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Genomic Island Location in *Acinetobacter baumannii* Strains by tRIP-PCR Technique

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Abstract: This study was performed to detect the presence of genomic islands which usually insert in the tRNA genes and other non-coding RNA genes, in this study eight strains of *Acinetobacter baumannii* (AYE, A457, A14, A424 A473, A92, ACICU, A25) were tested by used of tRIP-(tRNA site Interrogation for Pathogeni city islands, prophases and other GIs)-PCR method. The results of PCR and agarose gel electrophoresis for eight strains of two loci #7, #24 were: the results of #7 loci screening showed that all strains were positive except *A. baumannii* A457 strain was negative. While the results of #24 loci showed presence of foreign DNA in *A. baumannii* AYE, A457, A14, A424, A473, A92 except the results of (ACICU, A25) was positive.

Keywords: *Acinetobacter baumannii*, genomic islands, tRIP-PCR

INTRODUCTION

Acinetobacter baumannii has become an progressively more important human pathogen because of the increase in the number of infections caused by this organism and the emergence of Multidrug-Resistant (MDR) strains. The majority of infections caused by *A. baumannii* are hospital-acquired, most commonly in the intensive care setting in severely ill patients. In addition, *A. baumannii* has developed as a cause of infections acquired in long-term care facilities, in the community and in wounded military personnel (Sengstock *et al*., 2010).

Many types of infection were produced by *A. baumannii* include, but are not limited to, bacteremia, endocarditic, pneumonia (both hospital and community acquired), skin and soft tissue infections, urinary tract infections and meningitis (Munoz-Price *et al*., 2010). In addition, the emergence of pan-drug resistant strains magnifies the problem by reducing viable treatment options and effectively increasing the mortality rate associated with *Acinetobacter* infections (Mortensen and Skaar, 2012).

The phenomenon of Multidrug-Resistant (MDR) pathogens has increasingly rabidly and become a cause for serious concern with regard to both nosocomial and community-acquired infections related to equation of *A. baumannii* of the GEI (discrete DNA segments differing between closely related bacterial strain) that plays a critical role in its path biology and antibiotic resistance.

The synteny or co linearity of bacterial chromosomal genes is usually well conserved between strains of the identical species. Preserved regions along the chromosomes of individual strains are referred to as the genomic backbone, Horizontal gene transfer events have led to the integration of alien Genomic Islands (GIs) into these backbones. This additional complement of DNA, which can differ considerably between members of the same species, frequently lies within recognized insertion 'hotspots' tRNA and translocations hereafter referred to collectively as 'tRNA' gene (Hacker and Kaper, 2000; Bassetti *et al*., 2011). Recently, tRIP-PCR-based screening methodwas investigated to locate putative Genomic Islands (GIs) lying downstream of tRNA genes, known hotspots for the integration of horizontally acquired DNA (Zhang *et al*., 2011).

MATERIALS AND METHODS

Bacterial strains: All strains of *Acinetobacter baumannii* A92, A473, A424, A457, A14, AYE, A25, ACICU were obtained as stock in freez-glycerol solution from University of Leicester/UK and used for isolation of genomic DNA. The bacterial strains were refreshed by striking on LB agar and then incubated at 37°C for 18 h and followed by taking of single colony that cultured on LB broth in 37C° for 18h in shaker at 200 rpm, finally LB agar plate was cultured and after incubation, the samples were kept in 4°C for use in experiments.

Chromosomal DNA extraction: Chromosomal DNA was isolated by using the DN easy Blood and Tissue kit (Qiagen) by using the protocol for bacterial cells according to the manufacturer's instructions:

- **Cell lysis:** 500 µL cell suspension (overnight culture) was added to 1.5 mL microfuge tube on ice, then, the sample was centrifuge at 13,000-16,000 xg for 5 sec to pellet cells then carefully, the supernatant was remove by a pipet. After that, 300 µL cellists solution was added and

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Table 1: tRIP-PCR primer and its results

Num	Primer name	Primer sequence	Orientation	Band size in bp
1	#24-1F	TATGGCAGCGAATCAACAAA	F	#24-1F+#24-1R 1112 bp
2	#24-1R	CTGCACCTGTAGCAACCAAA	R	
3-	#24-2F	CTGAGCTATTCCCGCAATGT	F	#24-2F+#24-2R 94 bp
4-	#24-2R	GCGTCGTGCTGATTAAACG	R	#24-1F+#24-2R 2047 bp
5-	#7-1F	ATCGTCATGCCATTGTTCAG	F	#7-1F+#7-1R 59 bp
6-	#7-1R	TGTCAGAAACTGCGCCTTTA	R	
7-	#7-4F	GTTGACCACGTTCTAGATGC	F	#7-4F+#7-2R 202 bp
8-	#7-2R	TCATTTTCAGGCCTTTCTCG	R	#7-1F+#7-2R 1848 bp

mix by pipet it up and down until cells are suspended. The sample was incubated at 80°C for 5 min to lyse cells.

