

How to Cite:

Jaber, A. N., Rostamzad, A., & Alzubaidi, A. F. A. (2022). Evaluation of class 1, 2 and 3 integrons among multidrug resistant isolates of *Acinetobacter baumannii*. *International Journal of Health Sciences*, 6(S9), 3376–3386. <https://doi.org/10.53730/ijhs.v6nS9.13332>

Evaluation of class 1, 2 and 3 integrons among multidrug resistant isolates of *Acinetobacter baumannii*

Atheer Nadhim Jaber

University of Al-Qadisiyah / College of Education Pure Sciences

Asst. Prof. Dr. Arman Rostamzad

University Of Ilam / Faculty of Basic Sciences

Asst. Prof. Dr. Adawia Fadhel Abbas Alzubaidi

University Of Diyala / College of Education Pure Sciences

Abstract--The goal of this study was to determine the prevalence of class 1, 2, and 3 integrons in clinical *Acinetobacter baumannii* isolates from hospitalized patients. A total of 100 *A. baumannii* isolates were collected from several Imam Khomeini hospitals in Ilam, Iran. *A. baumannii* was discovered by traditional biochemical research. Following that, PCR was employed to detect several integron classes. Biochemical testing was used to identify *Acinetobacter* species. The samples obtained from patients (patients aged 21 to 69 years old, including 55 males and 45 women): *Acinetobacter baumannii* was found in 100 samples, *Acinetobacter lufii* in 25, and other *Acinetobacter* species in 12. 42 samples from the critical care unit were separated, 28 from the infectious department, 23 from the emergency department, and 7 from other departments. Furthermore, 44 blood samples, 23 trachea samples, 11 wound samples, 10 urine samples, and 12 unknown samples were isolated. Class 1 Integrons were found in 87 of 100 isolates, Class 2 Integrons in 74 of 100 isolates, and Class 3 Integrons in none of the isolates.

Keywords---integrons, *Acinetobacter baumannii*, integron classes, Multidrug Resistant Isolates of *Acinetobacter baumannii*.

Introduction

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, *A. baumannii* has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged (> 90 d) hospital stay (Peleg *et.al.*,2008; Commonly

associated with aquatic environments, it has been shown to colonize the skin as well (Howard *et al.*, 2012). As being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals (Marks *et al.*, 2014). In recent years, it has been designated as a “red alert” human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum. Several exchangeable genetic elements such as plasmids, transposons, and integrons are the most important genetic elements responsible for the transmission of antibiotic resistance genes in different gram-negative bacteria. Various studies showed that integrons are significantly in relation to the presence of MDR in *A. baumannii* isolates (Marks *et al.*, 2014). These elements play a major role in the dissemination and rearrangement of resistance determinants called mobile genetic cassettes. So far, several classes of integrons have been described in gram-negative bacteria. All integrons have a 5′ conserved segment, consist of the integrase gene, and the cassette integration site (attI), but have a distinct 3′ conserved segment. The most prevalent integrons belong to class I and play an important role in the development of antimicrobial resistance and the emergence of MDR profiles in gram-negative bacteria (Partridge *et al.*, 2018). As for the class I integrons, the 3′ conserved sequence area (3′CS) includes three open reading frames: Integrons of class II are commonly found associated with the Tn7 transposon family and its 3′ conserved segment containing five *tns* genes, which are responsible for the mobility of transposons (Ike *et al.*, 2021). Class II integrons have been described most often in isolates within the Enterobacteriaceae family. Integrons of class III have also been reported but its 3′ conserved segment has not been characterized (Zeighami *et al.*, 2019).

The prevalence of different classes of integrons and their relationship with antibiotic resistance in clinical isolates of *A. baumannii* is not clear in Ilam. Therefore the objectives of this study will to investigate the prevalence of class I, II and III integrons among *A. baumannii* isolates collected from hospitalized patients. (Naylor *et al.*, 2018)

2. Materials and Methods

2.1. Study Population

In order to obtain the most accurate assessment of the types and amounts of *Acinetobacter baumannii* present multiple samples from several areas of the Meningitis, Blood infection. Urinary tract infections, Nosocomial pneumonia, Nosocomial infections. Were collected frequently in the first few days to weeks following injury in the proper way to avoid any possible contamination. The study was conducted in the Department of Microbiology at Ilam University.

