Microbes and Infectious Diseases 2021; 2 (2): 343-351

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Association between mutations in GyrA/B and ParC/E genes of *Acinetobacter baumannii* clinical isolates and fluoroquinolones resistance

Mohammed Shees Ahmed Shahhat [1], Essam Abo El Yazed Gaballa [1], Raafat Zaher Abd El Rohman [2], Mohammed Nabil Mohammed [1], Ayman Sabry Yassin [*1]

1- Depatrment of Microbiology and Immunology, Faculty of Medicine, Al-Azhar University.
2- Department of Microbiology and Immunology, Military Medical Academy.

ARTICLE INFO

Article history:
Received 7 February 2021
Received in revised form 11 March 2021
Accepted 22 March 2021

Keywords:
A.baumannii
Quinolones resistance
RFLP-PCR
Molecular mechanisms
Mutations

ABSTRACT

Background: *Acinetobacter baumannii* (*A. baumannii*) is an important organism in hospital acquired infections. *Acinetobacter baumannii* has emerged as quinolones resistant organism. **Objectives**: Determination of mutations of gyrA/B and parC/E genes using Restriction Fragment Length Polymorphism PCR (RFLP - PCR) among fluoroquinolones resistant clinical isolates of *A. baumannii* showed in ICU patients at Al-Azhar University hospitals. **Methods**: The study was conducted on 100 isolates of *A.baumannii* which were subjected to molecular study of mutations of gyrA, gyrB, parC and parE genes beside determination of minimal inhibitory concentration (MIC). **Results:** Most *A. baumannii* isolates had MIC >128 µg/ml (61.1%). The resistant isolates showed combind mutations in both gyrA& parC genes most frequently (79.2%) then gyrA (9.7%), parC (8.3%) and gyrB (2.8%). **Conclusions**: Mutations in gyrA and parC are the most common in fluoroquinolones resistant *A. baumannii* isolated from Al-Azhar University hospitals.

Introduction

Acinetobacter species are distributed in nature, and have been determined in soil, water, animals, and humans. Some of them can exist for weeks in environment & cause hospital acquired infections [1]. They inhabits in human pores and skin and are often isolated from the throat and respiratory tract of hospitalized patients [2].

More than thirty known and unknown *Acinetobacter* species had been described [3]. Some *Acinetobacter spp have* medical importance, which includes Acinetobacter baumannii (*A. Baumannii*), Acinetobacter gen3, and *Acinetobacter Gen. Sp. 13TU*, at the same time a different species, like *Acinetobacter johnsonii, Acinetobacter schindleri* and *A. Ursingii*, additionally can incidentally associated to cause infections [4].

Acinetobacter baumannii has emerged in the ultimate years as a completely important infectious agent inflicting fitness care related infections. It's one among what is called "ESKAPE" microorganism (*Staphyllococcus aureus, Enterococcus faecium, Acinetobacter baumannii, Klebsiella pneumoniae*, Enterobacter species and *Pseudomonas aeruginosa*) [5].

In addition, *A. baumannii* resistant to carbapenem is one of the critical-precedence pathogens on the global Health Organization precedence listing of antibiotic-resistant microorganism for powerful drug development [6].

DOI: 10.21608/MID.2021.64414.1127
* *Corresponding author:* Ayman Sabry Yassin
E-mail address: asabry_2008@yahoo.com

Ciprofloxacin has wide spectrum bactericidal activity, good oral bioavailability, high tissue penetration, more safe and tolerable and used to treat a wide types of infections [7], ciprofloxacin will be taken as a model of fluoroquinolones in current study according to **Ardebili A et al.** [8] .

