

Role of Pseudomonas Quinolone Signal in Pathogenesis and Drug Resistance of *Acinetobacter baumannii*

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Abstract:

This study investigates the frequency of five essential efflux pump genes (*AdeM*, *AdeG*, *AdeF*, *AdeH*, and *AdeL*) in clinical isolates of *Acinetobacter baumannii* and how they relate to multidrug resistance (MDR). Using biochemical and molecular methods such as the API 20NE and VITEK 2 Compact systems, 26 isolates were obtained from hospitalized patients in Baghdad between November 2020 and February 2021 and identified as *A. baumannii*. 96% of isolates were also resistant to cefotaxime, colistin, piperacillin/tazobactam, and, amoxicillin/clavulanate, according to antibiotic susceptibility testing conducted on Mueller-Hinton agar in accordance with CLSI standards. High resistance rates were also noted for aminoglycosides (amikacin, gentamicin), ciprofloxacin, and trimethoprim-sulfamethoxazole. Resistance rates to imipenem, tetracycline, and meropenem ranged from 65% to 85%. Polymerase chain reaction (PCR) amplification with certain primers and gel electrophoresis were used to identify efflux pump genes. All isolates (100%) had the *AdeM* gene, whereas 23% had *AdeG*, 38% had *AdeL*, and 7.6% had both *AdeF* and *AdeH*. Most of the isolates had a clonal origin, according to genomic studies, and a shared genetic profile suggested that they spread throughout hospital environments. These results demonstrate the vital role efflux pump systems play in mediating MDR in *A. baumannii* and the pressing need for focused approaches to lessen the effects of these mechanisms and lower the number of hospital-acquired infections.

Keywords: *Acinetobacter baumannii*, QS genes, Efflux Pump, MDR.

دور جينات مضخات التدفق في الأمراض ومقاومة المضادات في بكتيريا

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مستخلص البحث:

تهدف هذه الدراسة إلى الكشف عن خمسة جينات أساسية لمضخات التدفق (*AdeM*)، *AdeG*، *AdeF*، *AdeH*، و *AdeL*) في العزلات السريرية لبكتيريا *Acinetobacter baumannii* وعلاقتها بمقاومة الأدوية المتعددة (MDR). تم جمع 26 عزلة من المرضى في مستشفيات بغداد خلال الفترة من تشرين الثاني 2020 إلى شباط 2021، وتم تحديدها على أنها *A. baumannii* باستخدام الطرق البيوكيميائية والجزئية مثل أنظمة API 20NE و VITEK 2 Compact. أظهرت اختبارات حساسية المضادات الحيوية وفقاً لمعايير CLSI على وسط Mueller-Hinton أن 96% من العزلات مقاومة لمضادات مثل السيوفوناكسيم، الكولستين، البيراسيلين/تازوباكتام، والأموكسيسيلين/كلافولانات. كما لوحظت معدلات مقاومة عالية للأمينوغليكوزيدات (مثل أميكاسين وجنتاميسين)، السيبروفلوكساسين، والتريميثوبريم-سلفاميثوكسازول، بينما تراوحت معدلات المقاومة للإيميبينيم، التتراسيكلين، والميروبينيم بين 65% و 85%. تم تحديد جينات مضخات التدفق باستخدام تقنية تفاعل البلمرة المتسلسل (PCR) والترحيل الكهربائي في الهلام وأظهرت النتائج أن جميع العزلات وبنسبة (100%) تحتوي على جين *AdeM*، بينما كان جين *AdeG* موجوداً في 23% من العزلات و *AdeL* في 38% و *AdeF* و *AdeH* في 7.6% من العزلات فقط. أظهرت الدراسات الجينية أن معظم العزلات تعود إلى نسيلة واحدة مما يشير إلى انتشارها في البيئات داخل المستشفيات من خلال نمط جيني مشترك. أذ تؤكد النتائج الدور الحيوي لمضخات التدفق في تعزيز مقاومة الأدوية المتعددة في *A. baumannii* مما يستدعي تطبيق استراتيجيات مركزة لتقليل تأثير هذه الآليات والحد من العدوى المكتسبة في المستشفيات.

الكلمات المفتاحية: *Acinetobacter baumannii*، استشعار النصاب، مضخة التدفق، مقاومة الأدوية المتعددة.