- **RNase treatment:** 1.5 μL RNase Asolution was added to the cell lysate and then the sample was mixed by inverting the tube 25 times and then the sample was incubated at 37°C for 15-60 min.
- **Protein precipitation:** the sample was cooled to room temp by placing it on ice for 1 min then 100 μL protein precipitation solution was added to the cell lysate, after that, the sample was mixed by vortex it vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate, then, the sample was centrifuged at 13000-16000 xg for 3 min lead to the precipitate proteins that lead to form tight pellet. If the protein pellet isn't tight step 3 should repeated then followed by incubated on ice for 5 min and then repeat step 4.
- **DNA precipitation:** The supernatant that contain the DNA (leaving behind the precipitation protein pellet) was poured in to a clean 1.5 mL microfuge tube containing 300 mL 100% isopropanol, then the sample was mixed by inverting gently 50 times. After that the sample was centrifuged at 13000-16000 xg for 1 min, the DNA should be visible as small white pellet. Then, the supernatant was discard and then the tube was drained on clean absorbent paper, following that, 300 mL of 70% ethanol was added and then the tube was inverted several times to wash the DNA, after that, the sample centrifuged at 13000-16000 xg for 1 min, then the ethanol pellet was poured and then invert and the tube was drained on clean absorbent paper and allow to air dry 5-10 min.

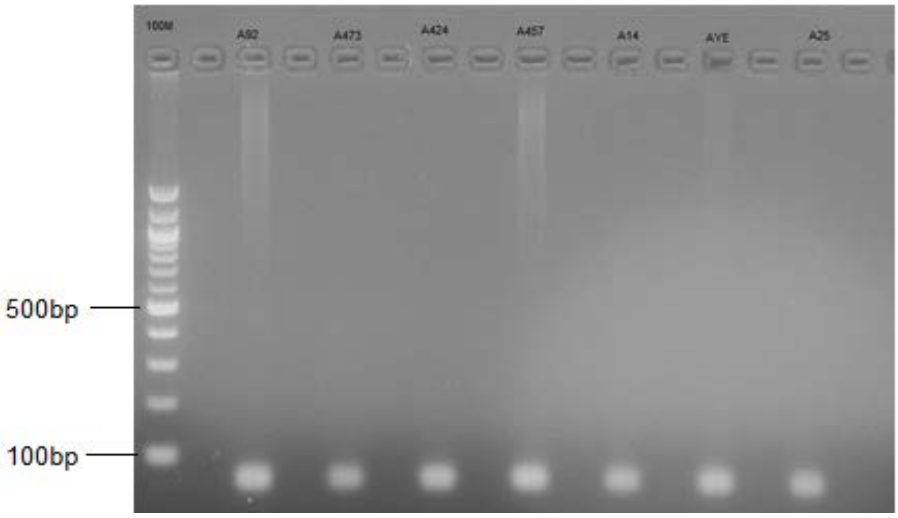
DNA hydration: 50 μL of DNA hydration solution was added to the sample, and then DNA was rehydrated by incubating sample for 1hr at 65°C and or overnight at room temp, Finally, DNA was stored at 4°C, long term storage at -20° C.

Primer design: tRIP-PCR primers of this technique were designed according Mobilize Finder tool (Table 1), Extract Flank method that was used to extract and align 2 kb of conserved flanking sequence upstream and downstream of genomic islandloci (because it depends on that DNA sequences immediately flanking the empty sites and/or corresponding islands are found to highly conserved between strains), then the process was completed by used of *in silico* PCR program that confirm the right binding sites of primers, four sets of primers were designed for two loci #7, #24 of *A. baumannii* strains.

