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MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCE GENES OF *ACENITOBACTER BUMANNII*

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ABSTRACT

This study aims to detection antibiotic resistance gene in *Acenitobacter bumannii* isolates collected from different diseases. It was included 276 specimens collected from different sources including patient suffer from burn, wound, upper respiratory tract inflammation, urinary tract inflammation, osteocytes, endocarditis and blood infection. 52 of these isolates belonged to italic pleas which have been identified through several laboratory tests including the morphology characterizes, biochemical, API 20 E and VITK -2 compact system technique. The distribution of isolates was burn (34.6 %), wound (28.8 %), urine (19.2), blood (9.6%), sputum (5.7%), plural fluid and CSF (1.9 %). The molecular study revealed that *A. baumannii* have the gene of ESBL, oxacillance and MBLs *bla OXA like 51, bla OXA like 24, bla PER, bla TEM* is (94%, 89%, 84%, 96%) respectively also the *gene aadA* and *aadB is* (76% and 84%) respectively fluroquinolone *gyrA* (66%) and finally all isolates have the gene *bla TEM*.

Keyword: ESPL, Extended spectrum beta lactamase

INTRODUCTION

The genus of *Acinetobacter* appeared in the last years in number of types that are considered as opportunistic pathogen for many diseases acquired from hospitals including the pneumonia, urinary tract infection, wound infection, burn infection, septicemia and meningitis. These bacteria considered the ranked second non lactose fermenting bacteria after the *Pseudomonas areuginosa* that isolated from hospitals This genus has more than 47 types but the type of the *Ainetobacter bumannii* considered more important pathogen than others, the *A. bumannii* gram negative, not fermented, aerobic, cocobacilli bacteria opportunistic pathogen is associated with nosocomial infection especially in immune-compromised *Acinetobacter baumannii* has emerged as an important and problemic human pathogen it is causative agent of several types of infection (Zarrilli *el al.*, 2004, Fatahillah, et al., 2016, Rahim, 2017). The Acinetobacter bumannii has many virulence factors that enables it to facilitate from invasion to the tissue human and increase the pathogen including: having the capsule polysaccharide, adhesion factors in the epithelial cells of human enzymes production, having sidrophors and high-water efficiency facilitates adhesion on surface of unliving body's (Goel and Kapil, 2001). Furthermore *A. bumannii* is multidrug resistance (MDR), the strains which are resistant to wide range of antibiotic including broad spectrum B-lactamase, aminoglycosides and fluoroquinolones, resistance to B-lactamase appears to by primary causes by B-lactamase production including the extended spectrum B-lactamase (ESB) (*bla TEM, bla PER*) metallo –B-lactamase (MB) and most commonly, oxacillinases (*bla OXA 51, bla OXA 24*) antibiotic target site alteration confer resistance to fluoroquinolones (*gyrA*) and aminoglycosides (*aadA1, aad B*)(Goel and Kapil, 2001).

Materials and Methods

DNA Extraction: Genomic DNA was extracted using a commercial extraction system (Genomic DNA Minni Kit favorgen biotech corp. Taiwan).

Molecular Identification: Gel electrophoresis was used for detection of DNA by UV transilluminator. The PCR assay was performed to detect the antibiotic resistance genes for *Acenitobacter bumannii* shown in table 2. This primer was designed by Alpha DNA company, Canada as in table 1. Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µl of 10 mg/mL ethidium bromide (Sigma, USA) and it run at 75v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK) and bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products (Levy *et al*., 2008).