1. Introduction

Acinetobacter baumannii is a prototypical non-fermentative Gram-negative, bacilli shaped, aerobic and a non-flagellated bacterium making it a major opportunistic infection well known for its multiple antibiotic resistance in the control of infections particularly in the Intensive care unit (Ali, IR and Majeed, SH.2021; Abed, E., and Ali, M. 2020). This bacterium is also a member of the 'ESKAPE' group of pathogens which are among the most notorious drug resistant pathogens which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species* (Jain *et al* ,2024). This group of organisms can avoid the action of microbes which is the reason behind outbreaks of all infections in hospitals. *A. baumannii* exhibits resistance to key antibiotic classes, including carbapenems, cephalosporins, aminoglycosides, fluoroquinolones, and polymyxins. This resistance results from mechanisms such as enzymatic inactivation, efflux pumps, and target site modifications, making it one of the most challenging pathogens to treat and control. The antibiotic resistance and virulence of *A. baumannii* are driven by β -lactamases like OXA-type carbapenems (e.g., OXA-23 and OXA-24) and efflux pump proteins encoded by genes such as *AdeM* and *AdeG*. These enzymes degrade and expel antibiotics, while the capsule, pili, and other enzymes facilitate host cell destruction and infection. (Lee *et al* ,2017). *A. baumannii* is highly capable of acquiring resistance to antimicrobial agents, including carbapenems, which were previously considered first-line treatments. As resistance to these drugs has increased, therapeutic options have become more limited (Chen *et al* ,2020). Mechanisms of resistance involve the production of β -lactamase enzymes and efflux pumps. Additionally, the bacterium's ability to form biofilms further complicates eradication with conventional antibiotics (Lee *et al* ,2017).

Given the widespread resistance, researchers are exploring quorum sensing (QS) inhibition as a novel strategy to reduce biofilm formation and mitigate antibiotic resistance. Regarding alternative treatments, antibiotics from the tetracyclines class, such as doxycycline, minocycline, and tigecycline, show relative efficacy against *A. baumannii*, due to their bioavailability and low cost. Studies indicate that the flexible genetic structure of *A. baumannii* which allows for the transfer of resistance genes through horizontal gene transfer, enhances bacterial resistance to carbapenems by producing β -lactamase enzymes, such as Oxacillinase (Pagano *et al* ,2016). The aim of the study was to investigate the prevalence and role of flow pump genes in clinical isolates *A. baumannii*

and their relationship to multidrug resistance, and to provide insights into potential therapeutic interventions.

2. Materials And Methods

Isolation and identification

Between November 24, 2020, and February 15, 2021, a total of 27 bacterial isolates were collected from hospitalized patients at various hospitals within the Medical City complex in Baghdad. These hospitals included Child Protection Hospital, Burns Specialist Hospital, and Baghdad Hospital, along with educational laboratories. Among these, 26 isolates were identified as *A. baumannii*. Initial identification was performed using biochemical methods, including culture-based techniques and the API 20NE system (bioMérieux), which differentiates non-fermentative Gram-negative bacteria. Gram staining confirmed the isolates as non-motile, Gram-negative bacilli. To confirm species-level identification, the VITEK 2 Compact system with GN cards was employed, providing accurate and reliable differentiation at 99% confidence. Molecular confirmation was achieved using polymerase chain reaction (PCR). Oligonucleotide primers were prepared by dissolving 10 pmol/ μ L of stock solution in 90 μ L of nuclease-free distilled water. The mixture was vortexed and stored on ice at -20°C for further use. For the PCR process, 1 μ L of each primer was combined with 12.5 μ L of green master mix and 25 μ L of nuclease-free water, adjusting the final volume as required. The mixture was vortexed to ensure uniformity before proceeding with amplification. (Anwer *et al*, 2020).

Antimicrobial susceptibility test.

According to the Clinical and Laboratory Standards Institute (CLSI), the antimicrobial susceptibility test was conducted on Mueller–Hinton agar using various antibiotics, and the results were recorded as Susceptible, Intermediate, or Resistant (Ridha *et al*, 2019). The antibiotics assessed included beta-lactams such as ceftazidime (30 μ g), cefepime (30 μ g), cefixime (30 μ g), cefotaxime (500 μ g), amoxicillin/clavulanate, amoxicillin (25 μ g), piperacillin (100 μ g), and piperacillin/tazobactam (110 μ g). Carbapenems like imipenem and meropenem were also tested, alongside aminoglycosides including gentamicin (10 μ g) and amikacin. Fluoroquinolones such as ciprofloxacin (5 μ g) and tetracyclines like tetracycline were included, as well as colistin sulfate (10 μ g) from the polymyxin class. Additionally, trimethoprim-sulfamethoxazole was evaluated. Antibiotic discs were sourced from Master UK.