The maximum crucial mechanism of fluoroquinolones resistance is the mutation in genes that encode the subunits of DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE). GyrA gene and its homologous region of the parC gene are most significant mutations incorporate mainly the quinolone resistance figuring out regions (QRDRs). On the opportunity hand, the mutations in gyrB and parE genes regions are of less significance [9,10]

Aim of study

This study aimed to detect the presence of gyrA/B and parC/E genes mutations by restriction fragment length polymorphism PCR (RFLP PCR) among fluoroquinolones resistant *A. baumannii* isolates in patients with infections in intensive care units at Al-Azhar university hospitals in Cairo**.**

Subjects and Methods

The study was done between February 2019 to July 2020. The study was approved by Al Azhar faculty of Medicine ethical committee. Samples collected from inpatients (each isolate from individual patient), including sputum, endotracheal tubes, urine, infected wounds and bed sores. The collected samples were subjected to: Direct microscopic examination of a Gram–stained smear, culture on MacConkey and blood agar media and biochemical tests [11]. Further identification to species level was done by VITEK 2 automated system [12].

The study was done on 100 clinical isolates of *A. baumannii* (68 males, 32 females) who developed clinical evidence of infection. Full history was taken including duration of hospital stay, underlying diseases, risk factors (e.g. Diabetes) & previous operation.

Consent was taken from the patient's family to be enrolled in the study.

***Acinetobacter* was identified by:**

1) Gram stain: short, Gram-negative rods, arranged in pairs or clusters**.**

2) Colony appearance: on MacConkey agar media colonies appear smooth, convex, glistening, sometimes pale yellow & mucoid.

3)Biochemical reaction: oxidase test negative and catalase test positive.

4)VITEK 2 automated microbiology system.

Antibiotic sensetivity test was done by modified Kirby Bauer disc diffusion method, according to CLSI guidelines [13].

Minimal inhibitory concentration for ciprofloxacin

Detection of MIC for ciprofloxacin among isolates of *Acinetobacter baumannii* was done by broth macrodilution approach consistent with the Clinical Laboratory Standards Institute instructions. Ciprofloxacin serial dilutions have been done using Muller Hinton broth and bacterial isolates with attention same to half McFarland have been brought to every tube then incubated for 18 hours at 37° C. Minimum inhibitory concentration is described as the least concentration without a seen growth. *Acinetobacter baumannii* isolates considered as intermediate resistant at MIC 2 µg/ml and full resistant at ⩾4 µg/ml [14].

DNA Extraction

Acinetobacter baumannii isolates had been subjected to extraction of DNA using boiling technique. At first colonies were suspended in 1ml of sterile distilled water then boiled for 10 minutes & then centrifuged for another 10 minutes. Then preserve supernatant in sterile eppendorf at -20 ºC till amplification [15].

Amplification technique

Amplification technique achieved by adding 2µl of the extracted DNA with 20 µl of amplification mixture (Qiagen) [16]. The used primers were summarized in **table (1)**.

The amplified products were purified by the use of QIA quick PCR purification kit according to the manufacturer's protocol. Then the eluant incubated at 37°C for 2.5 hours with 10 U of *HinfI* enzyme which used for the digestion. Then digests were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and photographed with UV illumination.

Table 1. Primers sequences of the target genes.

Target Gene	Primer sequence (5'-3')	Amplicon size
gyr A	**Forward** AATCTGCCCGTGTCGTTGGT	343 bp
	Reverse GCCATACCTACGGCGATACC	
gyrB	**Forward** GAAATGACCCGCCGTAAA	490 bp
	Reverse ACGACCGATACCACAGCC	
parC	**Forward** AATCTGCCCGTGTCGTTGGT	327 bp
	Reverse GCCATACCTACGGCGATACC	
parE	**Forward** CTGACCGAAAGCTACGTCAACC	417bp
	Reverse CGTTCGGCTTGCCTTTCTTG	

Results

Among the *Acinetobacter* group, *A.baumannii* was the main isolated_spp. (77.5%) while *A.lwoffii* (16.3%)and *A. Junii* were (6.2%) **(Figure 1)**.

Different types of samples were included in this current study, 36 wound, 38 sputum, 11 blood and 2 urine samples. While 13 isolates were recovered from miscellaneous samples (endotracheal tube (ETT), bronchial lavage and 1urinary catheter) **(Table2)**.

