. Overnight growth culture of five isolates subjected to plasmid curing and each of them inoculated into the tube containing 1 mL Muller Hinton broth and the incubated at 44°C for 24 hours. The minimal inhibitory concentration (MIC) of Ethidium bromide was determined then the highest concentration permitting growth (SIC) was considered as plasmid curing. The overnight culture was inoculated on Mac Conkey agar with sic of curing agents and incubated at 37°C for two days. The single colonies were picked up by sterile toothpicks and inoculated on nutrient agar plate (30 colonies/plate). The plates was incubated at 37°C for 24 hours and used as master plates. These colonies were inoculated on nutrient agar with ciprofloxacin (5 µg) and on nutrient agar with meropenem (5 µg) and on nutrient agar with ceftriaxone (30 µg) and then incubated at 37°C for 48 hours. The colonies that did not grow on selective medium, were known as cured colonies. At the same time, a non-cured culture on selective medium as a control for curing of each marker was performed. The loss of plasmid DNA was confirmed by gel electrophoresis.

Figure 1. Antibiotic Resistance of the Isolates



AN, Amikacin; AMC, Ampicillin-clavulanic acid; CIP, Ciprofloxacin; CTX, Cefotaxime; CTR, Ceftriaxone, IMP, Imipenem; MEM, Meropenem; COT, Co-trimoxazole; T2P, piperacillin/Tazobactam; CAZ, Ceftazidime.

Figure 2. Plasmid Profile of Isolates



Line 1, Smart marker (100-3000bp); Line 2 to 5, *A. baumannii*; Line 6 and 7, *P. aeruginosa*; Line 8 and 9, *K. pneumoniae* harbored two plasmid bands; Line 18, 19 and 20: *P. fluorescens*.

4. Results

4.1. Identification and Antibiotic Susceptibility of the Isolates

Totally 117 Gram-negative bacteria were isolated and the most common were *P. aeruginosa* (37.6%), *P. fluorescens* (25.6%), *A. baumannii* (20.5%) and *K. pneumoniae* (7.6%), respectively. The isolates were resistant against ceftazidime and co-amoxiclavate with high frequency (100%) and against amikacin with low frequency (70%) (Figure 1).

4.2. Plasmid Isolation

Nineteen resistant strains against all antibiotics were subjected for plasmid isolation (Figure 2).

4.3. Plasmid Curing

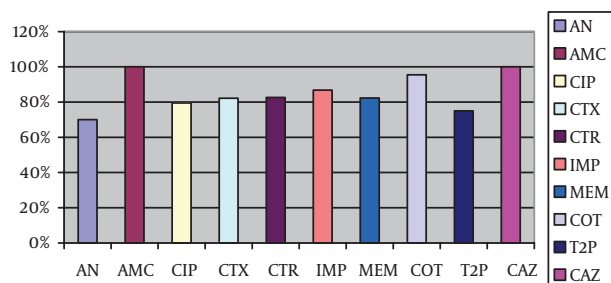
Five isolates of bacteria that showing resistance to all antibiotics were selected for plasmid curing. The results showed that *A. baumannii* and *P. aeruginosa* were cured (with 60% frequency) whereas; *P. fluorescens* was not cured (60%).

Table 1. Susceptibility of the Bacterial Strains to Ethidium Bromide

Bacteria of Isolates	SIC, µg/mL	MIC, µg/mL
<i>A. baumannii</i>	62.5	125
<i>A. baumannii</i>	250	500
<i>P. fluorescens</i>	1000	2000
<i>P. aeruginosa</i>	1000	2000
<i>A. baumannii</i>	125	250

Table 2. Sensivity of Cured Strains to the Marked Antibiotics With Ethidium Bromide

Antibiotics	Susceptibility of Cured Strains, %
Ceftriaxone	66
Meropenem	33
Ciprofloxacin	83

**Figure 3.** Verification of the Existence of Plasmid in Cured Strains of *P. aeruginosa* and *A. baumannii*

4.4. Determination of Plasmid Mediated Antibiotic Resistance

Our findings showed that 83% to 66% of the cured strains were sensitive to ciprofloxacin and ceftriaxone. However, 33% of the isolates were sensitive to meropenem (Table 2 and Figure 3).

5. Discussion

Recently, high frequency antibiotic resistance bacteria, especially in the hospitals, culminated a serious problem for treatment of infectious diseases. In this regards, several resistant strains of *S. aureus*, *K. pneumonia* and *P. aeruginosa* strains against ceftazidime and imipenem and carbapenem respectively were isolated from hospitals (3, 13). Parallel to these reports the present study isolated antibiotic resistant Gram-negative bacteria from burned patient in hospitals. Some of the isolates showed resistance to ceftazidime and Amoxicillin-clavulanic acid. In a similar study *P.seudomonas aeruginosa* isolated from burned patient in Sanandaj, Iran were 100% resistant to ceftazidime (14). On the other hand, parallel to our finding several reports showed that amikacin has

effective antibiotic features against burned infectious agents (15-17).

In the present study, the most common Gram-negative bacteria isolated from the patients were *P. aeruginosa*, *Acinetobacter* spp and *K. pneumoniae*. Out of all, *Acinetobacter* spp and *P. aeruginosa* isolates exhibited common antibiotic resistance pattern (3, 18, 19). In different investigation in Brasilia *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* were introduced as collective nosocomial infectious agents (20). Generally, antibiotic resistance markers located in the chromosomes, plasmids, integron and transposons (21). Of all, antibiotic resistance markers encoded in plasmid play an important role for transferring the resistance marker among different bacteria (22, 23). In the present study 60% of the strains harbored plasmid and they showed resistance property to ciprofloxacin and ceftriaxone.