Table 1: Primers used in this study

Genes	Primer type	Sequence	Product size (bp)	Reference
bla TEM	TEM -F	5'- GCA CGA GTG GGT TAC ATC GA -3'	310	Hujer KM el., 2006
	TEM –R	5'- GGT CCT CCG ATC GTT GTC AG -3'		
bla PER	PER-F	5'- ATG AAT GTCATT ATA AAA G -3'	927	Hujer KM el., 2006

	PER-R	5'- TTG GGC TTA GGG CAG -3'		
bla $_{CTX-M}$- 15	CTX- M-15- F	5'-C GCG ATG GGC AGT ACC AGT AA -3'	510	Hakemi Vala M., el 2013
	CTX-M- 15- R	5'- TTA CCC AGC GTCAGA TTC CG -3'		
bla $_{OXA}$-51 like	OXA – 51-F	5'- TAA TGC TTT GAT CGG CCT TG -3'	353	Woodford N el., 2006
	0XA -51-R	5'- TGG ATT GCA CTT CAT CTT GG -3'		
bla $_{OXA-24}$ like	OXA-24-F	5'- GGT TAG TTG GCCCCCTTA AA -3'	246	Woodford N el 2006
	OXA-24-R	5'- AGT TGA GCG AAA AGG GGA TT -3'		
bla aadA1	aadA-F	5' ATG AGG GAA GCG GTG ATC G -3'	624	Hujer KM el..2006
	aadA-R	5'- TTA TTT GCC GAC TAC CTT GGT G -3'		
bla aadB	aadB-F	5' -ATG GAC ACA ACG CAG GTC GC -3'	495	Hujer KM el..2006
	aadB-R	5'- TTA GGC CGC ATA TCG CGA CC -3'		
bla gyrs A	gyrs-A-F	5' AAA TCT GCC CGT GTC GTT GGT -3'	285	Srinivasan VB el., 2009
	gyrs-A –R	5'- GCC ATA CCT ACG GCG ATA CC -3'		

Table 2: PCR program of antibiotic resistance primer that apply in the thermocycler

Gene	Initial denaturation	No.of cycles	Denaturation	Annealing	Extension	Final extension
bla$_{TEM}$	94 Cº for 5 min.	30	94 Cº for 30 sec.	55 Cº for 30 sec.	72 Cº for 1 min.	72Cº for 4 min.
bla $_{PER}$	94 Cº for 5 min.	30	94 Cº for 1 min.	44 Cº for 45 sec.	72 Cº for 1 min	72Cº for 16 min.
bla$_{CTX-M}$-15	94 Cº for 5 min.	35	94 Cº for 1 min.	55 Cº for 1 min.	72 Cº for 1 min	72Cº for 7 min.
bla gyrs A	95 Cº for 5 min.	30	95 Cº for 30 sec.	63 Cº for 30 sec.	72 Cº for 30 sec.	72Cº for 5 min.
bla aadA$_1$	94 Cº for 5 min.	30	94 Cº for 30 sec.	62 Cº for 30 sec.	72 Cº for 1 min	72Cº for 10 min.
bla aadB	94 Cº for 5 min.	30	94 Cº for 1 min.	68 Cº for 30 sec.	72 Cº for 1 min	72Cº for 10 min.
Bla OXA-24 like	94 Cº for 5 min.	33	94 Cº for 1 min.	53 Cº for 1 min.	72 Cº for 1 min	72Cº for 7 min.
bla OXA-51 like	94 Cº for 5 min.	33	94 Cº for 30 sec.	53 Cº for 1 min.	72 Cº for 1 min	72Cº for 10 min.

RESULTS AND DISCUSSION

Prevalence of *A. baumannii* encoded gene among bacterial isolates: According the results obtainned, the *bla* OXA like -51 was (83%) and the *bla* TEM, *bla* PER, *bla* OXA like 24, *aad*A1, *gyrsA*, aadB (775, 67%, 65 %, 52%, 48 %, 44%), the CTX -M-15 showed the 17% the compared genes show in Table 3. Inclusion of virulence factors was summarized by combination of virulence factors expression by each *A. baumannii* isolates tend to determine the specific syndrome accompanying an infection, however, in clinical cases it is often difficult to distinguish between simple colonization infection, and no diagnosis tool id available to assess the virulence potential of given isolate. The result finding the high rate genes found in burn isolates and the others isolates low rate. The result that *A. baumannii* encoded genes that resistance to antibiotic in most isolates but high rate in wound and urine isolates (Alexander, 2009).

