Rwanda Medical Journal RMJ

ORIGINAL ARTICLE Open Access

Distribution of Ten Virulence Genes Along the Chromosome of Acinetobacter baumannii Isolated from Various Clinical Samples

Authors: M.A. Ali Ghaydaa[1,*]; Ghada A. Mohammad[1]

Affiliation: *[1]Department of Biology, College of Science, University of Mosul, Mosul, Iraq*

ABSTRACT

The current study focused on Acinetobacter baumannii because of its increasing importance as a causative agent of infection in hospitals and its resistance to many antibiotics. A total of 204 samples were collected from various cases from main hospitals in Mosul City (Iraq) and were cultured on selective media (HiCromeTM Acinetobacter Agar Base and MacConkey Agar). Bacteria were identified by conventional and molecular methods by detecting the blaOXA-51-like gene, which is genetically considered a diagnostic gene because it is present in all strains of A. baumannii. Results: A total of 18 sample isolates of A. baumannii were positive out of the 204 collected samples distributed in burned skin, respiratory tract infections, wounds and urine. Ten virulence genes (las I, lasR, RhlI, RhlR, cvaC, iutA, kpsMTII, PAI, ibeA, traT) were also detected in our local A. baumannii, the results showed the presence of Quorum Sensing genes (lasI, lasR, RhlI, RhlR) with percentages 84.2%, 84.2%, 89.4%, 26.3%, respectively. PAI gene showed in the 19 bacterial strains (including the standard) with 94.7%. kpsMTII, traT, and iutA genes with percentages of 31.5%, 26.3%, and 5.2% respectively, while cvaC and ibeA genes were not recorded in any of the isolates under study.

Keywords: Acinetobacter baumannii, bla_{OXA-51} gene, Quorum Sensing genes, Virulence factor genes

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is Gram-negative aerobic non-lactose-fermenting coccobacilli, considered an opportunistic bacterium responsible for nosocomial infections due to their virulence factors and its resistance to antibiotics. This bacterium can survive in the hospital environment together with Pseudomonas aerogenosa and causes pneumonia, urinary tract infections, oozing wounds, burns and infections bacteremia, skin infections and infections in intensive care units (ICU), especially in immunocompromised patients. It was classified by the World Health Organization (WHO) as one of the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.) groups [1,2].

The Acinetobacter genus was discovered in the early twentieth century and its name was associated with the Iraq war Iraqibacter. It is

***Corresponding author:** Ghaydaa M.A. Ali, Department of Biology, College of Science, University of Mosul, Almajmoaa Street, 41002, Mosul, Iraq, email: kaysbio106@uomosul.edu.iq; **Potential Conflicts of Interest (CoI):** All authors: no potential conflicts of interest disclosed; **Funding:** All authors: Research funding from Universitas Trisakti No. 0303/PUF/FK/2021-2022; **Academic Integrity.** All authors confirm that they have made substantial academic contributions to this manuscript as defined by the ICMJE; **Ethics of human subject participation:** The study was approved by the local Institutional Review Board. Informed consent was sought and gained where applicable; **Originality:** All authors: this manuscript is original has not been published elsewhere; **Review:** This manuscript was peer-reviewed by three reviewers in a double-blind review process; **Type-editor:** Peter (USA).

Received: 26st January 2024; **Initial decision given:** 28th April 2024; **Revised manuscript received:** 14th June 2024; **Accepted:** 27th July 2024.
Copyright: © The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY-NC-ND) (click here) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Publisher:** Rwanda Biomedical Centre (RBC)/Rwanda Health Communication Center, P. O. Box 4586, Kigali. ISSN: 2079-097X (print); 2410-8626 (online)

Citation for this article: M.A. Ali Ghaydaa; Ghada A. Mohammad et al. Distribution of ten virulence genes along the chromosome of Acinetobacter baumannii isolated from various clinical samples. Rwanda Medical Journal, Vol. 81, no. 3, p. 19-27, 2024. https://dx.doi.org/10.4314/rmj.v81i3.4

non-motile, oxidase negative, and catalase positive, and has a GC content of 38.76–39.7% [3]. It has many species, the most common is A. baumannii. It is found in many environments, including soil, water, and food, in addition to the hospital environment and medical equipment [4]. The diagnosis of bacteria depends on its ability to grow on MacConkey agar, blood agar and other differential media. Also, the Gram stain is considered a beneficial tool for diagnosis. Differential biochemical tests such as Oxidase, Oxidative/Fermentation glucose test, Triple Sugar Iron test, Methyl Red, Vogas–Proskauer, Citrate Utilization, Motility and its ability to grow at a temperature of 44°C are also dependent [5].