2.2. Collection of specimens

Total 120 samples were collected from different hospitals in Ilam. The samples included samples collected from blood, wounds, urine, trachea, etc. These samples were collected from patients who had been hospitalized for at least three days and had acquired the infection on the hospital premises.

2.3. Isolation of bacteria

BHI broth medium containing the sample was transferred to the laboratory medium and then each sample was cultured on Blood agar and MacConkey agar media. The cultures were then kept in an incubator at 37 ° C for 24 hours. After 24 hours, all cultures were transferred to nutrient agar medium and kept again for 24 hours at 37 ° C. Then, from the nutrient media, the desired colony was placed on the slide with a loop. After drying, the spread on the slide was stabilized with heat and hot staining was performed (Kumar *et al.*, 2013).

Thus, the presence of gram-negative coccobacilli of *Acinetobacter* was confirmed microscopically. Then differential tests were performed: urea, MRVP, SIM, OF, TSI, as well as catalase, oxidase and simon citrate tests. Growth at 37 and 42 degrees Celsius was also one of the tests performed to detect different species of *Acinetobacter* (Pendleton *et al.*, 2013). The absence of a loop line in the SIM medium after 24 hours of culture indicated that the bacterium was moving negatively.

Failure to form a red ring after adding coaxial reagent to the SIM medium after 24 h of culture indicated that the bacterium was endol negative. The absence of red color in the MRVP medium after the addition of phenol-Red reagent after 24 h of culture indicated that the bacterium was MR negative (De Oliveira *et al.*, 2020). The absence of red color in the MRVP medium after 24 h of culture after addition of barite reagent indicated that the bacterium was VP negative.

In the TSI medium using a loop, the bacteria were cultured at depth and surface and kept in an incubator at 37 ° C for 24 hours. In this environment, if the tube turns yellow / yellow (ie, completely yellow), acidic / acidic, if red / red (ie, completely red), alkaline / alkaline, and if red / yellow (ie, the upper part red and the lower part yellow) Alkaline / acidic was reported (Lucidi *et al.*, 2018).

3. Genome extraction

To extract the genome by boiling, the following steps must be performed in order:

- 1) 150 µl of physiological serum was poured into a 1.5 ml sterile microtube.
- 2) A loop of pure bacterial culture was transferred to the microtype.
- 3) Vertex is done manually for 1 minute to create a one-handed mixture of bacteria and physiological serum.
- 4) The microtube was placed in boiling water at 100 degrees Celsius for 20 minutes.
- 5) The microtube was quickly placed in a centrifuge at 13,000 rpm for 15 minutes.
- 6) The supernatant containing the bacterial DNA is carefully separated with a sampler and transferred to another sterile microtype.
- 7) Finally, the extracted product was electrophoresed on 1% agarose gel.

4. Polymerase Chain Reaction

4-1. DNA template

The DNA extracted by boiling is the template DNA that is stored at -20 ° C until PCR is performed.

4-2. Master Mix:

Master Mix from Ampliqon III was used. This product had a concentration of x2 containing Taq DNA polymerase, PCR buffer, a mixture of MgCl₂ 1.5Mm and dNTPS, which was purchased from Sinagen. In fact, this product contained all the necessary components for PCR except primer and template DNA.

Primer:

The primers used included oligonucleotides from nucleotide sequences synthesized in the selected region, which were selected by the studied articles and prepared by Sinagen Tehran Company. Primers F and R are lyophilized and separated in small tubes kept at -20 ° C. First, the lyophilized primer must be dissolved, so according to the instructions given on the primer, add some distilled water to it and put it in a pan at 37 degrees Celsius for 10 minutes, and then this solution was kept at -20 degrees. It was used during PCR(Tewari *et al.* , 2018).

The sequence of primers used includes that were designed using primer 3

Primer name	Sequence	Product size
Integron Class 1 F	TCGTGATGCCTGCTTGTCT	360 bp
Integron Class 1 R	AACTGCGGGTCAAGGATCTG	360 bp
Integron Class 2 F	GGATTAGCTTTGCGGCCATT	506 bp
Integron Class 2 R	GTCCACTCTTTAAAATACGGCG	506 bp
Integron Class 3 F	AGTGGGTGGCGAATGAGTG	600 bp
Integron Class 3 R	TGTTCTTGTATCGGCAGGTG	600 bp

5- PCR implementation method

Remove the material needed for PCR and DNA samples from the freezer and refrigerator to bring it to room temperature, but it should be noted that the primer will break if left out for -20 long. Number the PCR microtubes Positive and negative controls were considered(Sato *et al.* , 2019).