Detection of Efflux Pump Genes.

All isolates were grown in nutrient broth for 24 hours at 37°C. Scratched agar plates yielded about two loops of biomass, which was then suspended in 100 microliters of deionized water and boiled for 10 minutes. After 10 minutes of 10000g centrifugation at 4°C, around 5 microliter (as a PCR temple) was transferred to a PCR (Ali, MR., and Khudhair, AM (2019). the identification of EP genes was accomplished by PCR amplification of the genes. Table 1 shows the Efflux Pump primers and PCR amplification program (Cotar *et al*,2010).

Table 1: The primers utilized in this study:

NO	Pri mer name	(5'-3') Sequence	Size product	Reference
1	<i>AdeL</i>	F- CACGTCCACGTACTGAC ACA R- AGACTTGGTTGGAGAAG CGG	266 bp	(9)
2	<i>AdeF</i>	F- CGATGCAAAAGCTGCAC CAA R- CCTTCGCGAGTAAACCC TGT	684 bp	
3	<i>AdeG</i>	F- AACTTGAAGACCGAGGT GCC R- TTTTACAGGCTAGCCCG ACC	774bp	
4	<i>AdeH</i>	F- CTTGCTACCGCACAAAC CAC R- TGGCGACGCTTTCCTCA TAA	442 bp	
5	<i>AdeM</i>	F- CGTCCCTACGCGAATGG TTA R- AGCGTTACGAGGCTATT CCGG	449bp	



Gel electrophoresis:

After PCR amplification, the resulting products were subjected to electrophoresis on 1% (w/v) agarose gels (Promega, USA), stained with 5 g/100 ml ethidium bromide in 1x TBE buffer. The gels were then photographed under UV light to document the results following electrophoresis (Sallman *et al*,2018).

3. Results And Discussion:

Isolation and identification of *A. baumannii*

Obtained 26 isolates of *A. baumannii*. 12 blood isolates were part of this collection and formed the bulk (44.44%) of sources. Isolates from burn represented 18.51% of the total with 5 isolates while wound isolates made up 14.81% with 3 isolates. Also, 3 isolates were obtained from CSF and sputum which represents 11.11% of the total number as shown in Fig.1.

The identification of *A. baumannii* was conducted using advanced biochemical and molecular techniques. The VITEK 2 Compact system, equipped with GN cards from Biomerieux France, was instrumental in species-level confirmation. This system employed 46 biochemical reactions to provide reliable differentiation and diagnostic accuracy (Fallah *et al*,2017). The isolates were confirmed with a high degree of confidence, achieving a 99% probability of correct identification. In addition to the VITEK 2 system, intragenomic multiplex PCR with Biomos Biomatic instruments was utilized to validate the isolates genetically. This method complemented the biochemical testing by confirming the presence of genetic markers specific to *A. baumannii*. Such a combination of biochemical and molecular tools ensures robust and precise identification, minimizing the risk of misdiagnosis. These findings not only reflect the high prevalence of *A. baumannii* in clinical settings but also highlight the critical need for accurate diagnostic methods to guide effective treatment strategies, particularly in the context of multidrug resistance commonly associated with this pathogen.