A. baumanii diagnosis is definitively based on the Oxa-type genes, especially the subgroup of Carbapenemase gene Oxa-51 using the PCR technique [6]. A. baumannii has many virulence factors: quorum sensing, biofilm formation, endotoxin, iron ligand production and cytotoxic necrotizing factor. Bacterial virulence factors are compounds that increase their potency, such as attaching to the host cell surface and evading the host's immune response [7]. In comparison to virulence factors identified for other Gram-negative bacteria, some virulence factors had been shared with A. baumannii. Virulence factors like efflux pumps, hemolytic factors, iron acquisition systems, lipopolysaccharides, and OmpA can trigger host immunological responses or bacterial adhesion to epithelial cells [5]. Due to the importance of this bacteria in terms of the increase in the spread of its infections due to the different virulence factors it possesses and its high resistance to antibiotics, we decided in this study to conduct a rapid survey to estimate the percentage of its strength from different clinical samples to reveal the extent of its dominance in clinical cases and then investigate what these isolates possess Virulence factors by detecting the genes in its genome through the Multiplex PCR technique.

METHODS

Specimens Collection and Culturing: 204 samples were collected from different clinical cases in Mosul City hospitals (Iraq). These hospitals include: Al-Salam Teaching Hospital, Al-Khansaa Teaching Hospital, Al-Zahrawi Teaching Hospital, Mosul General Hospital, Ibn-Sina Teaching Hospital, Albatool Teaching hospital Public Health Laboratory, and Mosul Specialized Center for Burns and Plastic Surgery

The samples included 89 burns, 40 respiratory tract infections, 33 wounds, 17 blood, 13 vaginal swabs, and 12 urine, in addition to using the standard strain of A. baumannii 19606, kindly provided by Medya Diagnostic Center in Erbil (Iraq).

Samples were transferred under aseptic conditions and cultured on MacConkey agar (Figure 1A) and selective medium Hicrome Acinetobacter agar Base (Figure 1B). The plates were incubated at 37oC for 24 h. The necessary biochemical tests were conducted for the initial diagnosis of A. baumannii. The tests included Oxidase, Catalase, Triple Sugar Iron test, Citrate Utilization and growth at a temperature of 44°C.

Colonies of A. baumannii appeared in the MacConkey medium as pale yellow colonies due to not fermenting the lactose, with a smooth round shape, about 2-3 mm in diameter, While its colonies appeared on the HiCromeTM Acinetobacter Agar Base as a glossy violet colour and smooth round shape with 2-3 mm in diameter.

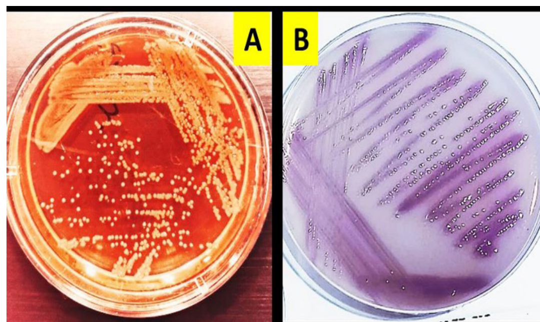

Figure 1: Colonies of cultured A. baumannii on (A) MacConkey agar and (B) Hicrome agar

Genetic extraction and detection: The DNA of the suspected bacteria of being A. baumannii was extracted by using a DNA Extraction kit from (AddBio, Korea). A Nanodrop spectrophotometer was used to measure the concentration and purity, and finally, the electrophoresis was carried out in an agarose gel of 2% to detect the DNA.

Gene design and identification: The primers were blasted as forward and reverse with suitable specific base pair sizes as per Table 1. The presence of the bla oxa-51-like gene was investigated in all local isolates to ensure that they belong to the species A. baumannii, as this gene was relied upon as a diagnostic gene for this bacterium because it is present in the genome of all A. baumannii

bacteria. Therefore, it is differentiated from the rest species belonging to the genus Acinetobacter, this gene has a molecular size of 353 base pairs. Table 1 shows the primers used to identify the diagnostic gene [8]. The genes were investigated using the PCR technique, and the PCR mixture was prepared as per the manufacturer's instructions [9].