Table 3: Show the Prevalence of *A. baumannii* encoded gene among bacterial isolates.

Gene	Wound (n=18)	Burn (n=15)	Urine (n=10)	Blood (n=5)	Sputum (n=3)	Plural fluid and CSF. (n=1)	Total (n=52)
*Bla*OXA like -51	93%	94%	70%	80%	-	100%	83%
*bla*TEM	86%	83%	80%	60%	34%	-	77%
bla PER	80%	72%	70%	40%	33%	-	67%
Bla OXA like 24	73%	72%	70%	40%	33%	-	65%
*aad*A1	60%	67%	30%	40%	-	100%	52%
gyrsA	53%	61%	30%	40%	33%	-	48%
aadB	53%	55%	20%	40	33%	-	44%
CTX-M-15	27%	17%	10%	205	-	-	17%

The finding according to several of specimens of the wounds were recorded (100%) for biofilm formation and *A. baumannii* carring several genes related to MDR, such as (*bla* TEM, *bla* OXA like 51, *bla* OXA like 24, *bla* PER, gyrs *A* and aad *A1*) in wound specimens by burn and urine.

Detection of carbapenemase, metalo-B-lactamase and extended spectrum–B-lactamase (ESBLs)

genes (*bla OXA like- 51 enzyme, OXA like -24 enzymes, CTX -M -15 enzyme, bla TEM, bla PER):* The result of PCR analysis concerning of the found the OXA 51, showed that studies *A. baumannii* possess the gene *bla OXA like 51* from 52 isolated show 43 (83 %), were burn 17(94%), wound 14 (93 %), blood 4 (80%), urine 7 (70%), sputum 0 (0 %) and plural & CSF 1 (100%) (figure 2).

The study revealed the gene *bla OXA like 24* showed that from the 52 isolates was 34 (65 %) were burn 13 (72 %), wound 11 (73%), urine 7 (70 %), blood 2(40), sputum 1 (33%) and plural and CSF 0(0%) as shown in figure 3.

Regarding to gene *bla TEM* showed that from the 52 isolates 40 (77%) were burn 15 (83%), wound 13 (86 %), urine 8 (80%), blood 3(60), sputum 1 (33%) and plural & CSF 0(0%) as presented in figure 4.

The results of PCR analysis concerning the presence of gene *bla PER* showed that from the 52 isolations 36 (67 %) show the burn 12 (72 %), wound 14 (80%), urine 6 (70%), blood 3(40), sputum 2 (33%) and plural &CSF 0(0%) as figure (5).

The finally of PCR analysis concerning the presence of gene *CTX-M -15* showed that from the 52 isolations 9 (17 %) were burn 3 (17 %), wound 4 (27%), urine 1 (10 %), blood 1(20), sputum 0 (0 %) and plural & CSF 0(0%) as in figure 1. The group of B-lactamses identified so far in *A. baumannii* including more than 50 different enzymes or their allelic form, according to their nucleotides sequences the can be detected in four groups named class A, B-lactamases to class D, B-lactamases A, Band D have a serine at their active site, while class B enzyme have four zinc atoms at the active sit, some of the enzyme are intrinsically found in *A. baumannii* (Perez *et al.,* 2007) as shown in figure 6.