Most effective antibiotics against *A. baumannii* were imipenem (88%), ciprofloxacin (33%), ceftriaxone (11%), ofloxacin (8 %), cefepime (7%) and amikacin (2%). While *A. baumannii* were 100% resistant to ampicillin/sulbactam, amoxicillin/clavulinate, trimethoprim/sulfamethozole, cefazolin and cefuroxime **(Table 3)**.

The sensitivity and specificity of disc diffusion method as compared to the MIC in detection of ciprofloxacin resistant were found to be 90% and 100%, respectively **(Table 4)**.

All resistant strains of *A. baumannii* to ciprofloxacin had mutations in gyrA ,gyr B and parC. The most frequent mutations were combined mutations in both genes (79.2%) then mutation gyrA (9.7%), parC(8.3%), gyrB (2.8%) and par E (0%) **(Table 5) (Figures 2,3&4)**.

Acinetobacter baumannii with resistance to ciprofloxacine had MIC ⩾4 µg/ml. Most isolates had MIC >128 µg/ml (61.1%) **(Table 6)**.

The isolates of *A. baumanii* with MIC more than 128 µg/mL; 44 (61.1%) had combined mutations in the gyrA and parC. These findings indicate that combined mutations are associated with high ciprofloxacin MIC **(Table 7)**. In relation to type of specimens it was found that *A. baumannii* with resistance to ciprofloxacine had MIC >128 µg/ml more in sputum and wounds culture **(Table 7)**.

Table 2. Distribution of *A. baumannii* isolates in clinical samples.

Samples	No.
Blood Culture	11
Sputum	38
Wounds Culture	36
Urine Culture	2
Others	13
Total	100

Table 3. Antibiotic resistance among isolates of *A. baumannii*.

Antimicrobial agent	Disc content	Zone diameter (mm)		Sensitive No.	Intermediate No.	Resistant No.
		Sensitive	resistant			
Ampicillin-sulbactam	(20 µg)	≥15	≤11	0	0	100
Amoxicillin-Clavulante	(30 µg)	≥18	≤13	0	0	100
Trimethoprim-sulfamethoxazole	(1.25/23.75µg)	≥15	≤12	0	0	100
Amikacin	(10 µg)	≥17	≤14	2	0	98
Ofloxacin	(5 µg)	≥16	≤12	8	15	77
Cefazolin	(30 µg)	≥18	≤14	0	0	100
Cefuroxime	(30µg)	≥18	≤114	0	0	100
Ceftriaxone	(30µg)	≥21	≤13	11	17	72
Cefepime	(30µg)	≥18	≤14	7	60	33
Ciprofloxacin	(5 µg)	≥21	≤15	33	19	48
Imipenem	(10µg)	≥22	≤18	88	3	9

Table 4. Comparison between disc diffusion test and MIC for detection of ciprofloxacin resistance.

Procedure used	CIP sensitive isolates		CIP resistant isolates	
	No.	%	No.	%
Disc diffusion test	33	33%	67	67%
MIC tube	28	28%	72	72%

Table 5. Genetic mutation among *A. baumannii* isolates.

Characteristics	No.	%
gyrA	7	9.7%
gyrB	2	2.8%
ParC	6	8.3%
ParE	0	0%
Combined gyrA and parC	57	79.2%
Total	72	100%