Electrophoresis was carried out after the preparation of agarose gel 2%. 8 µL of each sample of DNA amplification products resulting from PCR were added to agarose wells. 5 µL of DNA ladder was added in the first well, and the electrical migration was carried out by applying an electric potential difference to the tray of 50 volts for 60 minutes. Then, agarose was placed in a transilluminator apparatus. The Gel Documentation System was used to detect amplified products and the appearance of bands. To determine the presence or absence of some virulence genes: (LasI, LasR, rhlI, rhlR, cvaC, iutA, kpsMT II, PAI, ibeA, traT) in our local bacterial isolates, a PCR mixture was prepared as the total size 25 µL, includes 4 µL DNA (50 ng/µL), primers (10 µmol) for each forward and reverse primer (1 µL/ primer), 12.5µL master mix (2X) and 6.5 µL nuclease-free water. Multiplex PCR program (standard steps of primary denaturation, denaturation, annealing, extension, and final extension) is used to detect virulence genes optimized for each gene [10,11].

RESULTS

Results of identification: After collection of 204

Figure 1: The primers of diagnostic and virulence genes and their molecular sizes

	The gene	Primer sequences (5´-3´)	Gene size bp
Primers of virulence genes	LasI	F-TCGACGAGATGGAAATCGATG R-GCTCGATGCCGATCTTCAG	402 bp
	LasR	F-TGCCGATTTTCTGGGAACC R-CCGCCGAATATTTCCCATATG	401 bp
	rhlI	F- CGAATTGCTCTCTGAATCGCT R-GGCTCATGGCGACGATGTA	182 bp
	rhlR	F-TCGATTACTACGCCTATGGCG R-TTCCAGAGCATCCGGCTCT	208 bp
	cvaC	F-CACACACAAACGGGAGCTGTT R-CTTCCCGCAGCATAGTTCCAT	680 bp
	iutA	F-GGCTGGACATCATGGGAACTGG R-CGTCGGGAACGGGTAGAATCG	300 bp
	kpsMT II	F-GCGCATTTGCTGATACTGTTG R-CATCCAGACGATAAGCATGAGCA	272 bp
	PAI	F-GGACATCCTGTTACAGCGCGCA R-TCGCCACCAATCACAGCCGAAC	930 bp
	ibeA	F-AGGCAGGTGTGCGCCGCGTAC R-TGGTGCTCCGGCAAACCATGC	170 bp
	traT	F-GGTGTGGTGCGATGAGCACAG R-CACGGTTCAGCCATCCCTGAG	290 bp
Diagnostic target gene (bla_{OXA-51} gene)	bOXA-51-F bOXA-51-R	F-TAATGCTTTGATCGGCCTTG R-TGGATTGCACTTCATCTTGG	353 bp

samples from Mosul hospitals from different clinical cases, including 89 burns, 40 respiratory tract infections, 33 wounds, 17 blood, 7 vaginal swabs, and 12 urine (Table 2). The results showed that 93 samples had no growth on the culture media. These 112 samples had mixed growth on the culture media, these gave 136 bacterial isolates on MacConkey agar. Suspected colonies were subcultured using the streaking method to obtain pure bacteria.

A. baumannii isolates were negative for the oxidase test, catalase was positive, TSI was k/k, lack of gas production, positive for citrate utilization, and it succeeded in growth at temperatures of 37 °C and 44°C.

The results of the PCR technique revealed a band with a molecular weight of 353bp, as shown in Figure 2. According to this result, 18 bacterial strains belonging to the species of A. baumannii had an 8.7% isolation percentage.

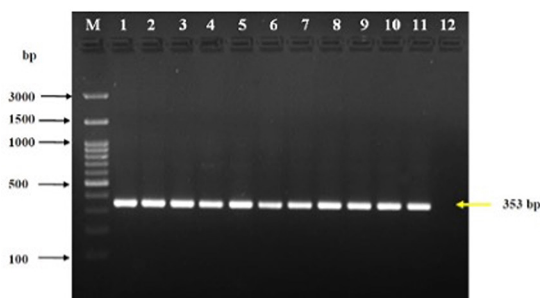

Figure 2: Electrophoresis of PCR product for bla_{oxa-51} like 353 bp for 11 A. baumannii isolates

Sample isolates: Eighteen isolates of A. baumannii were collected from different clinical settings of Mosul hospitals representing 8.8% positive samples from overall collected cases, distributed as 10.1% from burned skin, 12.5% from Respiratory tract infections, 6% from wounds and urine, while blood and vagina were negative cases (table 2). A. baumannii is prevalent in Mosul hospitals and medical attention is required to control and prevent the spread of this bacterium.