The result of gene *bla OXA like 51* from 52 isolates were high rate in the burn, wound and blood isolates, the low rate in isolates of urine, sputum and pleural fluid and CSF. The all isolates carried the results provide evidences that detection of *bla*OXA-51-like and showed that *bla*OXA-51-like can be used as a simple and reliable way for identifying *A. baumannii*. It has been found that *bla*OXA-51-like exists in all isolates of *A. baumannii* and those strains that show carbapenem resistance are almost possess blaOXA-51-like. The molecular analysis of the high rate in all the isolates carried the *bla* OXA like -51. The 52 isolates were carried the gene *bla* OXA like 24 show the high rate in burn, wound and urine, the low rate appear in blood, sputum and plural and CSF. The resistance rate to most antimicrobial agents, including carbapenems as first line drug agents against MDR *A. baumannii* isolates, are increasing in the world by (Asadollahi *el al.,* 2012) who some the isolates carried the gene. A recent study has reported that the prevalence of *bla*TEM genes analyzed by PCR-based, among *A. baumannii* isolates in Kaohsiung Armed Forces General Hospital. The same study has showed that in multidrug resistant *A. baumannii*, the presence of *bla*TEM predicts resistance to ceftazidime. Strain harboring only *bla* TEM was resistant to all β-lactams except carbapenems. The rate of *bla* TEM found in half of isolates (Ben *et al.,* 2011). The gene *bla PER* showed that from the 52 isolates the more of them have the gene, showed the isolates from specimens of burn, wound and urine have the high rate of the gene, while the blood, sputum and plural and CSF. The (Lee *el al.,* 2008) reveled that *A. baumannii* was observed that cell adhesiveness and biofilm formation on plastic is higher in strains harboring the *bla* PER gene than in those that do not harbor this genetic trait .Furthermore, the level of expression of this gene, as determined by reverse transcription-PCR is positively correlated with the level of biofilm formed on plastic and the adhesiveness of bacteria to human epithelial cells, Similar findings were reported by, who found a significant association between multidrug resistance and biofilms, although they believe that the presence of *bla* PER is more critical for cell adhesion than the formation of bacterial biofilms on abiotic surfaces (Rao *et al.,*2008). The result of gene *CTX-M -15* showed that from the 52 isolates only the 9 gave the gene that appear in the high rate in isolates of burn and wound, other isolates of urine, blood, sputum and plural and CSF gave low rate or not found in most of them. The showed that isolates gave the *CTX-M -15* gene from all isolates of *A. baumannii* only some gave the gene by (Al-Ajeeli 2013) the result revealed from all isolates only (5%) gave the gene (Al-Grawi, 2011).

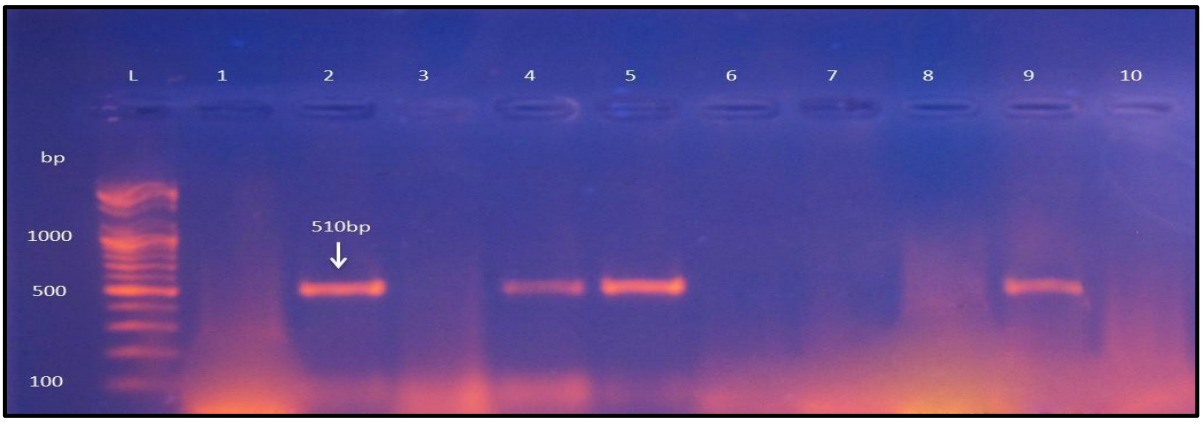

Figure 1: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *CTX-M -15*.

