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(RESEARCH ARTICLE)

Poly morphisms of *Adefg* Efflux Pump Genes in *Acinetobacter. baumannii* isolated from burn sample

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Abstract

A. baumannii is a multidrug-resistant opportunistic pathogen responsible for a range of human diseases. Much of this resistance is due to its efflux pump systems, the AdeFGH system composed of the AdeF, AdeG, and AdeH genes. This study focused on the adeG gene, which encodes the major transport protein of the AdeFGH system, with the aim of assessing the potential role of this gene in antibiotic resistance in burn-derived A. baumannii isolates. For this purpose, sixty bacterial samples were collected from burn patients at multiple hospitals in Baghdad. Biochemical methods were used to identify the bacteria, and disk diffusion was used to determine antibiotic resistance and sensitivity. The adeG gene was then identified by polymerase chain reaction. Resistance to amoxicillin was found to be 95% and to ciprofloxacin to 85%. The lowest resistance was found to imipenem, at 37%. The study aimed to clarify the relationship between the efflux pump resistance gene (adeG) and antibiotic resistance in bacteria. It was found that 70% (42/60) of A. baumannii isolates carried the adeG efflux pump gene. The results indicated sensitivity to antibiotics, and all strains exhibited varying degrees of resistance, with the highest resistance rate to amikacin (95%), tetracycline (65%), and ciprofloxacin (30%). In an analysis of the resistance of isolates containing the AdeG gene, resistance rates were lower, specifically 26.1% to ciprofloxacin, 42.8% to tetracycline, and 30.9% to amikacin.

Keywords: Burn Injuries; Efflux Pump; Encode Adeg Gene; Antibiotic Resistance

1. Introduction

Bacteria belonging to the enterobacteriaceae family have evolved to become highly virulent pathogens, including A. baumannii, these bacteria are Gram-negative and live in humid environments. (3, 4). They are also a leading cause of death in hospitals, especially among intensive care patients (1, 2). They cause numerous human diseases, including meningitis, pneumonia, urinary tract infections, and skin and wound infections (5, 6). Antibiotic resistance is a common cause of disease (8). One of the ways these bacteria resist a range of antibiotics is by producing enzymes that inhibit antimicrobials, including beta-lactamases and aminoglycosides, which target cell membrane permeability, increasing the expression of efflux pumps. (9). The RND family, which is frequently found in negative bacteria, is known to be essential for chemotactic efflux and antibiotic resistance (10) (11-13). The inner membrane, cytoplasm, and outer membrane contribute to determining the final confirmation of the RND pump, through which active substances are transported from the cytoplasm to the outside of the cell (12). Thus, the drug is forced to penetrate the low-permeability outer membrane (14, 15, 16). The *AdeFGH* efflux pump operon consists of three structural genes (*adeF, adeG, adeH*); however, the current study focused exclusively on the *adeG* gene, which encodes the major transporter protein and plays a central role in the efflux mechanism and development of antibiotic resistance in burn isolates from Baghdad.

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2. Material and methods

2.1. Bacterial isolates and species identification

in research gathered From October 2024 to December 2024, 88 A. baumannii isolates were discovered. in burn clinical specimens at Baghdad Hospital, Identification and isolation of strains by using common biochemical tests and techniques (17). After inoculation on MacConkey agar and blood agar, samples were cultured in 37°C. for 24 hours. Biochemical methods including oxidase, citrate, urease, sugar fermentation and oxidation, movement, to identify *A. baumannii* (18).

2.2. Antimicrobial susceptibility test

The sensitivity and resistance of bacterial isolates to five different antibiotics were tested using the Kirby-Bauer disk diffusion technique to test the following antibiotics (ceftazidime, ciprofloxacin, tetracycline, amikacin, and imipenem). The zone of inhibition was measured according to the Clinical, and Laboratory, Standards (CLSI) guidelines20.19.

2.3. DNA extraction

Genomic DNA was purified from the isolates using a DNA, extraction,kit provided, via Pioneer ,(Korea; catalog, no. K-3032.-2). according to the manufacturer's protocol.

2.4. Efflux Pump *adeg* and *bla* identification PCR-Like Genes for OXA-51

Table (1) The primer sequences employed in the current investigation are displayed. Polymerase chain reaction. (PCR) was performed to detect the *adeg* genes, to confirm the presence bacteria and identify them, primers from the study of Kaviani *et al* (4). were used, where the gene similar to blaOXA-51 was used. The thermal cycle criteria for identifying a gene similar to blaOXA-51 included the following: annealing, at 58°C for, 60, seconds (30, cycles), extension at 72°C, for 60 seconds (30 cycles), denaturation at 95.°C for 5 minutes, (1 cycle), denaturation at 95°C for 45 seconds (30 cycles), and final extension at 72°C for 5, minutes, (1 cycle).