Distribution of the virulence genes among local A. baumannii isolates: Ten genes were used for the investigation of virulence factors molecularly in A. baumannii (las I, lasR, RhlI, RhlR, cvaC, iutA, kpsMTII, PAI, ibeA, traT) in local strains by using the Multiplex PCR technique and specific primers for each. The molecular weight of the products of each gene was detected by ladder. Table 3 shows the distribution of virulence genes in local A. baumannii isolates as well as the standard strain ATCC A. baumannii 19606. The results show that our isolates have different genes of virulence factors.

Quorum sensing genes (QS): The genes of Quorum sensing (lasI, lasR, RhlI, and RhlR) were investigated in all isolates. The results showed the presence of these genes in local isolates by 15 isolates for each of lasI and lasR with 83.3%. The gene rhlI was found in 16 isolates, with 88.8%, while 4 isolates contained gene rhlR with 22.2%.

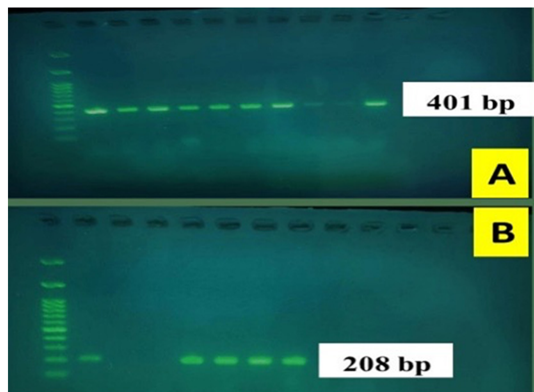

Figure 3: Electrophoresis of PCR product for (A) lasR gene 401 bp of A. baumannii isolates, (B) RhlR gene 208 bp of A. baumannii isolates.

Table 2: Sample sources and the number of A.baumannii isolates.

Sample source	Total number of sample	No.(%) of A.baumannii isolates	% of total samples
Burns	89	9 (10.1)	4.3
Respiratory tract infections	40	5 (12.5)	2.4
Wounds	33	2 (6.0)	1
Blood	17	0 (0.0)	0
Vaginal swab	13	0 (0.0)	0
Urine	12	2 (6.0)	1
Total of samples	204	18	8.7

Table 1: The distribution of virulence genes in A. baumannii isolates

Sample source	NO. of isolates	lasI	lasR	RhlI	RhlR	cvaC	iutA	kpsMTII	PAI	ibeA	traT
Burns	1	1	1	1	0	0	0	0	1	0	0
	2	1	1	1	0	0	0	0	1	0	0
	3	1	1	1	0	0	0	0	1	0	0
	4	1	1	1	0	0	0	0	1	0	0
	5	1	1	1	0	0	0	0	1	0	0
	6	1	1	1	0	0	1	0	1	0	1
	7	1	1	0	0	0	0	0	1	0	0
	8	0	1	1	0	0	0	1	1	0	0
	9	1	0	1	0	0	0	1	0	0	0
Respiratory tract infections	10	1	0	1	0	0	0	0	1	0	0
	11	0	0	0	0	0	0	0	1	0	0
	12	1	1	1	1	0	0	1	1	0	1
	13	1	1	1	1	0	0	1	1	0	1
	14	1	1	1	1	0	0	1	1	0	1
Wounds	15	1	1	1	1	0	0	1	1	0	1
	16	1	1	1	0	0	0	0	1	0	0
Urine	17	1	1	1	0	0	0	0	1	0	0
	18	0	1	1	0	0	0	0	1	0	0
Total (%)		15	15	16	4	0	1	6	17	0	5
		83.3	83.3	88.8	22.2	0	5.5	33.3	94.4	0	27.7
A. baumannii 19606	S	1	1	1	1	0	0	0	1	0	0

DISCUSSION

One of the most effective organisms causing nosocomial infections in the current healthcare system is A. baumannii because of their capacity to persist in a hospitalized setting for an extended period, biofilm development, lipopolysaccharide, outer membrane proteins, and the protein secretion system are all virulence characteristics that allow these bacteria to survive in the harsh conditions of a hospitalized environment [12].

A. baumannii is frequently isolated in nosocomial infections, especially in intensive care units, since they attack debilitated and immunocompromised patients; in addition, they have a high tolerance against antibiotics and an inherent ability to acquire antibiotic resistance genes, being, therefore, a serious emergent health problem. Their QS systems consist of homologues of the LuxR and LuxI proteins in Vibrio fischeri, known as AbaR receptor and AbaI (synthase) and play a role in biofilm formation and motility in Acinetobacter species. This QS system is an important virulence factor responsible for the outstanding antibiotic resistance and survival properties in the latter species [13].

