Investigated of *amp*C in Carbapenem Resistant Gram-Negative Bacteria Isolated from Burned Patients

*Leila Azimi [1, 2], Malihe Talebi [2], Parviz Owlia [3], Abdolaziz Rastegar Lari [1, 2]**

[1]*Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, IR Iran*

[2]*Deparment of Microbiology, Iran University of Medical Sciences, Tehran, IR Iran*

[3] *Molecular Microbiology Research Center, Shahed University, Tehran, IR Iran*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 10 Sep 2014
Revised: 11 Nov 2014
Accepted: 20 Nov 2014

Keywords:
Pseudomonas aeruginosa
Infection
Burn, Vaccine

ABSTRACT

***Background*:** Gram-Negative bacteria are the most cause of nosocomial infection especially in burned patients. Carbapenem resistant strains can limit seriously the choice of antibiotic therapy. *Amp*C can make resistance to carbapenem and detection of that can be useful for identification of carbapenem resistant. The aim of this study was identification of *amp*C in most prevalent cause of nosocomial infection.

***Methods*:** boronic acid combined with meropenem in combination disc method was used for phenotypic method and PCR was used for molecular identification of *amp*C.

Results: Fifty one strains showed positive results in phenotypic method but 119 strains were harbored *amp*C gene. Combination disc method with meropenem and boronic acid showed 34.4% sensitivity and 78.5% specificity according to the results of this study.

Conclusions: the results of this study were indicated the more prevalent of *amp*C in carbapenem resistant Gram-Negative bacteria. On the other hand use of boronic acid combined with meropenem showed low sensitivity for detection of *amp*C.

• ***Please cite this paper as:*** Azimi L, Talebi M, Owlia P, Rastegar Lari A. Investigated of *amp*C in carbapenem resistant Gram-Negative bacteria isolated from burned patients. *J Med Bacteriol.* 2014; **3**(3, 4): pp.20-25.

Corresponding Author: Abdolaziz Rastegar Lari, Professor of clinical microbiology, Iran University of Medical Sciences, Tehran, IR Iran. *Tel*: +98 21 82943183, *Email*: lari@iums.ac.ir, azizlari@gmail.com

.

Introduction

Pseudomonas aeruginosa, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are being reported increasingly as the causative nosocomial infections which occur in several countries (1- 5). Inappropriate antibiotic therapy and the overuse of broad-spectrum antibiotic have been selected antibiotic resistant bacteria (6). Antibiotic resistant can be association by different mechanism like production of different beta-lactamase enzymes and structurally modified antibiotic target, altered uptake of antibiotics (5, 7). One of the important mechanism of antibiotic resistance is the potential of extended-spectrum beta-lactamase (ESBL) and *amp*C β-lactamases (*amp*C) –producing (8- 10). *amp*C b-lactamases are Group I cephalosporinases that can involve in resistance to a wide variety of b-lactam antibiotics include narrow and broad spectrum cephalosporins, aztreonam and in over expression of *amp*C with porin loss and/or efflux pumps, we can observe resistant to carbapenems (11, 12). One of the *amp*C properties is poorly inhibition of this enzyme by bata-lactamases inhibitor like clavulanic acid (13). Antibiotic resistance in these bacteria can be a serious threat for antibiotic therapy interventions. Infections caused by ESBL and *amp*C-producing, Gram-Negative bacteria are often difficult for clinicians to treat and can increase morbidity and mortality especially in hospitalized burned patients. So, detection of *amp*C-producer microorganisms especially in burned patients that were infected with these antibiotic resistant microorganisms can be helpful for appropriate antibiotic therapy of them. The aim of this study was detection of *ampC* gene in carbapenem resistant *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* isolated from burned patients.

Material and method

Bacterial isolations

This cross sectional study was conducted during 2013. One hundred sixty one *P. aeruginosa* (n=65), *A. baumannii* (n= 63) and *K. pneumoniae* (n=33) were collected from burned wound infection of hospitalized patients from different wards in Motahari Hospital, a burn care teaching hospital in Tehran, Iran. The plate of bacteria sent to laboratory. These isolates were identified with conventional biochemical and microbiological tests. Like, oxidase, TSI, urea, SIM and citrate etc.

