Research Article
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Survey of aminoglycoside acetyl transferase genes in multi-drug resistance *acinetobacter*

Abstract

Acinetobacter baumannii is a gram-negative, non-fermenting cocobacills that its species are opportunistic pathogens and cause nosocomial infections**.** Bacteria achieve their resistance to the antibiotics through three mechanisms. aminoglycoside acetyl transferase are member of GCN5 super family as an AMEs for example AAC(3) and AAC(6) N acetylate aminoglycoside on the amine grope on position 3and 6 amino hexose respectively**.** In this is study we are survey present of AAC (3, 6) gen in *Acinetobacter baumannii* isolated from Iranian hospital patient. A total of 43 non-duplicated *Acinetobacter* clinical isolates were collected from the Tehran hospital in 2016. DNA extraction carried out by gram negative DNA extraction kit. Two target gens and their primers used for PCR amplification. Result show that aac (3) IIa gene with 740bp was 68.4% and in gentamicin resistant strain was 66.9% aac (6) Ib gene with 482bp present in 76% of resistant strain (43/33) and in amikacin resistant strain was 72.2% and in gentamicin resistant strain was 70.6%. Preview study showed presence of high variety of resistance gene especially aminoglycoside. This variety explains that other genes may be have role in *Acinetobacter* resistance for aminoglycoside**.** It believes that the ability of this pathogen to harbor diverse genetic elements parallels the experience with *P. aeruginosa*. Genome wide analysis will provide critical insights into this ability.

Keywords: *acinetobacter*, aminoglycoside acetyl transferase, aminoglycoside, pcr

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Introduction

Acinetobacter baumannii is a gram-negative, non fermenting cocobacills that its species are opportunistisic pathogens and cause nosocomial infections among patients in intensive care unit (ICUs). This bacteria cause various infections such as pneumonia, meningitis, endocarditis, and urinary tract.[1] Three decades ago, *Acinetobacter baumannii* infections treated with traditional antibiotics but today its show resistance to major classes of antibiotics including aminoglycosides, tetracyclines, fluroquinolones, carbapenms, cephalosporins, etc… At last year's multidrug resistance (MDR) clinical isolates have shown global distribution.[2] Since Discovery and use of antibiotics, resistance to these agents has been observed. That has negative effect on the treatment of infectious disease.[3] Bacteria achieve their resistance to the antibiotics through three mechanisms: (1) efflux of the antibiotic from the cell via membrane-associted pumping proteins. (2) modification of antibiotic binding target molecule such as special protein or ribosomal RNA or by reprogramming of biosynthetic pathways.(3) by modifying enzymes that selectively modified and destroyed of antibiotic activity. These mechanisms require new programming by the cell in response to the presence of antibiotics.[4] Acetyl transfer, is a common mechanism for in activation of antibiotic that employed by bacteria. O-acetylation or N-acetylation is biologically stable. The aminoglycoside antibiotics bind to the A-site of the ribosome and as e result, impire the codon-anticodon decoding mechanism and blocking of translation fidelity. Aminoglycoside antibiotics bind to 16s rRNA molecule.[5] Aminoglycoside acetyl transferase are member of GCN5 super family of protein include the histon acetyl transferase that are classified based on their region specificity of acetyl transfer on the aminoglycoside structure. For example AAC (3) and AAC(6) N acetylate aminoglycoside on the amine grope on position 3and 6aminohexose respectively. Genes encoding these enzymes are widespread in plasmids, transposons, and integrons (Figure 1).[6] In this is study we are survey present of AAC (3, 6) gen in *Acinetobacter baumannii* isolated from Iranian hospital patient.