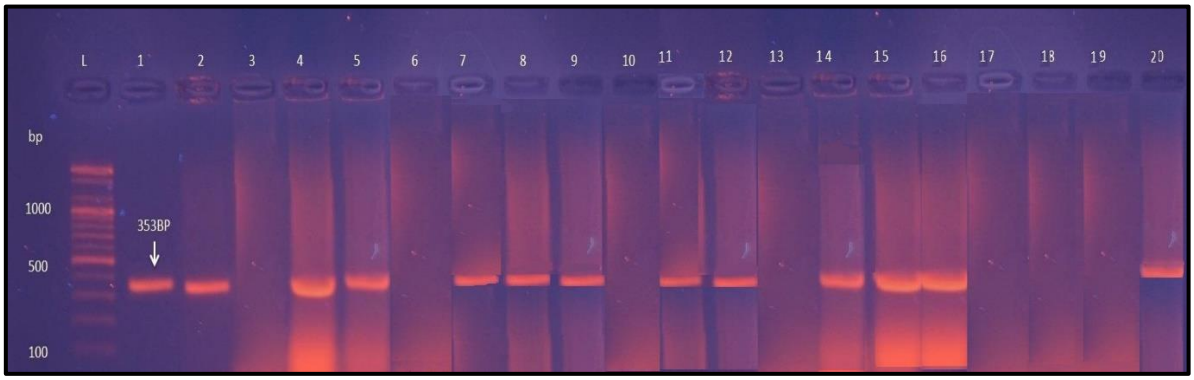

Figure 2: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *bla OXA like 51*

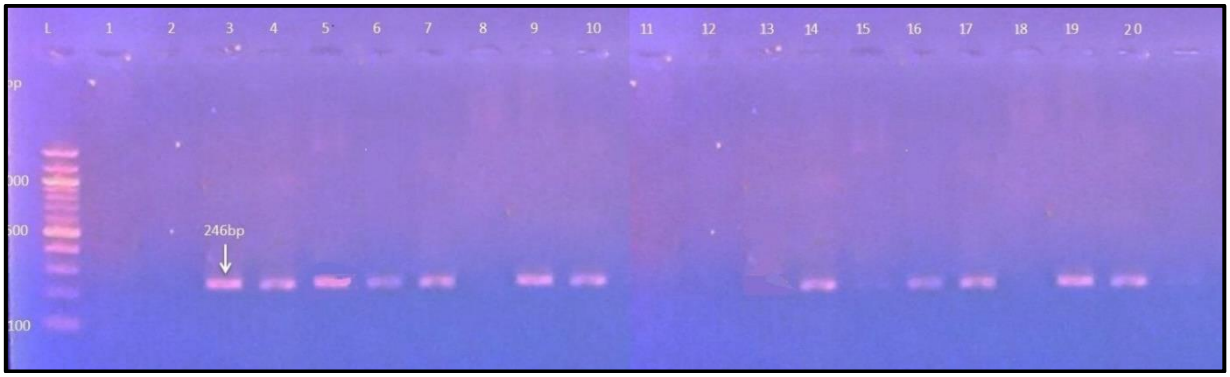

Figure :3 Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *bla OXA like 24*.