Antibiotic susceptibility testing

Confirmation of carbapenem resistant including imipenem (10µg), ertapenem (10µg) and meropenem (10µg) was done by disc diffusion agar method according to CLSI guide line (14). Standard antibiotic discs were prepared from MAST Company (Mast Diagnostics, UK).

Phenotypic detection of ampC

Two phenotypic tests were used for identification of *amp*C: The increasing inhibition zone at least 5mm around meropenem (10µg) plus 600 µg/disc boronic acid and meropenem (10µg) plus 750 µg/disc cloxacillin simultaneously in comparison to meropenem (10µg) alone. The stock of boronic acid was prepared 30mg/ml and the stock of clocacillin was prepared 37.5 mg/ml (15).

Genotypic detection of ampC

PCR was used as a molecular test for detection of *ampC* gene with primer showed in table 1. The polymerase chain reaction was performed in following condition:

The first denaturation at 94°C for 5 minutes and 30 cycles of 94°C for 60 seconds, annealing at 66°C, 45°C and 52°C for *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* respectively, for

60 seconds extension at 72°C for 60 seconds and at last the final extension at 72°C for 5 minutes. Isolates which showed specific band after PCR and electrophoresis have been sent for sequencing to Genfanavaran, Macrogen, and Seoul, Korea.

Result

Table 1. Sequence of primers

Primer *AMP*	Sequence (5`>>>3`)	bp	Ref.
CF/*A.baumannii*	ACTTACTTCAA CTCGCGACG	662	16
CR/*A.baumannii*	TAAACACCACA TATGTTCCG		
CF/*P. aeruginosa*	ATGCAGCCAAC GACAAAGG	1223	17
CR/*P. aeruginosa*	CGCCCTCGCGA GCGCGCTTC		
CF/*Kpeunomoniae.*	ATTCCGGGTAT GGCCGT	835	18
CR/*K.peunomoniae*	GGGTTTACCTC AACGGC		

Carbapenem resistant of 161 tested isolates were confirmed by disc diffusion agar. Fifty one strains showed at least 5mm around meropenem with boronic acid in contrast with meropenem alone. On the other hand, 119 strains (60 P. aeruginosa, 55 A. baumannii and 4 K. pneumoniae) showed specific band after gel electrophoresis. The results of sequencing were confirmed the positive results of PCR assay. Nine strains with positive phenotypic test showed negative result in molecular method and 78 isolates with negative phenotypic test and positive PCR were observed (Table 2). Combination disc method with meropenem and boronic acid showed 34.4% sensitivity and 78.5% specificity according to the results of this study. Bacterial strains and the percent homology between phenotypic and genotypic were showed in Tabel 3. Bacterial strains and the percent homology between phenotypic and genotypic methods.

Table 2. Results of phenotypic and genotypic *amp*c detection

Test	Positive phenotpic /negative genotypic	Negative phenotypic /positive genotypic	Positive phenotypic /positive genotypic	Negative phenotypic /negative genotypic
No. strains	9	78	41	33

Discussion

P. aeruginosa is the most common Gram - negative bacteria in nosocomial infections in burned patients (1-3) and *A. baumannii* is the second. *K. pneumonia* is one of the important enterobacteriaceae which can cause nosocomial infection. Thus, emergence of multi antibiotic resistant isolates led to therapeutic challenge for clinicians (1, 2). These Gram-Negative strains can produce variety type of beta – lactamase enzymes like *amp*C (1, 2, 4). *amp*C is one of important betalactamses can cause of resistant not only to cephalosporins but also can cause resistance to carbapenems (11, 12). In regards, sequencing results indicated the high prevalent of *amp*C – producer, more than 85% of *P. aeruginosa* and *A. baumannii* in this study. But we observed low prevalence with the rate of 12% in *K. pneumonia* according to sequencing results.