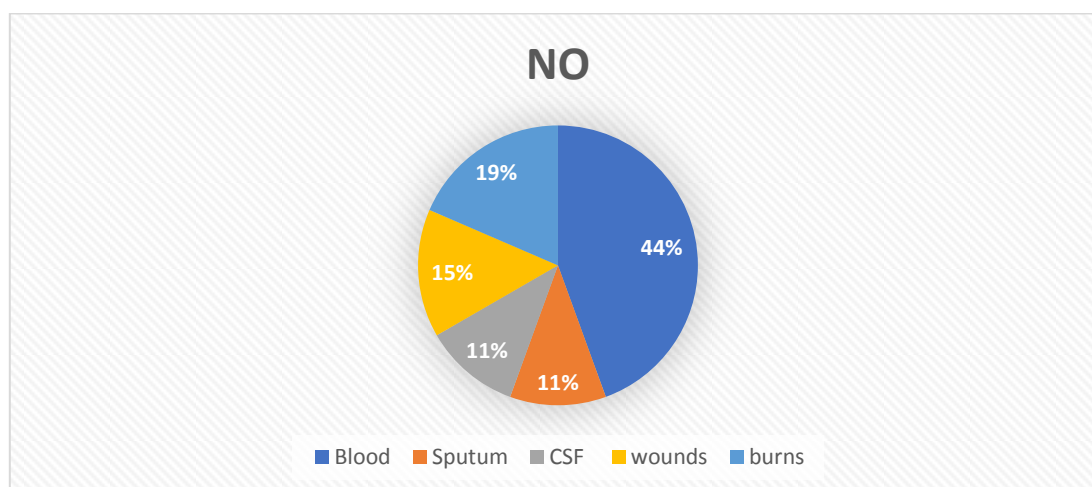


Fig.1. The distribution of *Acinetobacter baumannii* isolates based on clinical sources (N = 26).

Antibiotic Susceptibility Patterns:

The bacterial isolates exhibited varying resistance levels to the 16 antibiotics tested. All isolates (100%) demonstrated resistance to ceftazidime, cefixime, cefepime, cefotaxime, colistin sulfate, piperacillin/tazobactam, and amoxicillin/clavulanate. Resistance to imipenem, tetracycline, and meropenem was observed in 96% of the isolates. Additionally, 69% of the isolates were resistant to gentamicin and amikacin, while 80% exhibited resistance to ciprofloxacin and trimethoprim-sulfamethoxazole. High resistance rates were also noted for amoxicillin and piperacillin, at 92% and 88%, respectively. These results indicate a high level of multidrug resistance among the *A. baumannii* isolates under study. These findings highlight the increasing threat of MDR *A. baumannii* in clinical environments, especially considering the essential function of carbapenems and cephalosporins as last-resort therapeutic alternatives. Resistance to colistin, often considered a drug of last resort, is especially alarming and highlights the diminishing therapeutic arsenal against this pathogen. The high resistance rates to aminoglycosides, fluoroquinolones, and β -lactam/ β -lactamase inhibitor combinations further complicate treatment options, leaving few alternatives for managing infections caused by these isolates. The 96% resistance to carbapenems corresponds with global patterns, indicating the extensive spread of carbapenem-resistant *A. baumannii* (CRAB) strains (Abed, E., and Ali, M. 2020). The mechanisms underlying this resistance may include the overexpression of efflux pump systems,



acquisition of carbapenemase genes, and alterations in outer membrane proteins. Additionally, the observed resistance to aminoglycosides and fluoroquinolones may be attributed to the presence of specific resistance genes, such as *AdeM* and *AdeG*, as identified in this study. Due to the significant incidence of multidrug resistance identified in this study, regular monitoring and molecular analysis of resistance mechanisms are crucial for informing effective antibiotic stewardship and enhancing treatment protocols. The global rise of MDR *A. baumannii* highlights the pressing need for coordinated international efforts to address this emerging public health challenge.

Dendritic classification of QS genes:

The dendritic classification of quorum sensing (QS) genes in *A. baumannii* isolates offers crucial insights into the genetic relationships and epidemiological patterns of hospital-acquired infections (HAIs). Two main groups were identified by the analysis: Cluster A, which included 22 isolates, and Cluster B, which included 4 isolates. These clusters showed that the isolates in each group shared a high degree of genetic similarity. Given Cluster A's dominance, it is highly likely that a single bacterial strain with little genetic variation caused the infections in the hospitals under study. The outbreak's clonal structure emphasizes a limited source of infection as opposed to several, unconnected pathogen invasions. The discovery of a smaller group (Cluster B) with little genetic variations suggests that there have been few mutations or adaptations, which may have been brought on by environmental stressors such as exposure to antibiotics or the development of biofilm on medical equipment. These results are consistent with other studies showing that *A. baumannii* has a high risk of clonal outbreaks, which are frequently caused by cross-contamination or insufficient sterilization. The clustering found in this study is in line with reports of clonal complexes, in which isolates exhibit genetic markers linked to virulence and resistance, predominating in hospital settings across the globe. This data emphasizes how important it is to implement focused infection control measures to stop the spread of these genetically linked isolates in hospital settings (Li *et al* ,2023) .As shown in Fig .2.