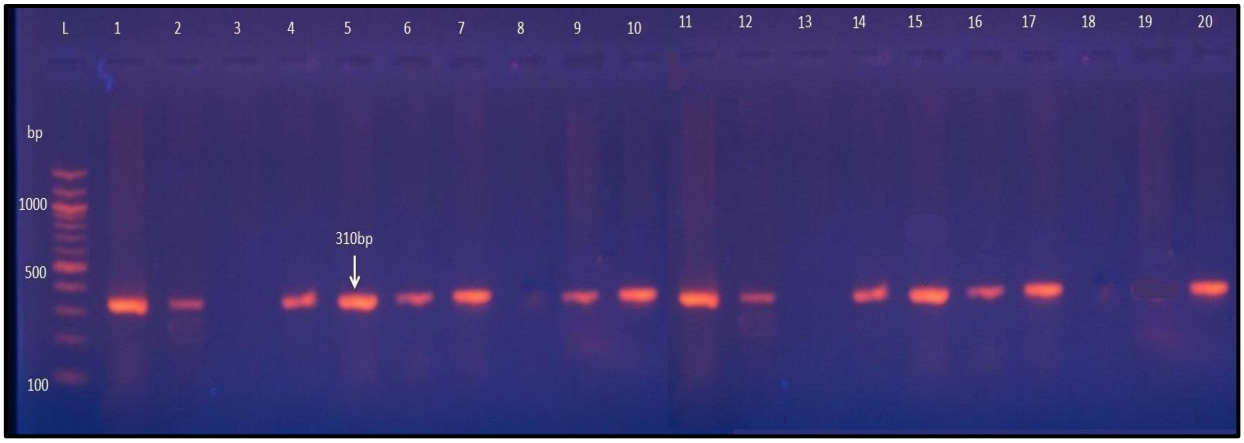

Figure 4: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *bla TEM* .

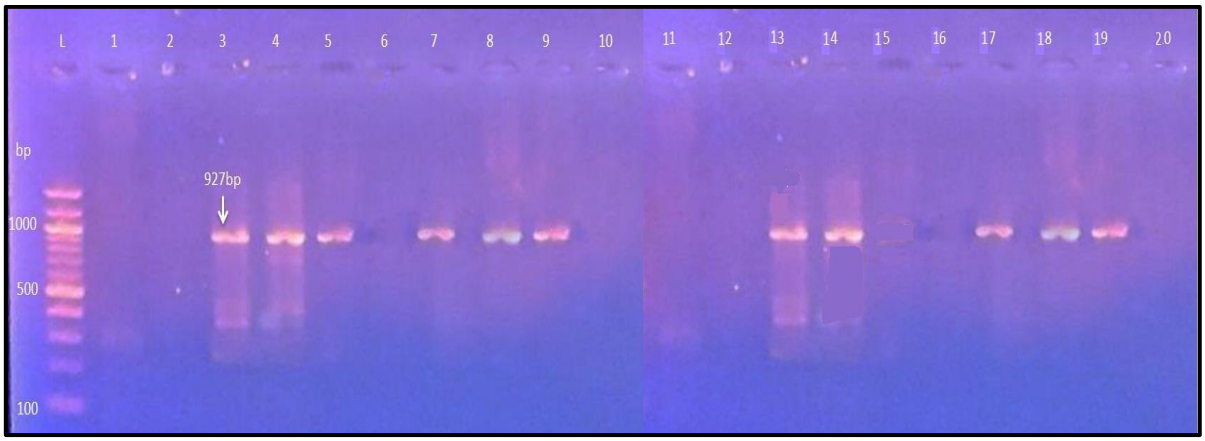

Figure 5 Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *bla PER* .

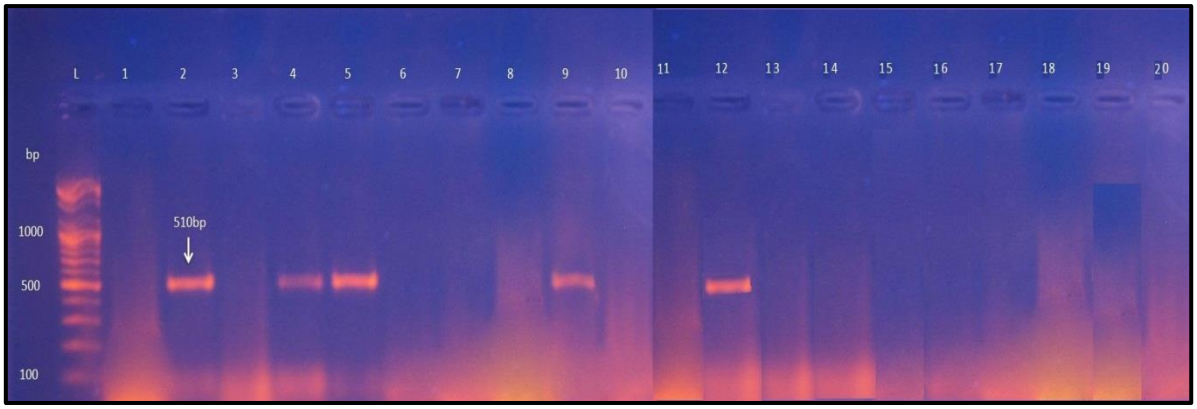

Figure :6 Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *CTX-M -15*.