These results indicated that over production of *amp*C have important role *in P. aeruginosa* and *A. baumannii*. The results of this study can indicate that one of the most mechanisms in carbapenem

resistant strains which isolated from burned patients in Tehran. The results of study was conducted in Turkey in west-north of Iran were showed the prevalence of 8% *amp*C-producer *K. pneumoniae* isolated from blood culture (19). This prevalence is less than our results. This can related to *K. pneumoniae* were isolated from hospitalized burned patients in our study. In Pakistan that is in east – south border of Iran the rate of *amp*C – producer – *K. pneuminiae* was 12% (8). The results of study in China were showed 12% *amp*C – producer – *K. pneuminiae* (18). In a another study that was conducted in India six out of 44 Klebsiella spp. (13.6%) were positive for *amp*C (21).These isolates were collected from different clinical specimens (21). The results of these three studies are similar to ours. Six of 22 *K. pneumoniae* (27.2%) were positive for *Amp*C in 2014 (22). These two studies (21, 22) were conducted in the same country but the frequency of *K. pneumoniae* – *amp*C producer is different between them. It can relate to the influence of antibiotic treatment and their pressure for selection of resistant bacteria can be different in different health care settings. In 2014, in the other study 16% of *A. baumannii* isolated from burned patients were identified as an *amp*C-producer strain (23). But in our study 87% of *A. baumannii* isolated from burned patients were positive strains for *amp*C. Another study in India and in 2014 was conducted on *P.aeruginosa* isolated from different clinical specimens. In that study none of the strains were *amp*C-producer. (24). However, we identified 92% *amp*C-producer in our tested *P. aeruginosa*. Of course, our strains were carbapenem resistant and this high prevalence of *amp*C-producer *A. baumannii* and *P. aeruginosa* can be reasonable. Generally, over use of broad spectrum antibiotics for treatment of infection and the pressure of antibiotic can lead to select more resistance bacteria and it can be reason of high prevalence of *amp*C-producer in *A. baumannii* and *P. aeruinosa* in our study. On the other hand, *apmC*- producer strains should be considered because of plasmid mediated *ampC* gene can transfer to other bacteria and make complication in treatment of patients (13).

Conclusion

The results suggest that anti-*P. aeruginosa* OprF antibodies elicited in burn wound sepsis model by active immunization are protective against infection with *P. aeruginosa*, and provide a rational for further development of the vaccine for prevention against *P. aeruginosa* infection in burn patients.

Acknowledgements

This study was supported by a grant (M/T 91-04-134-20187) from Iran University of Medical Sciences, Tehran, Iran.

Conflict of interest

None declared conflicts of interest.

References

1. Alaghehbandan R, Azimi L, Rastegar Lari A. Nosocomial infections among burn patients in Teheran, Iran: a decade later. *Ann Burns Fire Disasters* 2012; **25** (1): 3-7.

2. Azimi L, Motevallian A, Ebrahimzadeh Namvar A, *et al*. Nosocomial infections in burned patients in motahari hospital, Tehran, Iran. Dermatol Res Pract 2011; ID: 436952. doi:10.1155/2011/436952.

3. Sar*f* AN, Biçmen M, Guälay Z. The First Report on the Outbreak of OXA-24/40-Like Carbapenemase-Producing

Acinetobacter baumannii in Turkey. *Jpn J Infect Dis* 2013; **66**: 439-442.

4. Lari AR, Alaghehbandan R. Nosocomial infections in an Iranian burn care center. *Burns* 2000; **26** (8): 737-40.

5. Azimi L, Rastegar Lari A, Talebi M, *et al*. Evaluation of Phenotypic Methods for Detection of Klebsiella Pneumoniae Carbapenemase-Producing K. Pneumoniae in Tehran. *J Med Bacteriol* 2014; **2** (3): 26-31.

6. Davies J, Davies D. Resistance Origins and Evolution of Antibiotic. Microbiology and Molecular Biology reviews. *Microbiol Mol Biol Rev* 2010; **74** (3): 417-33.

7. Rastegar Lari A, Azimi L, Rahbar M, *et al*. First report of Klebsiella pneumonia carbapenemase producing Pseudomonas aeruginosa isolated from burn patients in Iran: phenotypic and genotypic methods. *GMS Hyg Infect Control* 2014; **9** (1): 1-5.

8. Shafiq M , Rahman H, Qasim M, *et al*. Prevalence of plasmid-mediated *amp*C β-lactamase in *Escherichia coli* and *Kelebsiella pneumoniae* at hospital of Islamabad, European. *J Microbiol Immunol* 2013; **3**: 267-271.