The results revealed that 12 isolates shared the presence of each of the lasI, lasR, and rhlI genes, while 4 isolates shared the presence of the four genes combined. Elnegery et al. mentioned that lasR was detected in 47 (94%) out of 50 P. aeruginosa isolates collected from infected burn wounds and only three isolates did not harbour the gene [14]. The rhlR gene was detected in 45 (90%) out of 50 P. aeruginosa isolates collected, while only five 10% isolates did not harbour the gene.

Synthesis of N-(3-hydroxydodecanoyl)-L-HSL (3-hydroxy-C12-HSL) is catalyzed by abaI from A. baumannii. The completed genome sequence of A. baumannii strain ATCC 17978 indicates that

autoinducer synthase abaI (gene A1S_110) and acyltransferases may be the sole participants in the synthesis of AHL signals of variable chain length by the organism. Many strains of Acinetobacter 63% produce more than one AHL. None of the AHL signals can be specifically assigned to a particular species of the genus. Acinetobacter quorum signals are not homogenously distributed, and therefore, the distinction between virulent and non-virulent strains based on QS signals is difficult. Communication between bacteria concerning cell density is integral to the maturation of Acinetobacter spp. biofilm and efflux pump [15,16].

The iron uptake (siderophores) system consists of Yersinobactin (fyuA gene) and aerobactin (iutA gene). The ability of the two genes that encode for these two proteins to help the bacteria to survive when grown in low-iron conditions, the results of the study showed the presence of iutA in only one isolate from the total 18 A. baumannii by 5.5%. This finding may be because no limitation of iron is there, the results were similar to the study conducted by Ahmed and Mohammad, where the gene iutA was present in two isolates from a total of 19 pathological isolates [17]. Also, our results were consistent with the study that was not recorded in their isolates from cases of urinary tract infection [18]. Darvishi indicated that the percentage of gene isolation was 25% [4]. The reason for the non-existence of the iutA gene in our invaders may be due to its have of the types of iron carriers Siderophores other than it was targeted in our study. iutA gene works on encoding a membrane protein that is important in taking iron and converting it into Ferric iron through transferase enzyme and thus increases the growth of microorganisms in the tissue and body fluids of the host, which increases the pathogenicity of the bacteria.

The presence of the kpsMTII gene in our results was 6 isolates from a total of 18 A. baumannii 33.3%. Zeighami et al. mentioned that the percentage of the presence of genes was 57%, which is higher than the percentage of our study, and it may return to the difference in the numbers of isolates [19]. Adhesive Virulence Factors are subdivided into two parts; Fimbrial VF: which includes fim H, Sfa/ focDE, pap and Non fimbrial VF: which includes csg, fnb, kps MT. baumannii has many biofilm-related genes (bfmS, epsA, CsgA, pgaB, Kps MT, amp A, bap, bla PER-1, CsuE) [20]. The possession of the bacteria to the capsule it able to resist difficult environmental conditions such as heat and drought, and to survive on living bodies and nonliving surfaces The bacteria containing the kpsMT gene encodes the polysaccharide layer that surrounds the cell and thus form a capsule that is considered one of the adhesion factors [21].

Pathogenicity Islands transform genes that encode one or more virulence factors such as adhesins, toxins, and invasins. PAI is located on the bacterial chromosome or may be transmitted by plasmid [22]. The results of the study showed a distinct presence of the PAI gene in our local isolates, 17 isolates of A. baumannii were carriers of the PAI gene by 94.4%. Our results did not agree with the results of the researcher's study by Al Mahdi et al., in Babylon City (Iraq), as the percentage of gene presence was 28.5% with two isolates out of a total of 7 A.baumannii [23]. This percentage was considered low compared to our study, and the reason may be that PAI is not stable and is deleted at high frequencies, and it is possible to lose large parts of them or even lose them completely [24]. Because there have not been prior investigations on the PAI gene in A.baumnnii, other studies conducted by Bagaya et al. and Rezatofighi et al., indicate its presence in E. coli bacteria [25,26]. Therefore, its presence in our isolates may be attributed to its transmission between related isolates. PAI is part of the group of flexible genes, its prevalence has become richer than previously thought, and it has become one of the most common genetic formations found in the genomes of many types of bacteria, and the name (Pathogenicity Islands) has been changed to (Genomic Islands) encode a wide range of functions that carry genes useful for the survival and transmission of bacteria [27].