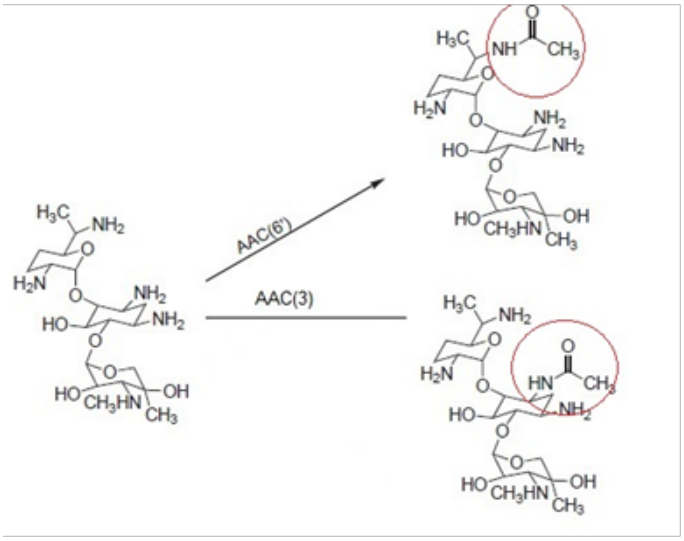

Figure1 Reaction catalysed by AACs.

Methods and materials

A total of 43 non-duplicated *Acinetobacter* clinical isolates were collected from the Tehran hospital in 2016.Biochemical test were used for identification at the species level in 43gram negative bacteria that had negative reaction on oxidase test and lack of lactose fermentation and TSI tests Alk/Alk.[7–9] The strains were isolated from trachea (60%) and sputum (40%). Multi drug resistance tests carried

out foe several antibiotics grope but in this analysis aminoglycoside (amikacin, gentamicin) resistance strains selected for study. Antibiotic susceptibility testing was performed using disc diffusion method (Kirby-bauer) on Muller Hinton agar. The criteria used were in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).[8] DNA extraction carried out by gram negative DNA extraction kit. Two target gens and their primers used for PCR amplification are listed in below Table 1.

Table 1 Two target gens and their primers used for PCR amplification

Primer name	Primer sequence (5to3)	Genes	Bp	Reference
aac(3)IIa	CGGAAGGCAATAACGGAG For TCGAACAGGTAGCACTGAG Rev	AAC(3IIa	sz740	10
aac(6)Ib	TTGCGATGCTCTATGAGTGGCT For CTCGAATGCCTGGCGTGTTT Rev	AAC(6Ib	482	11

PCR condition included denaturation at 94°C for 2min; 36 cycles consisting of 94°C for 45s, annealing at 58°C (for aac(6)Ib) and 54°C(for aac(3)IIa) for 45s, and 72°C for 45s;and a final extension at 72°C for 5mins.[7] PCR product electrophoresed on agarose gel 1% for aac(3)IIa and agarose 1.5% for aac(6)Ib. 100bp-1kbp DNA ladder was used to assess PCR product size and treatment 10min with ethtidium bromide and imaging with UV illuminator.[9]

Result

Acinetobacter spp isolated were recovered from 43 patient that in ICUs and some other part of hospital. The strains were isolated from trachea (60%) and sputum (40%). Multi drug resistance tests carried out foe several antibiotics grope. Isolated *Acinetobacter*s pp has high resistance to all groups of antibiotics. Aminoglycoside resistance for amikacin, was 95% and for gentamicin was 93%. Aac (3)IIa gene with 740bp was 68.4% and in gentamicin resistant strain was 66.9%. aac (6)Ib gene with 482bp present in 76% of resistant strain (43/33) and in amikacin resistant strain was 72.2% and in gentamicin resistant strain was 70.6%. Figure 2 & 3 show result of PCR product electrophoresis the last sample from right related to negative control and sample without band related to negative sample for *Acinetobacter* and band show present of gene in bacteria first column from left related to DNA ladder band.