9. Mohammadi-Barzelighi H, Talebi-Taher M, Adabi M, *et al*. Investigation of Class I, II and III Integrons among Acinetobacter Strains Isolated from Ventilator-Associated Pneumonia Patients in Intensive Care Unit of Rasoul Akram Hospital in Tehran, Iran. *J Med Bacteriol* 2012; **1** (2): 1-9

10. Maraskolhe DL, Deotale VS, Mendiratta DK, *et al*. Comparision of Three Laboratory Tests for Detection of *amp*C β Lactamases in Klebsiella Species and E. Coli. *J Clin Diagn Res* 2014; **8** (6): DC05-8. doi: 10.7860/JCDR/2014/8256.4432.

11. Sheyade SU, Agrawal GN. A Study on the *amp*C Production Amongthe Urinary Enterobacteriaceae Isolates. *J Clin Diagn Res* 2013; **7** (8): 1831-2.

12. Shayan S, Bokaeian M, Shahraki S, *et al*. Prevalence of *amp*C and ESBL Producing E. coli and Antibacterial Effect of Allim sativum on Clinical Isolates Collected from Zahedan Hospitals. Zahedan *J Res Med Sci* 2014; **16** (4): 6-10.

13. Subha A, Renuka Devi V, Ananthan S. *Amp*C b-lactamase producing multidrug resistant strains of Klebsiella spp. & Escherichia coli isolated from children under five in Chennai. *Indian J Med Res* 2003; 117: 13-18.

14. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: nineteenth informational supplement M100–S21. CLSI, Wayne, PA. 2013.

15. Giske CG, Gezelius L, Samuelsen Ø, *et al*. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in Klebsiella pneumoniae with the use of meropenem disks supplemented with aminophenyl boronic acid, dipicolinic acid and cloxacillin. *Clin Microbiol Infect* 2011; **17** (4): 552-6.

16. Andriamanantena TS, Ratsima E, Rakotonirina HC, *et al*. Dissemination of multidrug resistant Acinetobacter baumannii in various hospitals of Antananarivo Madagascar. Ann Clin Microbiol Antimicrob 2010; **30**: 9-17.

17. Rodríguez-Martínez JM, Poirel L, Nordmann P. Extended-Spectrum Cephalosporinases in Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2009; **35** (5): 1766-71.

18. Jones CH, Tuckman M, Keeney D, *et al*. Characterization and Sequence Analysis of Extended-Spectrum-_-Lactamase-Encoding Genes from Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis Isolates Collected during Tigecycline Phase 3Clinical Trials. *Antimicrob Agents Chemother* 2009; **53** (2): 465-75.

19. Sari An, Bimen M, G-Lay Z. Investigation of Plasmid Mediated *amp*C Beta-Lactamases Among Escherichia coli and Klebsiella pneumonia Isolated from Blood Cultures Mikrobiyol Bul 2013; **47** (4): 582-591.

20. Jikun Du, Peipei Li, Helu Liu, *et al*. Phenotypic and Molecular Characterization of Multidrug Resistant Klebsiella pneumoniae Isolated from a University Teaching Hospital, China. *PLOS ONE* 2014; **9** (4): e95181.

21. Oberoi L, Singh N, Sharma P, *et al*. ESBL, MBL and *amp*c β Lactamases Producing Superbugs - Havoc in the Intensive Care Units of Punjab India. *J Clin Diagn Res* 2013; **7** (1): 70-3.

22. Chelliah A, Thyagarajan R, Katragadda R, *et al*. Isolation of MRSA, ESBL and *amp*C - β -lactamases from Neonatal Sepsis at a Tertiary Care Hospital. *J Clin Diagn Res* 2014; **8** (6): 24-7.

23. Gupta V, Garg R, Garg S, *et al*. Coexistence of Extended Spectrum Beta-Lactamases, *amp*C Beta-Lactamases and Metallo-Beta-Lactamases in Acinetobacter baumannii from burns patients: a report from a tertiary care centre of India. *Ann Burns Fire Disasters* 2013; **26** (4): 189-92.

24. S S, Reddy A SK, SS, C A, V S, Ms K, Sk A, V V. Resistance Pattern of *Pseudomonas aeruginosa* in a Tertiary Care Hospital of Kanchipuram, Tamilnadu, India. *J Clin Diagn Res* 2014; **8** (5): 30-2.