In 2003, 95% of antibiotic resistant *P. aeruginosa* isolated from burn patients in Tehran hospitals harbored plasmid (24). In addition, plasmid curing confirmed the relationship between antibiotics resistance character and the existence of plasmid (25). In the present study cured *A. baumannii* and *P. aeruginosa* lost their resistance markers after losing their plasmids. Therefore, it can be interpreted that probably antibiotic resistance markers of these bacteria are plasmid mediated. Overall, our investigation showed the existence of the resistance markers in the plasmid of isolated bacteria from burned patients. Hence these markers might easily transfer among the bacteria in the hospitals.

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References

- Wenzel RP. The Lowbury Lecture. The economics of nosocomial infections. *J Hosp Infect.* 1995;**31**(2):79-87.
- Elwell LP, Inamine JM, Minshew BH. Common plasmid specifying tobramycin resistance found in two enteric bacteria isolated from burn patients. *Antimicrob Agents Chemother.* 1978;**13**(2):312-7.
- Song W, Lee KM, Kang HJ, Shin DH, Kim DK. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. *Burns.* 2001;**27**(2):136-9.
- Heritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2005;**49**(8):3198-202.
- Rajput A, Singh KP, Kumar V, Sexena R, Singh RK. Antibacterial resistance pattern of aerobic bacteria isolates from burn patients in tertiary care hospital. *Biomed Res.* 2008;**19**(1).
- Bogaerts P, Naas T, El Garch F, Cuzon G, Deplano A, Delaire T, et al. GES extended-spectrum beta-lactamases in *Acinetobacter baumannii* isolates in Belgium. *Antimicrob Agents Chemother.* 2010;**54**(11):4872-8.
- Ombui JN, Kimotho AM, Nduhiu JG. Antimicrobial resistance patterns and plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. *East Afr Med J.* 2000;**77**(9):463-7.
- Watanabe T, Fukasawa T. Episome-mediated transfer of drug resistance in Enterobacteriaceae. II. Elimination of resistance factors with acridine dyes. *J Bacteriol.* 1961;**81**:679-83.

9. Spengler G, Molnar A, Schelz Z, Amaral L, Sharples D, Molnar J. The mechanism of plasmid curing in bacteria. *Curr Drug Targets*. 2006;**7**(7):823-41.
10. Forbes BA, Sahm DF, Weissfeld AS. *Bailey and Scott's Diagnostic Microbiology*. Mosby: Elsevier; 2002.
11. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;**45**(4):493-6.
12. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*. 1979;**7**(6):1513-23.
13. Boyce JM, White RL, Causey WA, Lockwood WR. Burn units as a source of methicillin-resistant *Staphylococcus aureus* infections. *JAMA*. 1983;**249**(20):2803-7.
14. Kohanteb J, Dayaghi M, Motazedian M, Ghayumi MA. Comparison of biotyping and antibiotyping of *Pseudomonas aeruginosa* isolated from patients with burn wound infection and nosocomial pneumonia in Shiraz, Iran. *Pak J Biol Sci*. 2007;**10**(11):1817-22.
15. Revathi G, Puri J, Jain BK. Bacteriology of burns. *Burns*. 1998; **24**(4):347-9.
16. Estahbanati HK, Kashani PP, Ghanaatpisheh F. Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. *Burns*. 2002;**28**(4):340-8.
17. Signorini M, Grappolini S, Magliano E, Donati L. Updated evaluation of the activity of antibiotics in a burn centre. *Burns*. 1992;**18**(6):500-3.
18. Nasser S, Mabrouk A, Maher A. Colonization of burn wounds in Ain Shams University Burn Unit. *Burns*. 2003;**29**(3):229-33.
19. Towner KJ. *Acinetobacter*: an old friend, but a new enemy. *J Hosp Infect*. 2009;**73**(4):355-63.
20. Mendes C, Oplustil C, Sakagami E, Turner P, Kiffer C, Mystic Brazil Group. Antimicrobial susceptibility in intensive care units: MYSTIC Program Brazil 2002. *Braz J Infect Dis*. 2005;**9**(1):44-51.
21. Courvalin P. Transfer of antibiotic resistance genes between gram-positive and gram-negative bacteria. *Antimicrob Agents Chemother*. 1994;**38**(7):1447-51.
22. Brown TA. Plasmids. In: Talebatabaei Yazdi M, Sepehrizadeh Z editors. *Textbooks of Gene Cloning & DNA analysis*. 6th ed; 2010. pp. 33-9.
23. Amabile-Cuevas CF, Chicurel ME. Bacterial plasmids and gene flux. *Cell*. 1992;**70**(2):189-99.
24. Shahcheraghi F, Feizabadi MM, Yamin V, Abiri R, Abedian Z. Se-rovar determination, drug resistance patterns and plasmid profiles of *Pseudomonas aeruginosa* isolated from burn patients at two hospitals of Tehran (IRAN). *Burns*. 2003;**29**(6):547-51.
25. Baserisalehi M, Bahador N. A Study on Relationship of Plasmid with Antibiotic Resistance in Thermophilic *Campylobacter* spp. Isolates from Environmental Samples. *Biotechnol J*. 2008;**7**(4):813-7.