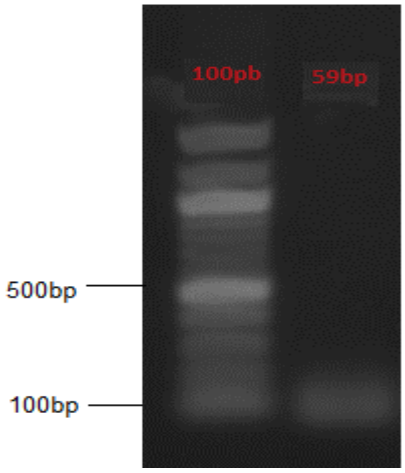
Polymerase Chain Reaction (PCR): For all experiments, PCR reactions were performed in a 20 μL reaction mixture. The components, volumes and concentrations were: 1 μL of primer in a final concentration 10 μM; 10 ng/μL (final concentration) of a plasmid or chromosomal DNA in 1 μL; 0.5 μL of a final concentration of 200 μMdNTPs (deoxyribonucleotide mix, 10 mM each); Expand *Taq* DNA polymerase 0.1 μL (3.5 U μL-1) and 5 μL of 5×PCR buffer. The reaction mixture was made up to a final volume of 20 μL with nuclease free deionised water. The PCR amplification conditions were: initial template denaturation step of 2 min at 95°C, followed by 33 cycles consisting denaturation step at 95°C for 30 sec, 30 sec of annealing at temperature that depends on the melting temperature of primers, 72°C (for *Taq* polymerase) for varying amounts of time according to the size of bands used (generally 1min/kb of expected product), followed by final extension step 72C° for 10 min, Finally Hold 15°C forever.

RESULTS AND DISCUSSION

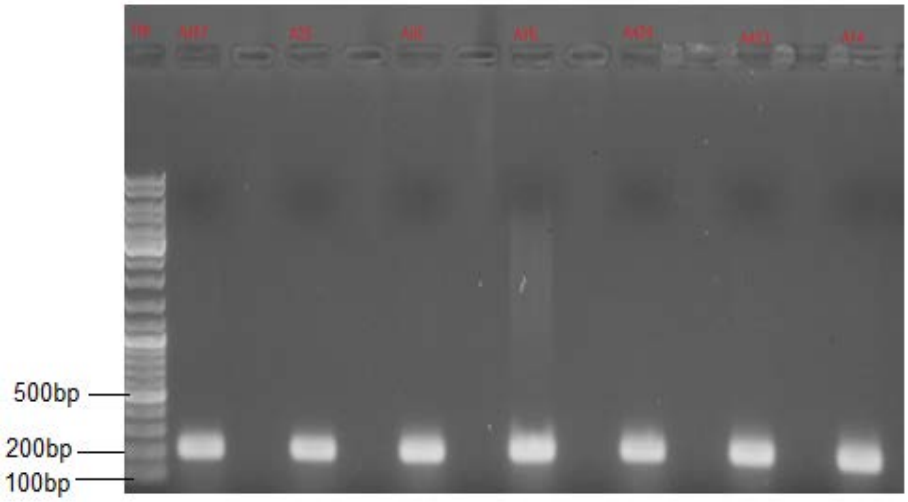
Many studies have been mentioned that GEIs found at the 63 variable loci were identified in the *A. baumannii* genomes (Di Nocerai *et al*., 2011). tRIP-PCR was used to detect the genomic islands in tRNAor non-coding RNA genes that consider a hotspot region, in this study tRIP-PCR has been performed of genomic islands that presence in two loci include #7 (that encode for proteins enriched in dipeptide motifs (valine-glycine repeats) hence call Vgr proteins are associated with ligand-binding proteins at the bacterial surfaced and #24 (DNA blocks which potentially encode typical phage products. These regions have all been classified as cryptic prophases (Giannouli *et al*., 2013). Eight strains of *A. bumannii* (A92, A473, A424, A457, A14, AYE, A25, ATCC, ACICU) were tested of two loci #7 and #24**,** After design four sets of primers two for #7 island and the others for #24 loci, then tRIP-PCR was