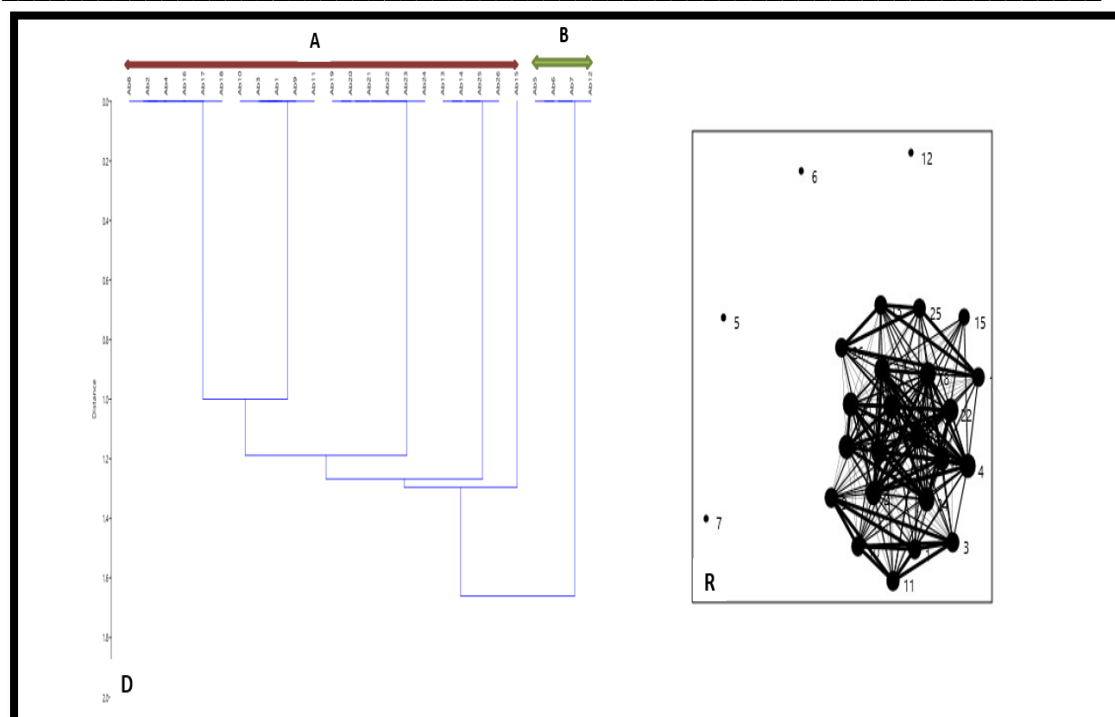


Fig. 2: The genetic relationships among *Acinetobacter baumannii* isolates based on dendritic clustering and genetic network patterns.

Detection of EP genes by conventional PCR techniques:

Important facets of *A. baumannii*'s multidrug resistance (MDR) mechanisms and its wider ramifications are highlighted by the identification and examination of efflux pump genes. The findings showed that the AdeM gene was present in all 26 isolates, suggesting that it plays a broad role in conferring resistance to fluoroquinolones and aminoglycosides but not β -lactams. This result is consistent with previous research indicating that AdeM contributes significantly to *A. baumannii*'s adaptive resistance. Additionally, the AdeG gene's potential as a target for therapeutic interventions meant to mitigate resistance to this class of antibiotics is highlighted by the fact that it was found in six isolates and that it was linked to fluoroquinolone resistance. Interestingly, the AdeF and AdeH genes were found in fewer isolates, which is consistent with previous research indicating their lower prevalence in MDR systems.

Overexpression of the AdeFGH efflux mechanism, which is known to be inherent to *A. baumannii*, has been associated to lower antibiotic sensitivity. This discovery highlights the dynamic regulation of efflux mechanisms and their role in resistance, which might vary depending on environmental or clinical circumstances. The found interaction between quorum sensing (QS) genes and efflux systems is one of the study's highlights. This finding suggests that quorum sensing may regulate or

augment efflux pump expression, hence contributing to bacterial virulence and persistence in clinical situations. Targeting QS pathways may have a dual effect of lowering virulence while also weakening efflux-mediated resistance, making it a potentially innovative treatment method. these findings are corroborated by gel electrophoresis data from both Uniplex and multiplex PCR which confirm the genetic profiles of the efflux pump genes. This comprehensive methodology improves the results credibility and applicability to treating MDR in *A. baumannii*. Finally, the work emphasizes the significance of ongoing monitoring and molecular characterization of resistance determinants in order to inform successful antimicrobial stewardship measures. (Fig 3 & Fig 4).