5-1. Isolation, culture, detection of bacterial samples

Biochemical tests include catalase, oxidase, urease, TSI, IMVIC, OF, and these tests were performed to detect *Acinetobacter* species and their results were obtained in the following order. The percentage and number of different species in the samples isolated from patients (Age of patients were 21-69 years old and sex distribution of patients was 55 male and 45 femal) : In this research, out of 137 samples of *Acinetobacter* isolated from 459 samples of hospitalized patients, 100 samples were identified as *Acinetobacter baumannii*, 25 samples were

Acinetobacter lufii and 12 samples were other *Acinetobacter* species. It is given in the diagram below. (Kumar *et al.*, 2021)

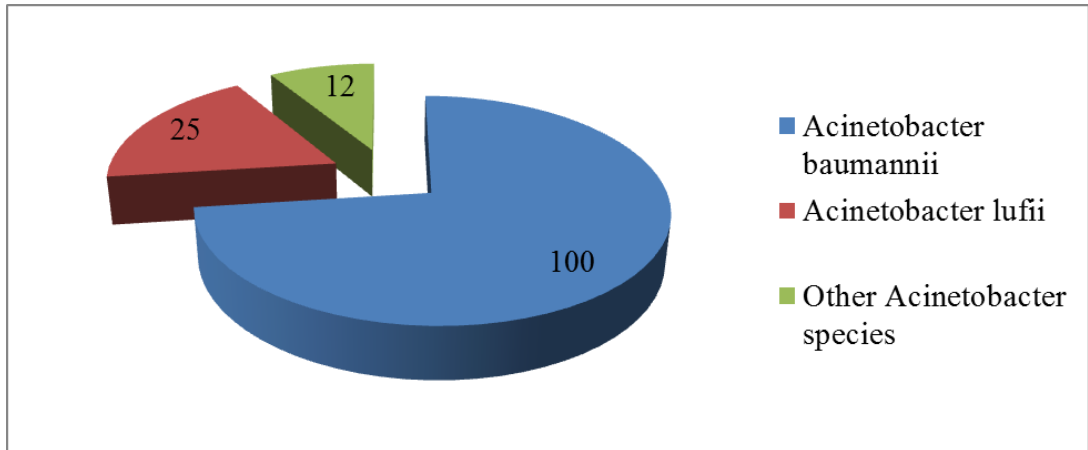


Figure 4-1, Frequency of *Acinetobacter* strains in the examined clinical samples

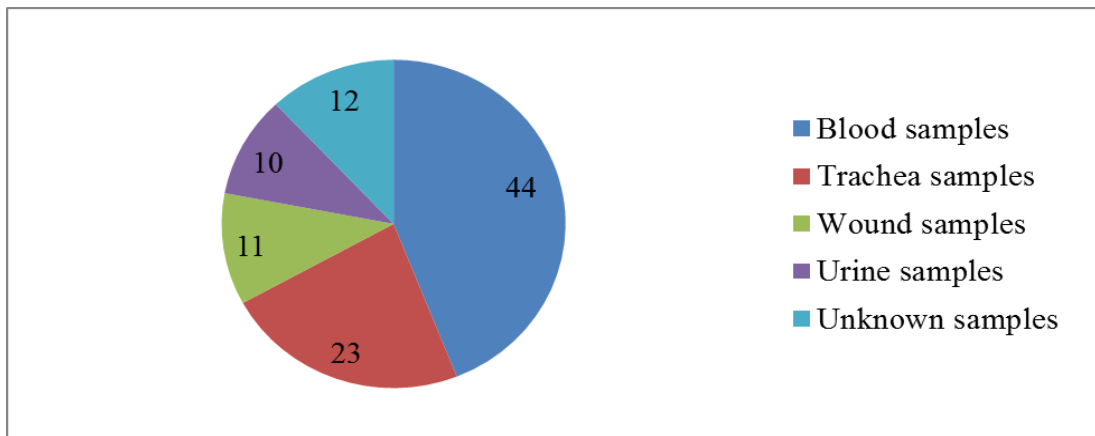


Figure 4-3: Types of samples

The PCR results of Different class of Integrons in all isolates. The DNA was extracted from all isolates by boiling method. To evaluate extracted DNA quality Gel electrophoresis was performed. The OD 260/280 of extracted DNA was approximately 1.8 that suggests best quality of DNA.