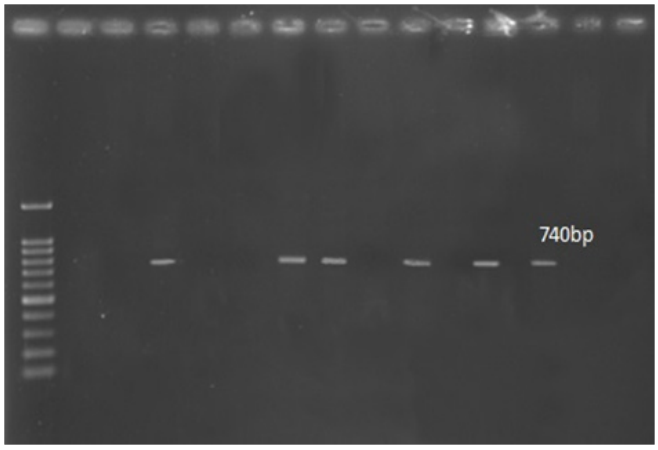

Figure 2 aac(3)IIa gene present in Acinetobacter with 740bp.

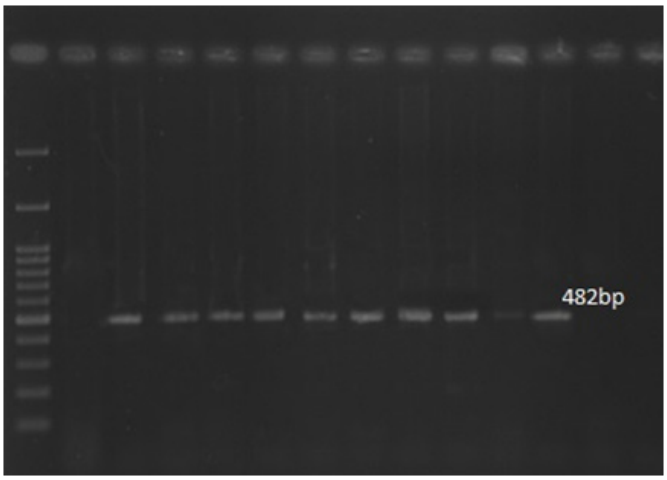

Figure 3 aac(6)Ib gene present in Acinetobacter with 482bp.

Discussion

*Acinetobacter*s pp isolated were recovered from 43 patients**.** The strains were isolated from trachea (60%) and sputum (40%). Antibiogram result showed high rate of resistance to all groups of antibiotics. Resistance to aminoglycoside due to inactivation of antibiotics by modifying enzyme such as acetyl transferase, phosphor transferase, and adenyl transferase.[10–12] Presence of aminoglycoside resistance gene such as aphA6 aacC1 and aadA1 in clinical isolated *Acinetobacter* was reported at below published.[13] A variety of aminoglycoside 3, 6_-*N*-acetyl transferase genes from *Acinetobacter* species have been reported to date.[14] In *Acinetobacter* species, AMEs are common especially AAC(6_)-I and APH(3_)-II.[15] Preview study showed presence of high variety of resistance gene especially aminoglycoside resistance gene in *Acinetobacter* and its reason for multi drugs resistance of *Acinetobacte* in this study resistance for Amikacin and gentamycin were 95% and 93% respectively and resistance gene rate were for tow gene aac(3)IIa gene with 740bp was 68.4% and in gentamicin resistant strain was 66.9%. aac(6)Ib gene with 482bp present in 76% of resistant strain (43/33) and in amikacin resistant strain was 72.2% and in gentamicin resistant strain was 70.6%. Its show that other genes may be have role in *Acinetobacter*

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resistance for aminoglycoside. Resistance to so antibiotics perhaps the intrinsic impermeability of these outer membranes coupled with the close relationship of *A. baumannii* to the soil and aquatic environment has made it possible for these organisms to acquire highly effective resistance determinants in response to multiple challenges.[16]

Conclusion

It believes that the ability of this pathogen to harbor diverse genetic elements parallels the experience with *P. aeruginosa*. Genome wide analysis will provide critical insights into this ability. Wasteful use of antibiotic cause to appearance of resistance strain of bacteria to the existence antibiotics and this makes treatment difficult also the cost and duration of treatment increased.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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