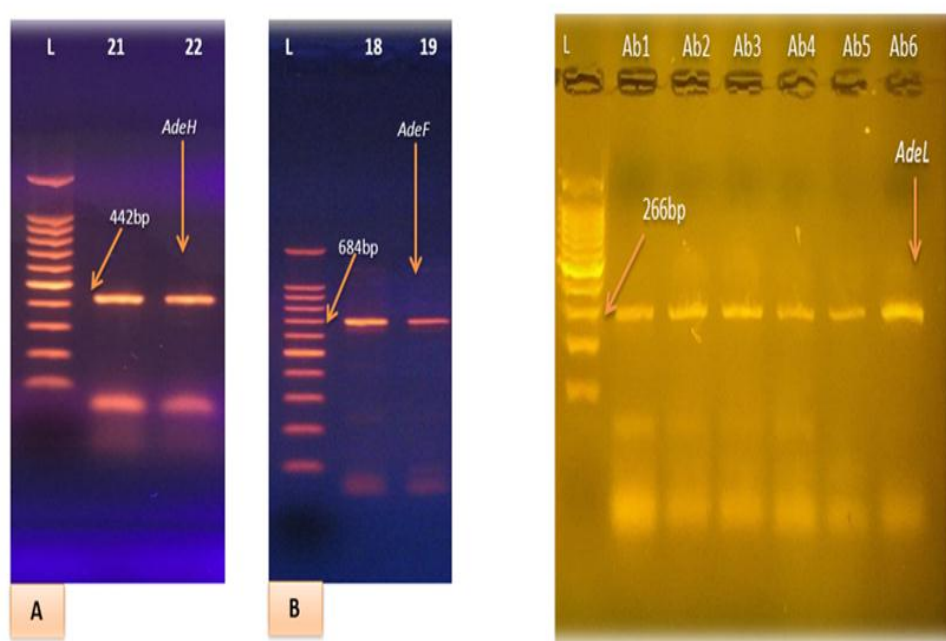


Fig.3. Uniplex PCR: Gel electrophoresis on 1% agarose gel shows *AdeH* at 442 bp (A) and *AdeF* at 684 bp (B) in *Acinetobacter baumannii* compared to the 100 bp DNA ladder. as well as *AdeL* at 266 bp.

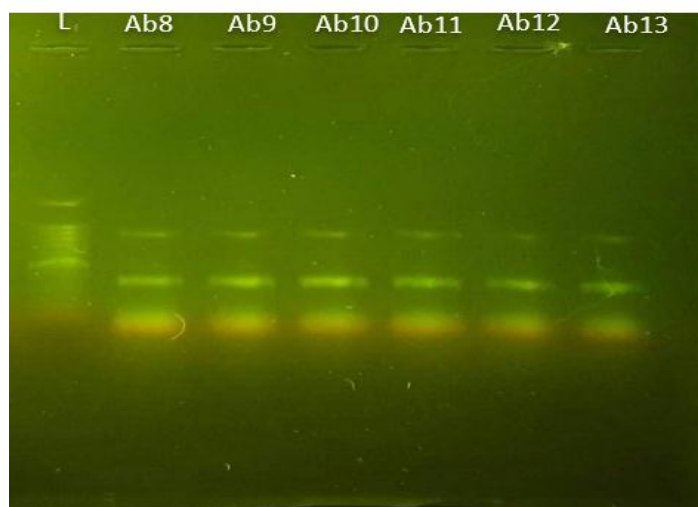


Fig. 4. Multiplex PCR: Gel electrophoresis on 1% agarose gel reveals AdeM at 774 bp and AdeG at 469 bp in *Acinetobacter baumannii*.

4. Conclusions

The study indicates that efflux pump genes, particularly *AdeM* (found in all isolates) play an important role in multidrug resistance (MDR) in *A. baumannii*. The relationship between quorum sensing systems and efflux pumps states a regulatory mechanism that increases bacterial virulence and persistence. These findings highlight the need of molecular observation and novel therapeutic techniques in combating MDR successfully.

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Conflicts of Interest: None

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