Figure 4-3, The quality of Extracted DNA

The frequency of class 1 integron as presented in Figure 4-4, Class 1 Integron was existed in 87 out of 100 isolates. The PCR product was 360bp. Our data showed that 87 percent of our studied patients were positive for class 1 integron.

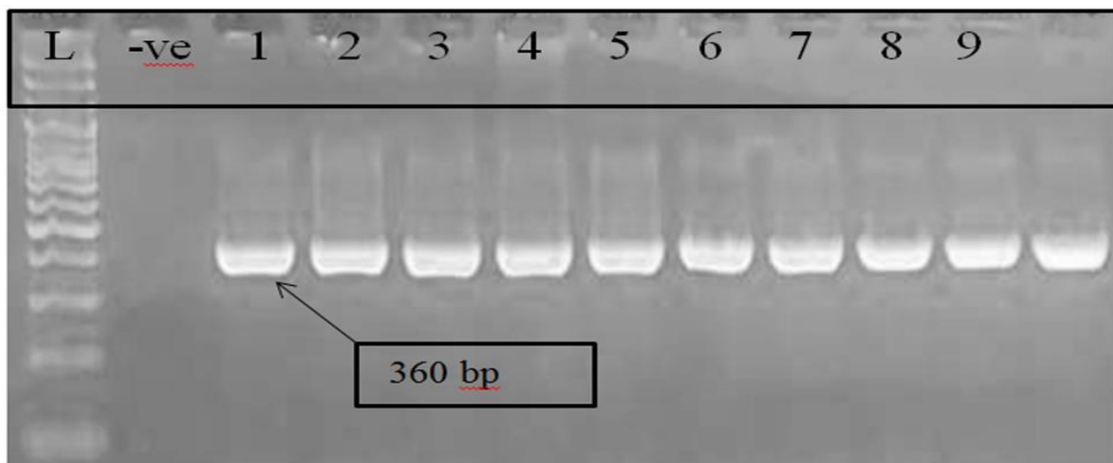


Figure 4-4, The Frequency of class 1 integron among isolates. Each number was stand for each isolates. The PCR product was 360 bp.

As presented in Figure 4-5, Class 2 Integron was existed in 74 out of 100 isolates. The PCR product was 506bp. Therefore our data showed that 74 percent of our studied patients were positive for class 2 integron.(Liebchen *et al.*,2020)

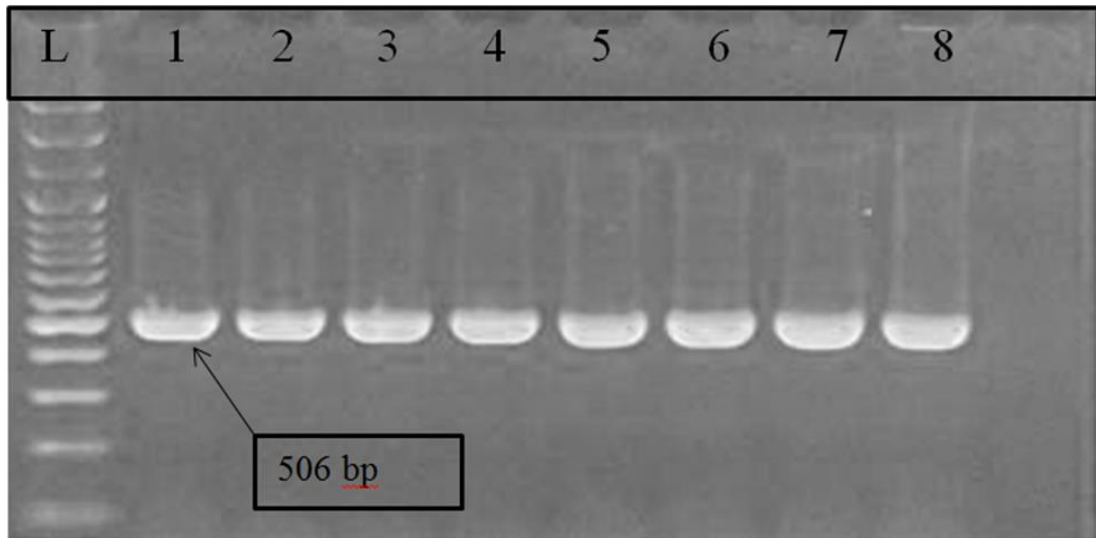


Figure 4-5, The Frequency of class 2 integron among isolates. Each number was stand for each isolates. The PCR product was 506 bp.