Detection of aminoglycoside modifying enzymes genes (*aab A1, aabB)*: The results of PCR analysis concerning the presence of gene *aab A1*showed that from the 52 isolates 27 (52 %) show the burn 12 (67 %), wound 9 (60 %), urine 3 (30%), blood 2(40), sputum 0 (0 %) and plural & CSF 1(100%).

The PCR results of gene *aab B* showed that from the 52 isolates 23 (44 %) were burn 10 (55 %), wound 8 (53 %), urine 2(20%), blood 2(40), sputum 1 (33 %) and plural & CSF 0(0%) as figure (7)(8). The gene *aab A1* from the 52 isolates 27 (52 %) show and the high rate of result have gene found in the isolates of the burn and wound the low content have gene show in isolates of urine, blood, sputum and plural & CSF. The genes of aminoglycoside (*aabA1* and *aabB*) play important role in mutation at the site of aminoglycoside attachment is responsible for reduction in the affinity of aminoglycoside for the ribosome and many interfere with ribosomal binding. Alteration of the ribosomal binding sites is mainaly responsible for the resistance to antibiotics because the antibiotic binding by the subunit of ribosomal the gene *aabB* were found from all isolates of *A. baumannii* showed half of the isolates have them, appear in the burn and wound have the most isolates the gene and the urine, blood, sputum and plural and CSF have the some of them (Galimand *el al.*, 2005).

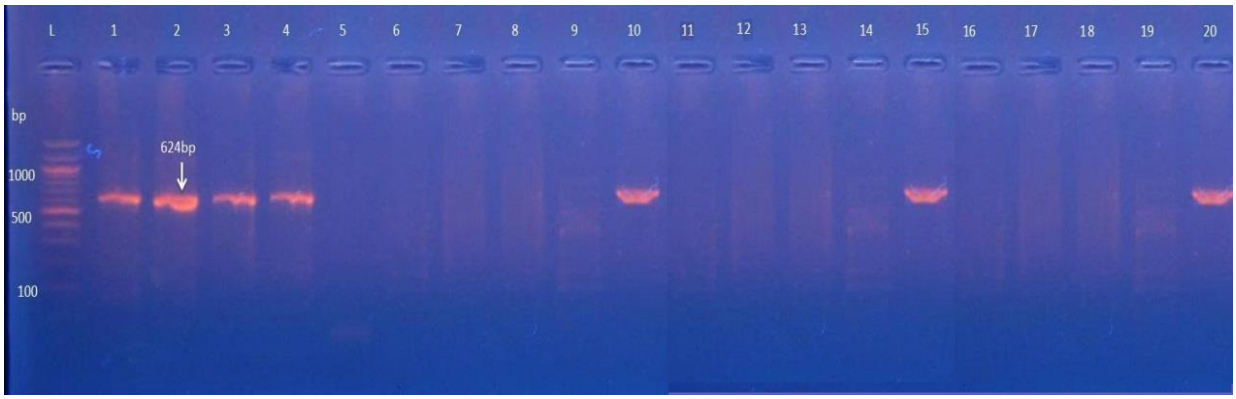

Figure 7: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *aab A1*.