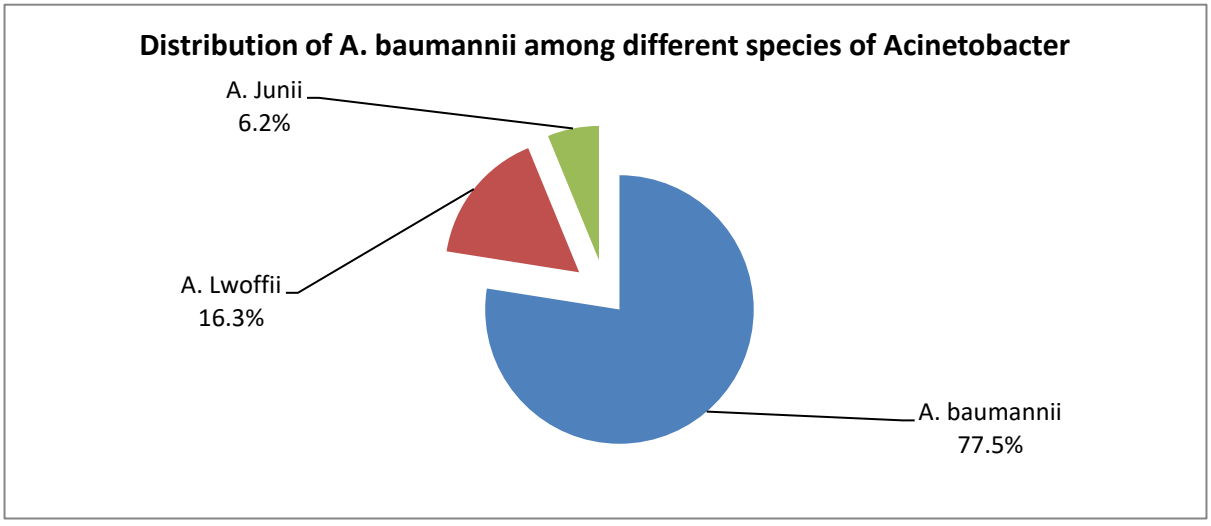
Table 6. *Acinetobacter baumannii* resistance by MIC.

	***A. baumannii* resistance by MIC (µg/ml)**													
	4		**8**		**16**		**32**		**64**		**128**		**> 128**	
	No	**%**	**no**	**%**	**No**	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**
A.baumannii isolates	11	15.2	6	8.3	2	2.8	2	2.8	3	4.2	4	5.6	44	61.1

Table 7. Genetic mutation among resistant *A. baumannii* isolate in relation to MIC (µg/ml).

	4		**8**		**16**		**32**		**64**		**128**		**> 128**	
	no	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**	**no**	**%**
gyrA	3	4.2	0	0	0	0	2	2.8	2	2.8	0	0	0	0
gyrB	0	0	2	2.8	0	0	0	0	0	0	0	0	0	0
parC	0	0	0	0	2	2.8	0	0	0	0	4	5.6	0	0
parE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Combined gyrA and parC	8	11.2	4	5.6	0	0	0	0	1	1.4	0	0	44	61.1

Figure 1. *Acinetobacter baumannii* distribution among different species of *Acinetobacter*.



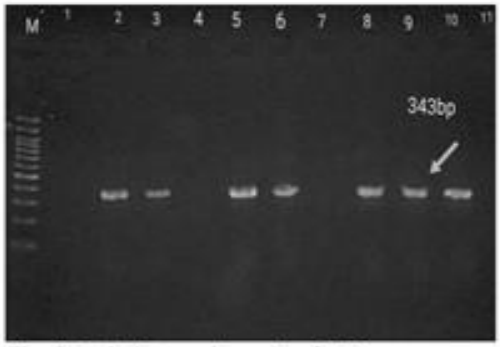


Fig. 2: Gel electrophoresis of PCR amplification for gyrA gene in *A. baumannii* isolates (343 bp):Lane2,3,5, 6,8,9,10 positive cases - Lane 1,4,7,11 negative cases.

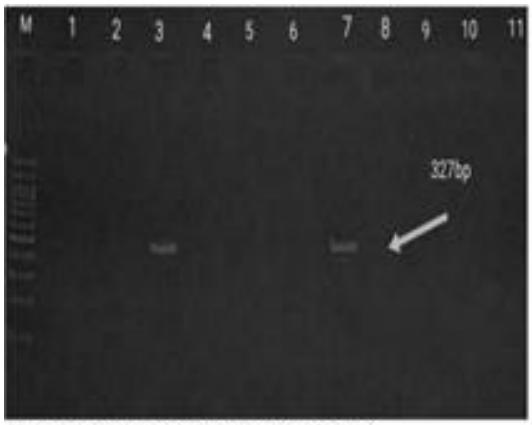


Fig.3: Gel electrophoresisof PCR amplification for par C gene in *A. baumannii* isolates (327 bp):lane3,7positive cases - Lane1,2,4,5,8,9,10,11 negative cases.