The ibeA gene encods to invasion protein A weighting 50KDa. The presence of the gene was not recorded in any isolates, and compared to what was stated in the study conducted by Momtaz et al., the percentage of presence was 12.39% of the total of 121 of A. baumannii, which is considered a low percentage [28]. Al-Mahdi et al., mentioned in their study on the isolated A. baumannii from the wounds in Hilla City (Iraq) that the percentage of the presence of ibe A was 71.4% with 5 isolates out of a total of 7, it highest compared to local studies, and the researcher may have to explain the different results to the different types of clinical samples (blood, burn, wounds--etc), as the presence of the gene determines the location of the sample [23].

Because there have not been prior investigations on the ibeA gene in A.baumnnii, we compare to studies that were conducted on E.coli . where ibeA encoded in E.coli a virulence factor responsible for meningococcal evasion in the newborn genome of 213 pathogenic E.coli (APEC), it can penetrate the microvascular endothelium cells and cross the blood to the brain; thus, ibeA gene is directly related to the pathogenic strains [29].

Our study did not show the presence of the cvaC gene in any of the local A. baumannii isolates under study. This result was not consistent with a study conducted in Baghdad City (Iraq) by Abdullah and Ahmed. In their study, the isolates containing cvaC reached 16 isolates out of a total of 38 A. baumannii [30], and our results did not agree with many studies, including Al Kadmy et al. and Momtaz [12,28]. cvaC gene is responsible for the production of Colicin protein, a protein produced by many bacterial strains such as E.coli and many Shigella, Citrobacter, to Enterobacteriaceae. Colicin is divided into types A, B, D, V, and I. It consists of 103 amino acids, it is an antibacterial peptide that kills some bacterial species that contain its receptors, and therefore it works to change the cell membrane [31].

The traT gene is a non-adhesive virulence factor responsible for encoding a protein that inhibits the classical pathway of complement activation. It is part of an F-like plasmid and is thus serum serum-resistant. The results showed the presence of traT of an operon in our local isolates in 5 isolates with a rate of 27.7%. A study conducted by Parviz et al. in Iran revealed 50 isolates of A. baumannii the presence of the gene in 40 isolates with 80% [11], which is considered a higher percentage compared to our study, while a study by Momtaz et al., showed that the presence of traT gene in 121 A. baumannii was only 1 isolate carrying the gene at a rate of 11.57% [28], and this percentage is lower than that of our study.

While the study contributes to our knowledge of the distribution of virulence genes in A. baumannii, it is important to acknowledge its limitations, such as the small sample size, limited gene selection, reliance on PCR techniques, lack of phenotypic analysis, and neglect of environmental factors. Future research should aim to address these limitations to obtain a more comprehensive understanding of the pathogenicity of this bacterium.

CONCLUSION

According to the results obtained, the bacteria are prevalent in our society with a percentage not to be underestimated, and the highest presence of these bacteria is among patients with burn injuries. Moreover, the spread of pathogenic genes in A. baumannii, especially the gene PAI and the genes lasI, lasR, rhlI, and rhlR, indicates their ability to acquire recombinants continuously, especially gene PAI, which is a jumping gene, as well as genes lasI, lasR, rhlI, rhlR, which is affected by the vicinity of bacteria. So, the necessary measures must be taken by health institutions to take these bacteria seriously and limit their spread.

REFERENCES

1. Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; et al. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. The Lancet Infectious Diseases 2018, 18, 318–327, doi:10.1016/S1473-3099(17)30753-3.

2. Antunes, L.C.S.; Visca, P.; Towner, K.J. Acinetobacter Baumannii : Evolution of a Global Pathogen. Pathogens Disease 2014, 71, 292–301, doi:10.1111/2049-632X.12125.

3. Peykov, S.; Strateva, T. Whole-Genome Sequencing-Based Resistome Analysis of Nosocomial Multidrug-Resistant Non-Fermenting Gram-Negative Pathogens from the Balkans. Microorganisms 2023, 11, 651, doi:10.3390/microorganisms11030651.

4. Darvishi, M. Virulence Factors Profile and Antimicrobial Resistance of Acinetobacter Baumannii Strains Isolated from Various Infections Recovered from Immunosuppressive Patients. Biomed. Pharmacol. J. 2016, 9, 1057–1062, doi:10.13005/bpj/1048.