5-2 The frequency of class 3 integron

Our data showed that Class 3 Integron was existed in zero out of 100 isolates.. Therefore our data showed that none of our studied patients was positive for class 3 integron.

6. Discussion

Acinetobacter species are gram-negative bacteria that exist widely in soil, water, and human skin. This bacterium is also one of the common contaminants of laboratory and hospital environments. Acinetobacter is similar to Neisseria in gram staining, with the difference that Neisseria is oxidase positive, but Acinetobacter is oxidase negative.(Yadav *et al.*,2020) This organism has been reported as an important factor in hospital infections in recent years. *Acinetobacter baumannii* is the most common species that can be isolated from blood, sputum, pleural fluid, skin and urine. Acinetobacter infection is very dangerous especially in patients who are hospitalized in special care units of hospitals. The most common bacterium of the Acinetobacter group is *Acinetobacter baumannii*, which has recently been recognized as one of the main causes of hospital-acquired infections due to its high level of antimicrobial resistance, including pneumonia, septicemia, urinary tract infections, wound infections, and meningitis. *Acinetobacter baumannii* strains have shown resistance to all antibiotics that have been reported so far. Today, resistance mechanisms in different Acinetobacter species appear in different ways, one of which is the production of broad-spectrum beta-lactamases. (He *et al.*,2011)

Similar with our study Taherikalani *et al* (2011)(19) used a total of 100 non-duplicate *Acinetobacter baumannii* isolates that were collected from different hospitals in Tehran and were confirmed as *A. baumannii* by conventional biochemical and API testing. The isolates were then detected as carrying class 1

and 2 integron gene cassettes by PCR evaluation and then genotyped by REP-PCR. The results showed that more than 80% of all multidrug resistant *A. baumannii* strains carry a class 1 integron. Distribution of Int 1 and Int 2 among *A. baumannii* isolates was 58% and 14%, respectively. Analysis of a conserved segment of class 1 integron showed a range from 100 bp to 2.5 kb (Fluit *et al.*,1999). Other researchers also showed that *Acinetobacter baumannii* is a bacterium with low virulence, which can be found in patients with neutropenia, cystic fibrosis, people undergoing chemotherapy, as well as in Immunocompromised people in hospital environments, especially in intensive care units (ICU), burns and surgery. Being hospitalized can cause severe infections. Infections caused by this bacteria include: hospital infections, urinary tract infections, wound infections, respiratory tract infections (pneumonia), meningitis, endocarditis, peritonitis, skin and soft tissue infections, bacteremia or septicemia (Taherikalani *et al.*,2011).

Similarly, Mehrdad Halaji et al using a cross-sectional study showed that in two teaching hospitals in Isfahan, Iran, from October 2015 to October 2016 and a total of 147 non-duplicate *A. baumannii* isolates using standard microbiological methods and confirmed by genotyping showed that out of 147 confirmed *A. baumannii* isolates, 97.3% of isolates were extensive drug-resistant (XDR) and 2.7% were multidrug-resistant (MDR). Class I and II integrons were detected in 63.9% and 78.2% of the *A. baumannii*, respectively. Class III integron was not detected in any of the isolates (Antunes *et al.*, 2014).

One of the important species of this genus is *Acinetobacter baumannii*, whose colonization rate is increasing in hospitalized people, especially patients who have been hospitalized for a long time or who are being treated with broad-spectrum antibiotics or anti-cancer drugs. (Halaji *et al.*,2018) *Acinetobacter bomani* has been isolated from different environments such as the air of hospitals and most of the equipment used in the hospital, including faucets, ventilation devices and ventilators, etc., also *Acinetobacter bomani* can survive on dry surfaces for days and its resistance Compared to the environmental conditions (11 days in 31% relative humidity and 4 days in 10% relative humidity), the possibility of the presence of this bacterium in hospital environments has increased.(Kempf *et al.*, 2012) This bacteria is found in human secretions such as phlegm, urine, feces and vaginal secretions, 25% of people have this bacteria on the skin surface and 7% of adults and babies have it in the throat area. *Acinetobacter baumannii* is highly resistant to antimicrobial agents, and this resistance can be inherent or acquired through genetic factors. Most *Acinetobacter baumannii* strains are resistant to ampicillin, amoxicillin/clavulanic acid, anti-staphylococcal penicillins, broad-spectrum cephalosporins (except ceftazidime and cefepime), tetracycline, macrolides, rifampin and chloramphenicol. The resistance of this bacterium to non-carbapenemic β -lactams is commonly associated with overproduction of cephalosporinase (Aliramezani *et al.* , 2019)