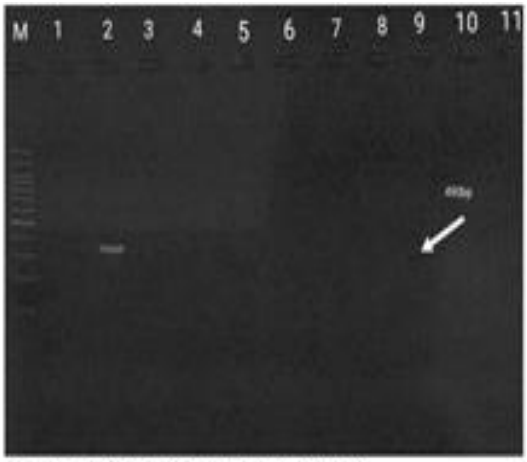
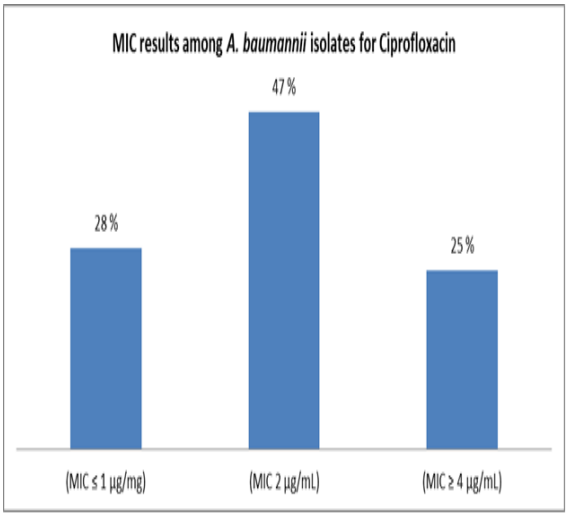


Fig. 4: Gel electrophoresis of PCR amplification for gyrB gene in *A. baumannii* isolates (490 bp):Lane2 positivecase- Lane 1,3, 4,5,6,7,8,9,10,11 negative cases.

Figure 5. Minimum inhibitory concentration results among *A. baumannii* isolates for ciprofloxacin.



Discussion

Acinetobacter baumannii has emerged as an essential organism in hospital acquired infections in last few years. The importance of *A. baumannii* came from its extensive antimicrobial resistance that make little antibiotic alternatives for treatments of its infections [17].

In the current study the most effective antibiotics against *A. baumannii* were imipenem (88%), ciprofloxacin (33%), ceftriaxone (11%), ofloxacin (8 %), cefepime (7%) and amikacin (2%). While *A. baumannii* were 100% resistant to ampicillin/sulbactam, amoxicillin/clavulinate, trimethoprim/sulfamethozole, cefazolin and cefuroxime. This results is consistent with **Enas et al.** and **Nowroozi et al.** [18,19]. In the current study all *A. baumannii* isolates (100 %) were significantly multidrug resistant isolates based on definition of multidrug resistance [15]. This result is also matching with **Nowroozi et al.** [19].

In our study from a total 72 *A. baumannii* isolates resistant to ciprofloxacin it was found that 44 (61.1%) isolates showed ciprofloxacin MICs > 128 µg/mL, indicating that these isolates were highly resistant to ciprofloxacin, in addition to 28(38.9%) isolates had MICs <128 µg/mL. Ciprofloxacin resistance among *A. baumannii* was 75 % in a study conducted in Egypt in 2014 [20]. This points to that there is increase in ciprofloxacin resistance in the last years in Egypt among *A. baumannii* isolates. Meanwhile resistance were 95.6 % in a study from Iran [8], 97.3 % in a study from Pakistan [21], and 96.2 % in a study from Saudi Arabia [22].

In the current study the sensitivity and specificity of disc diffusion method as compared to

the MIC in detection of ciprofloxacin resistance showed that 90% and 100%, respectively, this results concomitant with **Karami et al.** [23]. These results differed from **Stone et al.** [24], who reported that disc diffusion technique turned into a great fast screening technique to MIC for detection of antimicrobial sensitivity. Our results were matching with **Maysaa et al.** and **Valentine et al.** [25, 26].