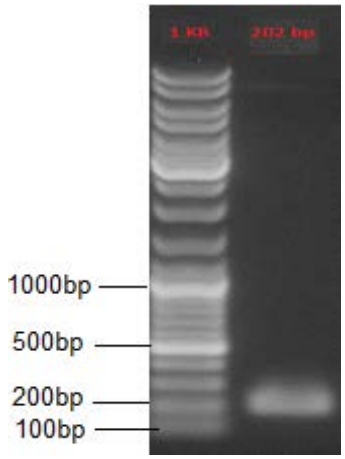
#7-1F+1R 59bp



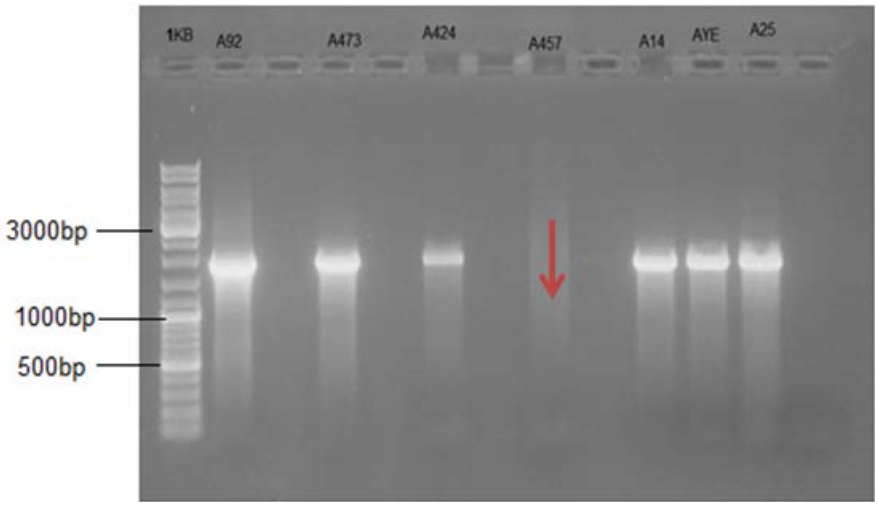
#7-1F+1R(59 bp)-ACICU



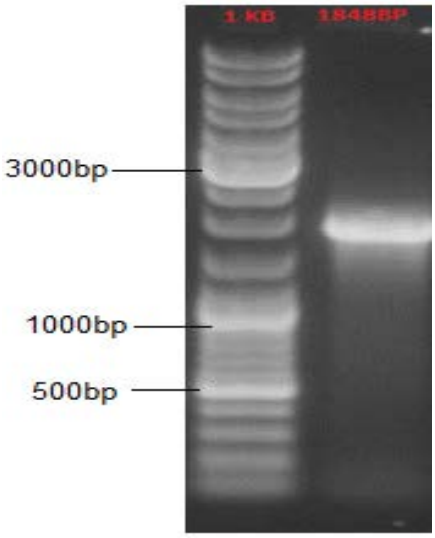
#7-4F+2R 202bp



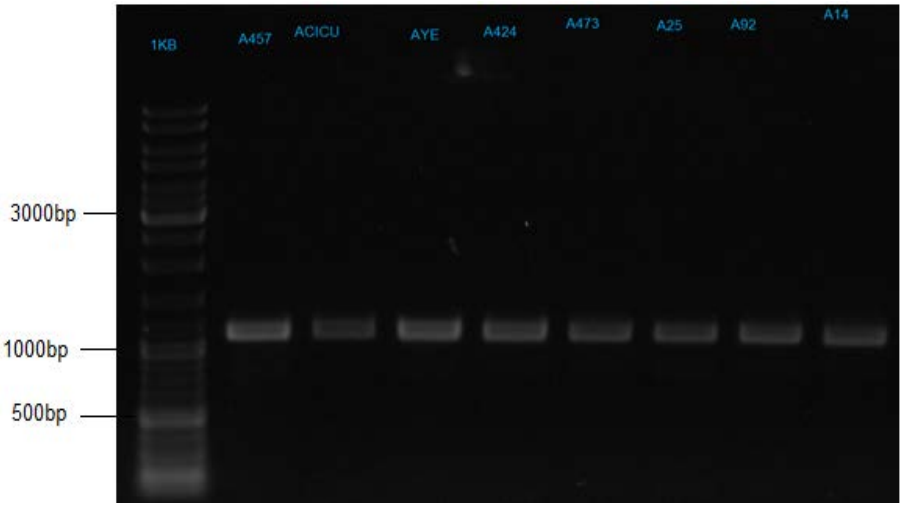
#7-4F+2R (202bp) ACICU



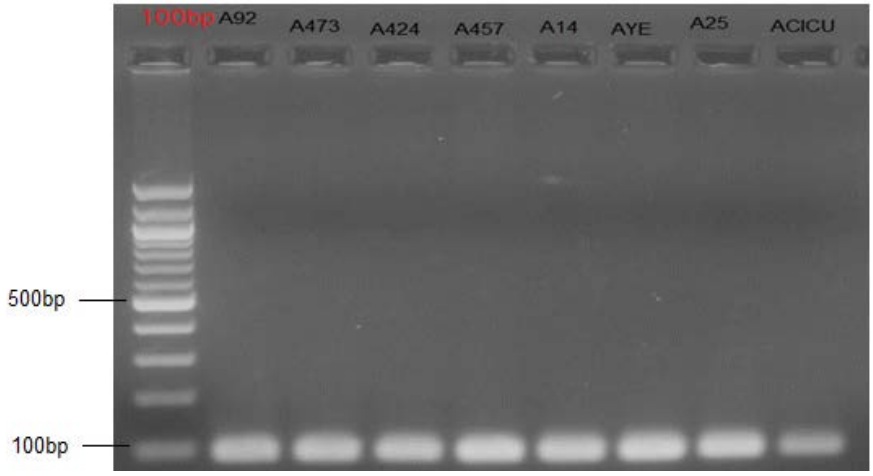
#7-1F+2R 1848



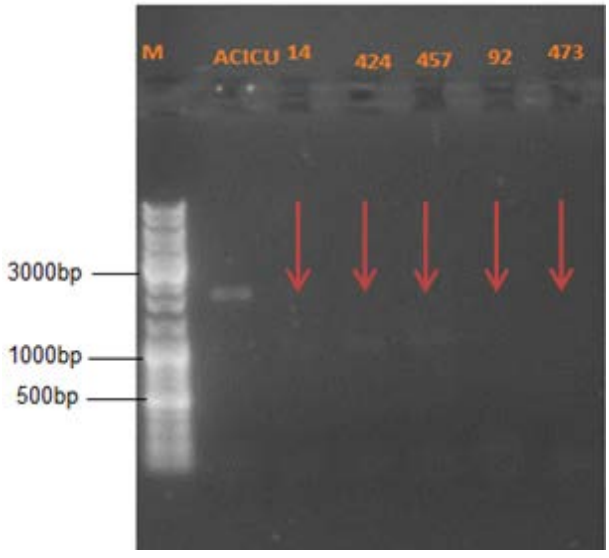
#7-1F+2R (1848 bp) ACICU



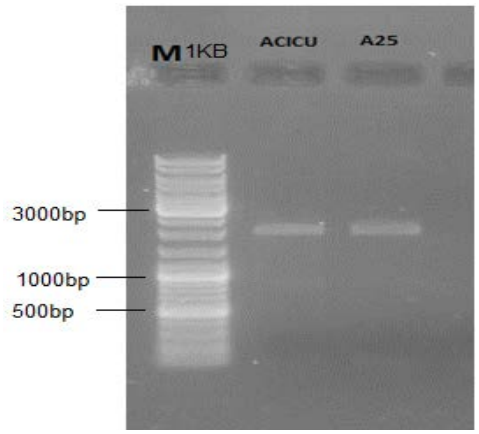
#24-1F+1R 1112bp



#24-2F+2R 94bp



#24-1F+2R-2047bp



#24-1F+2R 2047bp

Fig. 1: The results of tRIP-PCR of eight strains of *A. baumannii* on 1% agarose, at 100 V for 1 hr

Table 2: The results of tRIP-PCR

Primers pairs	A92	A473	A424	A457	A14	AYE	A25	ACICU	Size in BP
#7-1F+1R	+	+	+	+	+	+	+	+	59 bp
#7-4F+2R	+	+	+	+	+	+	+	+	202 bp
#7-1F+2R	+	+	+	NEG	+	+	+	+	1848 bp
#24-1F+1R	+	+	+	+	+	+	+	+	1112 bp
#24-2F+2R	+	+	+	+	+	+	+	+	94 bp
#24-1F+2R	NEG	NEG	NEG	NEG	NEG	NEG	+	+	2047 bp

#7-AYE positive control, #24-ACICU positive control

performed and the results of PCR and agarosegel electrophoresis were (Fig. 1).

The results of tRIP-PCR method for eight strains (A92, ACICU, A25, A457, AYE, A14, A424A473) of #7 loci were (Table 2): all strains give positive results (band) except A457 strain that was negative that confirm the presence of foreign DNA, while the results of #24 loci were: the presence of foreign DNA in (AYE, A457, A14, A424A473, A92) that lead for negative results, while other strains was positive (ACICU, A25) (Pilhofer *et al*., 2008; Di Nocera *et al*., 2011).

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