In addition, Fahimeh Nourbakhsh and coworkers showed that Among the 100 *A. baumannii* studied isolates, the highest and the least resistance was seen for the of Cefepime (89%), Ciprofloxacin (95.4%) Ceftazidime (91.3%); and Chloramphenicol (3.7%) and Nitrofurantoin (2.9%) antibiotics, respectively. The frequency of Class I, II and III integrons was 100%, 44% and 3%, respectively

(Nourbakhsh *et al.*, 2016).

The resistance of clinical isolates to antimicrobial agents may make the treatment of infections related to this bacteria difficult and is associated with unfavorable prognosis and increased treatment costs. Although resistance to carbapenem is increasing, carbapenem is currently used as the drug of choice in the treatment of MDR (multi drug resistance) infections. Carbapenem-resistant strains first caused an increase in hospital infections in 1991, so that these strains have been reported in different countries. This bacterium is the third most common pathogen isolated from hospitalized patients with pneumonia in Brazil. Also, in Turkey, *Acinetobacter baumannii* was the second most common organism in ICU in 2005. Studies show that the percentage of isolates resistant to carbapenems in *Acinetobacter baumannii* is gradually increasing in the last ten years in Europe, North America, Latin America, and the Middle East (Turkey, Lebanon, Iran, Iraq, and the United Arab Emirates). (Liebchen *et al.*, 2020)

Recently, there are reports of *Acinetobacter baumannii* infections resistant to carbapenems in military and civilian people who have returned to their country from the war in Iraq and Afghanistan. During the last decade, the increase of MDR strains of *Acinetobacter baumannii* was probably the result of the wide use of this class of antimicrobial agents. (Yadav *et al.*, 2020) Infection with MDR strains of *Acinetobacter baumannii* has been associated with increased length of hospitalization and higher mortality than infection with sensitive strains. Among different countries, a great difference in the pattern of resistance to antibiotics in *Acinetobacter baumannii* strains has been observed, which also includes developing countries. Drug resistance is spread by a group of identical strains among different cities and countries, and one of the reasons for the resistance of *Acinetobacter baumannii* is the acquisition of CHCDs (carbapenem-hydrolyzing class D β lactamase) genes surrounded by IS (Insertion sequence) elements. The stable presence of antibiotic resistance in *Acinetobacter baumannii* strains. Production of carbapenem-hydrolyzing β -lactamases (carbapenemases) is the most common mechanism of resistance to carbapenems in *Acinetobacter baumannii* (Boucher *et al.*, 2012).

References

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev.* 2008;21(3):538-82.
2. Howard A, O'Donoghue M, Feeney A, Sleator RD. *Acinetobacter baumannii*: an emerging opportunistic pathogen. *Virulence.* 2012;3(3):243-50.
3. Marks LR, Reddinger RM, Hakansson AP. Biofilm formation enhances fomite survival of *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Infection and immunity.* 2014;82(3):1141-6.
4. Jaisankar AI, Girija AS, Gunasekaran S, Priyadharsini JV. Molecular characterisation of *csgA* gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University-Science.* 2020;32(8):3380-7.
5. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. *Clinical microbiology reviews.* 2018;31(4):e00088-17.