5. Aliramezani, A.; Soleimani, M.; Fard, R.M.N.; Nojoomi, F. Virulence Determinants and Biofilm Formation of Acinetobacter Baumannii Isolated from Hospitalized Patients. Germs 2019, 9, 148–153, doi:10.18683/germs.2019.1171.

6. Kim, M.H.; Jeong, H.; Sim, Y.M.; Lee, S.; Yong, D.; Ryu, C.-M.; Choi, J.Y. Using Comparative Genomics to Understand Molecular Features of Carbapenem-Resistant Acinetobacter Baumannii from South Korea Causing Invasive Infections and

Their Clinical Implications. PLoS ONE 2020, 15, e0229416, doi:10.1371/journal.pone.0229416.
7. Amala Reena, Aa.; Subramaniyan, A.; Kanungo, R. Biofilm Formation as a Virulence Factor of Acinetobacter Baumannii: An Emerging Pathogen in Critical Care Units. J Curr Res Sci Med 2017, 3, 74, doi:10.4103/jcrsm.jcrsm_66_17.
8. Baker, A.; Makled, A.; Salem, E.; Salama, A.; Ajlan, S. Phenotypic and Molecular Characterization of Clinical Acinetobacter Isolates from Menoufia University Hospitals. Menoufia Med J 2017, 30, 1030, doi:10.4103/mmj.mmj_452_17.
9. Khalid, I.; Sobhi Nayyef, N.; M. Merkhan, M. A Taxonomic Study Comparing the Two Types of Medicinal Leeches Available in Iraq. RJPT 2022, 1119–1122, doi:10.52711/0974-360X.2022.00187.
10. AlQumaizi, K.I.; Kumar, S.; Anwer, R.; Mustafa, S. Differential Gene Expression of Efflux Pumps and Porins in Clinical Isolates of MDR Acinetobacter Baumannii. Life 2022, 12, 419, doi:10.3390/life12030419.
11. Mohajeri, P.; Sharbati, S.; Farahani, A.; Rezaei, Z. Evaluate the Frequency Distribution of Nonadhesive Virulence Factors in Carbapenemase-Producing Acinetobacter Baumannii Isolated from Clinical Samples in Kermanshah. J Nat Sc Biol Med 2016, 7, 58, doi:10.4103/0976-9668.175071.
12. AL-Kadmy, I.M.S.; Ali, A.N.M.; Salman, I.M.A.; Khazaal, S.S. Molecular Characterization of Acinetobacter Baumannii Isolated from Iraqi Hospital Environment. New Microbes and New Infections 2018, 21, 51–57, doi:10.1016/j.nmni.2017.10.010.
13. Stacy, D.M.; Welsh, M.A.; Rather, P.N.; Blackwell, H.E. Attenuation of Quorum Sensing in the Pathogen Acinetobacter Baumannii Using Non-Native N -Acyl Homoserine Lactones. ACS Chem. Biol. 2012, 7, 1719–1728, doi:10.1021/cb300351x.
14. Elnegery, A.A.; Mowafy, W.K.; Zahra, T.A.; Abou El-Khier, N.T. Study of Quorum-Sensing LasR and RhlR Genes and Their Dependent Virulence Factors in Pseudomonas Aeruginosa Isolates from Infected Burn Wounds. Access Microbiology 2021, 3, doi:10.1099/acmi.0.000211.
15. Castillo-Juárez, I.; Maeda, T.; Mandujano-Tinoco, E.A.; Tomás, M.; Pérez-Eretza, B.; García-Contreras, S.J.; Wood, T.K.; García-Contreras, R. Role of Quorum Sensing in Bacterial Infections. WJCC 2015, 3, 575, doi:10.12998/wjcc.v3.i7.575.
16. He, X.; Lu, F.; Yuan, F.; Jiang, D.; Zhao, P.; Zhu, J.; Cheng, H.; Cao, J.; Lu, G. Biofilm Formation Caused by Clinical Acinetobacter Baumannii Isolates Is Associated with Overexpression of the AdeFGH Efflux Pump. Antimicrob Agents Chemother 2015, 59, 4817–4825, doi:10.1128/AAC.00877-15.
17. Ahmad, N.H.; Mohammad, G.A. Identification of Acinetobacter Baumannii and Determination of MDR and XDR Strains. Baghdad Sci.J 2020, 17, 0726, doi:10.21123/bsj.2020.17.3.0726.
18. Braun, G.; Vidotto, M.C. Evaluation of Adherence, Hemagglutination, and Presence of Genes Codifying for Virulence Factors of Acinetobacter Baumannii Causing Urinary Tract Infection. Mem. Inst. Oswaldo Cruz 2004, 99, 839–844, doi:10.1590/S0074-02762004000800010.
19. 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, 629, doi:10.1186/s12879-019-4272-0.
20. Eze, E.; Chenia, H.; El Zowalaty, M. Acinetobacter Baumannii Biofilms: Effects of Physicochemical Factors, Virulence, Antibiotic Resistance Determinants, Gene Regulation, and Future Antimicrobial Treatments. IDR 2018, Volume 11, 2277–2299, doi:10.2147/IDR.S169894.
21. Tomaras, A.P.; Dorsey, C.W.; Edelmann, R.E.; Actis, L.A. Attachment to and Biofilm Formation on Abiotic Surfaces by Acinetobacter Baumannii: Involvement of a Novel Chaperone-Usher Pili Assembly System. Microbiology 2003, 149, 3473–3484, doi:10.1099/mic.0.26541-0.
22. Levine, M.M.; Kotloff, K.L.; Nataro, J.P.; Muhsen, K. The Global Enteric Multicenter Study (GEMS): Impetus, Rationale, and Genesis. Clinical Infectious Diseases 2012, 55, S215–S224, doi:10.1093/cid/cis761.
23. AL Mahdi, Z. A., Bunyan, I. A., & AL Shukri, M. S.(MOLECULAR STUDY FOR SOME VIRULENCE FACTORS OF ACINETOBACTER BAUMANNII ISOLATED FROM PATIENTS WITH WOUND INFECTION IN HILLA CITY.
24. Gal-Mor, O.; Finlay, B.B. Pathogenicity Islands: A Molecular Toolbox for Bacterial Virulence. Cell Microbiol 2006, 8, 1707–1719, doi:10.1111/j.1462-5822.2006.00794.x.
25. Bagaya, J.; Ssekatawa, K.; Nakabiri, G.; Nsubuga, J.; Kitibwa, A.; Kato, C.D.; Sembajwe, L.F. Molecular Characterization of Carbapenem-Resistant Escherichia Coli Isolates from Sewage at Mulago National Referral Hospital, Kampala: A Cross-Sectional Study. Ann Microbiol 2023, 73, 28,