A baumannii isolates with MIC >128 µg/mL had mixed mutations within the gyrA and parC. These findings suggest that mixed mutations are related to excessive MIC resistance to ciprofloxacin. The mixed mutations in those genes are related to remarkable decrease binding affinity for quinolones in the resistant organisms. Theses outcomes had been in settlement with **Maysaa et al.** and **Redgrave et al.** [25, 27].

Mixed mutations of gyrA & parC are mentioned to be related to a high-degree of resistance to quinolones, Six isolates in this study show mutations in parC without mutations in gyrA, suggesting that parC may have a role in ciprofloxacin resistance without mutation in gyrA. This result is consistent with **Ardebili et al.** [8]. While there had been two isolates in this result had mutations in gyrB gene alone those suggest that mutations in gyrB had little role in ciprofloxacin resistance, that is consistent with **Vila et al.** [28].

Conclusion

From this study we can conclude that resistance to ciprofloxacin was common in clinical isolates of *A. baumannii*. The most frequent mutations were present in gyrA and parC. However, mutations in gyrB or parC alone were not uncommon.

Conflict of interest: None.

Funding: None.

References

1-**Doughari H, Ndakiedemi P, Human I, Benade S.** The Ecology, Biology and Pathogenesis of Acinetobacter spp.: An Overview. Microbes Environ 2011; 26(2): 101-112.

2-**Fournier P., Richet H.**: The epidemiology and control of *A. baumannii* in health care facilities. Clin Infect Dis 2006.; 42: 692- 9.

3-**Nemec A, Musilek M, Maixnerova M, De Baere T, Van der Reijden T, Vaneechoutte M, et al.** *Acinetobacter beijerinckii* sp nov and *Acinetobacter gyllenbergii* sp nov., haemolytic organisms isolated from humans. International journal of systematic and evolutionary microbiology. 2009;59(Part 1):118-24. Int J Syst Evol Microbiol 2009; 59:1376- 1381.

4-**Dijkshoorn L, Nemec A, Seifert H.**: An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii.* Nat Rev Microbiol 2007;5: 939-951.

5-**Boucher HW, Talbot GH. Bradley JS., Edwards JE, Gilbert D, Ric LB, et al.** Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clinical Infectious Diseases 2009; 48: 1-12.

6-**Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al:** Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2017, (3):318.

7-**King DE, Malone R, Lilley SH.** New classification and update on the quinolone antibiotics. American family physician 2000;61(9):2741-8.

8-**Ardebili A, Rastegar Lari A, Beheshti M, Rastegar Lari E.** Association between mutations in gyrA and parC genes of *Acinetobacter baumannii* clinical isolates and ciprofloxacin resistance. Iran J Basic Med Sci 2015;18:623626.

9-**Sáenz Y, Zarazaga M, Briñas L, Ruiz-Larrea F, Torres C.** Mutations in gyrA and parC genes in nalidixic acid-resistant *Escherichia coli* strains from food products, humans and animals. J Antimicrob Chemother 2003; 51(4): 1001-5.

10-**Vila J, Ruiz J, Goñi P, Marcos A and**

Jimenez de Anta T: Mutation in the gyrA gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii.* Antimicrob Agents Chemother 1995; 39(5): 1201-3

11-**Wise KA, Tosolini FA:** Epidemiological surveillance of Acinetobacter species. J Hosp Infect 1990;16(4):319–329.

12-**Wang J, Ruan Z, Feng Y. et al**: Species distribution of clinical Acinetobacter isolates revealed by different identification techniques. PLoS ONE 2014; 9(8):e104882

13-**Clinical Laboratory Standards Institute, Wayne.** Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters. 5th ed. CLSI guideline M23. Wayne, PA: *Clinical and Laboratory Standards Institute*, 2018.

14-**Clinical Laboratory Standards Institute, Wayne.** CLSI Performance standards for antimicrobial susceptibility testing: twentieth Informational supplement; pp. M100–S20. CLSI document. J Infect Control 2013; 37:56-64.