6. Ike W, Adeniyi BA, Peters JE. Class 1 Integrons and Associated Gene Cassettes in Multidrug Resistant *Acinetobacter baumannii* from Southwest Nigeria: A Tertiary-Hospitals Study. *Nigerian Journal of Pharmaceutical and Applied Science Research*. 2021;10(1):61-9.
7. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients. *BMC Infect Dis*. 2019;19(1):1-9.
8. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, et al. Estimating the burden of antimicrobial resistance: a systematic literature review. *Antimicrobial Resistance & Infection Control*. 2018;7(1):1-17.
9. Kumar SG, Adithan C, Harish B, Sujatha S, Roy G, Malini A. Antimicrobial resistance in India: A review. *Journal of natural science, biology, and medicine*. 2013;4(2):286.
10. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. *Expert review of anti-infective therapy*. 2013;11(3):297-308.
11. De Oliveira DM, Forde BM, Kidd TJ, Harris PN, Schembri MA, Beatson SA, et al. Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev*. 2020;33(3):e00181-19.
12. Lucidi M, Runci F, Rampioni G, Frangipani E, Leoni L, Visca P. New shuttle vectors for gene cloning and expression in multidrug-resistant *Acinetobacter* species. *Antimicrob Agents Chemother*. 2018;62(4):e02480-17.
13. Tewari R, Chopra D, Wazahat R, Dhingra S, Dudeja M. Antimicrobial susceptibility patterns of an emerging multidrug resistant nosocomial pathogen: *Acinetobacter baumannii*. *The Malaysian journal of medical sciences: MJMS*. 2018;25(3):129.
14. Sato Y, Unno Y, Miyazaki C, Ubagai T, Ono Y. Multidrug-resistant *Acinetobacter baumannii* resists reactive oxygen species and survives in macrophages. *Sci Rep*. 2019;9(1):1-12.
15. Kumar S, Anwer R, Azzi A. Virulence Potential and Treatment Options of Multidrug-Resistant (MDR) *Acinetobacter baumannii*. *Microorganisms*. 2021;9(10):2104.
16. Liebchen U, Paal M, Jung J, Schroeder I, Frey L, Zoller M, et al. Therapeutic drug monitoring-guided high dose meropenem therapy of a multidrug resistant *Acinetobacter baumannii*-A case report. *Respiratory medicine case reports*. 2020;29:100966.
17. Yadav SK, Bhujel R, Hamal P, Mishra SK, Sharma S, Sherchand JB. Burden of multidrug-resistant *Acinetobacter baumannii* infection in hospitalized patients in a tertiary care hospital of Nepal. *Infection and drug resistance*. 2020;13:725.
18. He H, Li M, Guo Y, Luo L, Bhattarai R, Li W. Intracranial Infection Caused by Multidrug-resistant *Acinetobacter baumannii*. *Nepal Journal of Neuroscience*. 2021;18(4):59-63.
19. Taherikalani M, Maleki A, Sadeghifard N, Mohammadzadeh D, Soroush S, Asadollahi P, et al. Dissemination of class 1, 2 and 3 integrons among different multidrug resistant isolates of *Acinetobacter baumannii* in Tehran hospitals, Iran. *Polish Journal of Microbiology*. 2011;60(2):169.
20. Fluit A, Schmitz F. Class 1 integrons, gene cassettes, mobility, and epidemiology. *European Journal of Clinical Microbiology and Infectious Diseases*. 1999;18(11):761-70.

21. Taherikalani M, Maleki A, Sadeghifard N, Mohammadzadeh D, Soroush S, Asadollahi P, et al. Dissemination of class 1, 2 and 3 integrons among different multidrug resistant isolates of *Acinetobacter baumannii* in Tehran hospitals, Iran. *Polish Journal of Microbiology*. 2011;60(2):169.
22. Antunes L, Visca P, Towner KJ. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathogens and disease*. 2014;71(3):292-301.
23. Halaji M, Rezaei A, Zalipoor M, Faghri J. Investigation of class I, II, and III integrons among *Acinetobacter baumannii* isolates from hospitalized patients in Isfahan, Iran. *Oman Medical Journal*. 2018;33(1):37.
24. Aliramezani A, Soleimani M, Fard RMN, Nojoomi F. Virulence determinants and biofilm formation of *Acinetobacter baumannii* isolated from hospitalized patients. *Germs*. 2019;9(3):148.
25. Nourbakhsh F, Nourbakhsh V, Jafakesh MT. Prevalence of class I, II and III integrons in the antibiotic-resistant isolates of *A. baumannii* detected from patients hospitalized in medical centers of Shahrekord. *KAUMS Journal (FEYZ)*. 2016;20(5):461-8.
26. Kempf M, Rolain J-M. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *International journal of antimicrobial agents*. 2012;39(2):105-14.
27. Pogue JM, Mann T, Barber KE, Kaye KS. Carbapenem-resistant *Acinetobacter baumannii*: epidemiology, surveillance and management. *Expert review of anti-infective therapy*. 2013;11(4):383-93.
28. Boucher Y, Labbate M, Koenig JE, Stokes H. Integrons: mobilizable platforms that promote genetic diversity in bacteria. *Trends in microbiology*. 2007;15(7):301-9.