doi:10.1186/s13213-023-01732-9.
26. Rezatofighi, S.E.; Mirzarazi, M.; Salehi, M. Virulence Genes and Phylogenetic Groups of Uropathogenic Escherichia Coli Isolates from Patients with Urinary Tract Infection and Uninfected Control Subjects: A Case-Control Study. BMC Infect Dis 2021, 21, 361, doi:10.1186/s12879-021-06036-4.
27. Koga, V.L.; Tomazetto, G.; Cyoia, P.S.; Neves, M.S.; Vidotto, M.C.; Nakazato, G.; Kobayashi, R.K.T. Molecular Screening of Virulence Genes in Extraintestinal Pathogenic Escherichia Coli Isolated from Human Blood Culture in Brazil. BioMed Research International 2014, 2014, 1–9, doi:10.1155/2014/465054.
28. Momtaz, H.; Seifati, S.M.; Tavakol, M. Determining the Prevalence and Detection of the Most Prevalent Virulence Genes in Acinetobacter Baumannii Isolated From Hospital Infections. International Journal of Medical Laboratory 2015, 2, 87–97.
29. Ikeda, M.; Kobayashi, T.; Fujimoto, F.; Okada, Y.; Higurashi, Y.; Tatsuno, K.; Okugawa, S.; Moriya, K. The Prevalence of the iutA and ibeA Genes in Escherichia Coli Isolates from Severe and Non-Severe Patients with Bacteremic Acute Biliary Tract Infection Is Significantly Different. Gut Pathog 2021, 13, 32, doi:10.1186/s13099-021-00429-1.
30. Abdullah, R.; Ahmed, R. Sequencing Analysis of cvaC Gene in Acinetobacter Baumannii That Isolates from Different Infections. JASN 2021, 1, 24–31, doi:10.53293/jasn.2021.3782.1042.
31. Gérard, F.; Pradel, N.; Wu, L.-F. Bactericidal Activity of Colicin V Is Mediated by an Inner Membrane Protein, SdaC, of Escherichia Coli. J Bacteriol 2005, 187, 1945–1950, doi:10.1128/JB.187.6.1945-1950.2005.