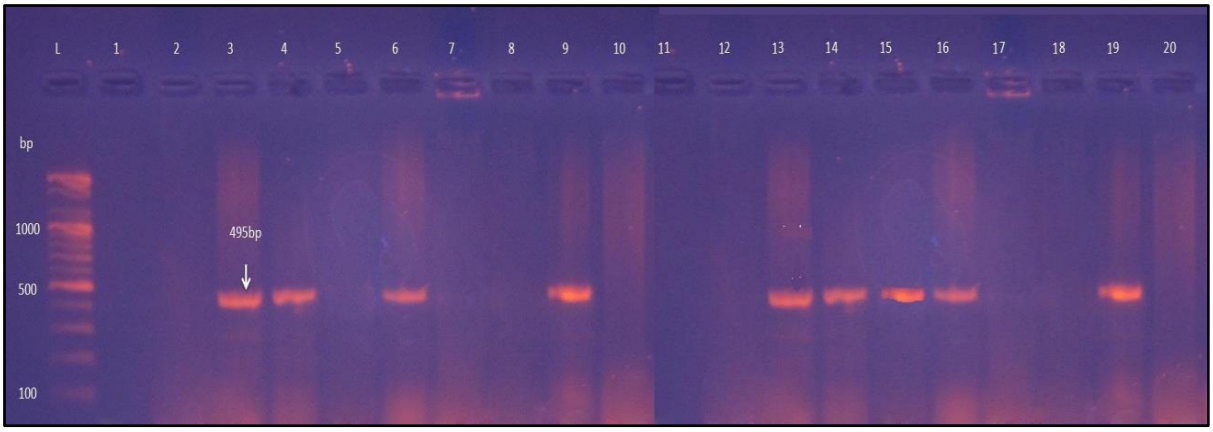

Figure 8: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *aabB.*

Detection of fluroquinolone resistance gene (*gyr A*): The results of PCR analysis concerning the presence of gene *gyr A* showed that from the 52 isolates 25 (48 %) were burn 11 (61 %), wound 8 (53 %), urine 3 (30%), blood 2(40), sputum 1 (33 %) and plural and CSF 0(0%) as in figure (9)**.** The result of gene *gyr A* showed that from the 52 isolates the half of them carried the gene Illustrates the burn, wound and sputum the high rate while the urine, blood and plural and CSF have low rate of the gene. The Resistance of *A. baumannii* to quinolones is often caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the *gyrA* and *parC* genes. These changes result in a lower affinity for the binding of the quinolone to the enzyme-DNA complex. A second mechanism of resistance to the quinolones is mediated by efflux systems that decrease intracellular drug accumulation (Galimand *el al.*, 2005).

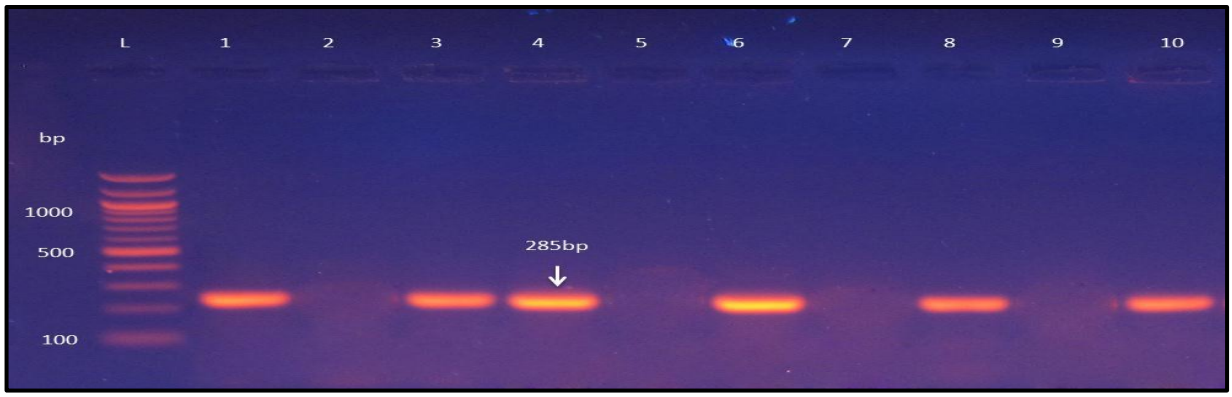

Figure 9: Ethidium bromide – stained 1.5% agarose gel showing the amplified product of *gyrs A.*

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