15-**Magiorakos AP, Srinivasan A, Carey RT, Carmeli Y, Falagas MT, Giske CT, et al.** Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012;18(3):268-81.

16-**Vila J, Ruiz J, Gon˜i P, Jimenez de Anta T.** Quinolone-resistancemutations in the topoisomerase IVparCgene of *Acinetobacter baumannii.* J Antimicrob Chemother 1997; 39(6):757e62

17-**Maragakis LL, Perl TM.** Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis 2009; 46(8):1254–1263.

18-**Newire EA, Ahmed SF, House B, Valiente E, Pimentel G.** Detection of new SHV-12, SHV-5 and SHV-2avariants of extended spectrum Beta- lactamase in *Klebsiella pneumoniae* in Egypt. Annals of Clinical Microbiology and Antimicrobials 2013; 12:16

19-**Nowroozi, J, Sepahi AA, Kamarposhti, LT, Razavipour R, Mazhar F.** "Evaluation of ciprofloxacin (gyrA, parC genes) and tetracycline (tetB gene) resistance in nosocomial *Acinetobacter baumannii* infections. Jundishapur J Microbiol 2014; 7: e8976.

20-**Gomaa FM, Tawakol WM, Abo El-Azm FI.** Phenotypic and genotypic detection of some antimicrobial resistance mechanisms among multidrug-resistant *Acinetobacter baumannii* isolated from immunocompromised patients in Egypt. Egypt J Med Microbiol 2014; 23. 99-111

21-**Joshi PR, Acharya M, Kakshapati T, Leungtongkam U, Thummeepak R, Sitthisak S.** Co-existence of bla OXA-23 and bla NDM-1 genes of *Acinetobacter baumannii* isolated from Nepal: antimicrobial resistance and clinical significance," Antimicrobial Resistance & Infection Control. Antimicrob Resist Infect Control 2017; 6: 21.

22-**Aly MM, Alsoud NA, Elrobh MS, Al Johani SM, Balkhy HH.** High prevalence of the PER-1 gene amon carbapenem-resistant *Acinetobacter baumannii* in Riyadh, Saudi Arabia. European Journal of Clinical Microbiology & Infectious Diseases 2016; 359110:1759-66.

23-**Karami A, Mazloom Zadeh S, Rastin A, Karami A, Shiri P.** The consistency of antibiotic resistance' results in two methods of disk diffusion and MIC in isolated organisms from patients with urinary tract infections. J Med Life 2015;8(Spec Iss 4): 282–286.

24-**Stone ND, O'Hara CM, Williams PP,**

McGowan JE Jr, Tenover FC. Comparison of disk diffusion, VITEK 2, and broth microdilution antimicrobial susceptibility test results for unusual species of Enterobacteriaceae. J Clin Microbiol 2007; 45(2): 340-6.

25-**Zaki ME, Abou ElKheir N, Mofreh M.** Molecular Study of Quinolone Resistance Determining Regions of gyrA Gene and parC Genes in Clinical Isolates of *Acintobacter baumannii* Resistant to Fluoroquinolone Open Microbiol J 2018; 12: 116–122.

26-**Valentine S, Contreras D, Tan S, Real LJ, Chu S, Xu HH.** Phenotypic and molecular characterization of *A. baumannii*clinical isolates from nosocomial outbreaks in Los Angeles,California. J Clin.Microbiol 2008; 46: 2499-507

27-**Redgrave LS, Sutton SB, Webber MA, Piddock LJ.** Fluoroquinolone resistance: mechanisms, impact on bacteria, and role inevolutionary success. Trends Microbiol 2014; 22(8): 438-45.

28-**Vila J, Ruiz T, Marco F, Jiminez de Anta MT.** Association between double mutation in gyrA of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. Antimicrob Agents Chemother 1994; 38: 2471–2479.

EL Shahhat M, Abo El Yazed E, Abd El Rohman R, Nabil M, Yassin A. Association between mutations in GyrA/B and ParC/E genes of *Acinetobacter baumannii* clinical isolates and fluoroquinolones resistance. Microbes Infect Dis 2021; 2 (2): 343-351.