Table 1 Study Primers Utilized

Gene	Primers sequence (5’ – 3’)	Product size	Reference
Adeg	F: TTCATCTAGCCAAGCAGAAG R: GTGTAGTGCCACTGGTTACT	468	23
blaO.XA-51	F TAAT,GCTTTGATCG,GCCTTG R TGGA,TTGC.ACTTCATCTTGG	342	27

3. Results and Discussion

3.1. Isolation of bacteria

Just 60 of the 88 bacterial strain isolates that were obtained from burn injuries in hospitals Baghdad for this investigation were identified as *A. baumannii*.

3.2. Antibiotic susceptibility test

As demonstrated in (Figure 1) Every isolation of *A. baumannii* displayed high antibiotic activity against amikacin (95%), ceftazidime (70%), and ciprofloxacin (85%), respectively, Tetracycline resistance reached 65%, and the carbapenems group of antibiotics, which includes imipenem, recorded the lowest resistance rate at 37%.

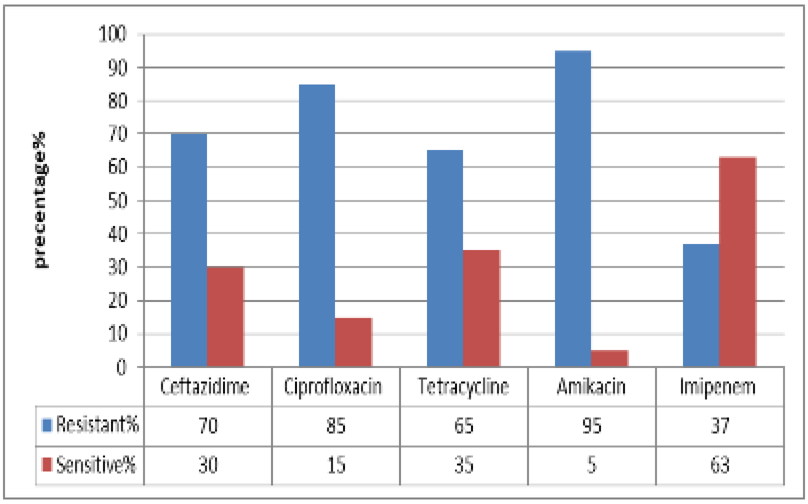

Figure 1 The Percentage of Resistance to Different Antibiotics in A. baumannii (N = 60)

Acinetobacter baumannii is consider negative bacteria and considered opportunistic pathogen. This bacterium is characterized by its high resistance to antibiotics due to its strong tendency to carry genes of resistance. It is thought to be a frequent cause of. infections, in hospitals (18). The widespread resistance in these bacteria is mainly driven by the improper use of broad-spectrum drugs among the population. Research indicates that they show reduced susceptibility to many beta-lactam and quinolone antibiotics, while resistance to aminoglycosides continues to rise. This extensive resistance is closely linked to the presence and transmission of various resistance genes (20). Our current results for amikacin, which recorded the highest resistance rate of 95%, were agree with a study, by Basatian-Tashkan *et al.* (19). who recorded a resistance rate of 96.6%? Our results differed from the researcher's results regarding the antibiotics ceftazidime (98.4%), tetracycline (91.6%), and imipenem (50%). The researcher explained that the variation in bacterial resistance to antibiotics may be related to the geographical distribution of bacteria and the diversity of patient sampling areas. Our findings were nearly in line with those of Talibi *et al.* (20) regarding tetracycline, which recorded a resistance rate of 55%. Our results were also consistent with a study by Maleki *et al.* (21). which recorded a high resistance rate of 86.66% for ciprofloxacin, in contrast to a research by Nouri *et al.* (22) demonstrated that every isolate was 100% resistant to ceftazidime, ciprofloxacin, and piperacillin [20]. Our results differed from A study was carried. by Rahabarnia *et al.* (23) in Iran, which reported that antibiotic resistance reached 95% to ciprofloxacin, 82% to imipenem, and 35% to gentamicin. Our results were also in approximately agreement with Abdour *et al.* (24), where ceftazidime resistance reached 93%. A study conducted in China by Jia *et al.* (25) demonstrated that the rate of ceftazidime resistance reached 92.2%, which is different from our current results, At the same time, a study carried out by Ranjbar *et al.* (26) found that the rate of ceftazidime antibiotic resistance reached 97.5%.

3.3. Relationships between antibiotic resistance and efflux pump (adeg) gene in bacteria .

The current study showed that 70% (42/60) of A. baumannii isolates carried the AdeG efflux pump gene. Antibiotic susceptibility results revealed varying levels of resistance among all isolates, with the highest resistance recorded against amikacin (95%), followed by tetracycline (65%) and ciprofloxacin (30%). When analyzing resistance specifically in isolates carrying the AdeG gene, lower resistance rates were recorded (26.1%, 42.8%, and 30.9% for ciprofloxacin, tetracycline, and amikacin, respectively) (Table 2).

Table 2 Relationships between antibiotic resistance and efflux pump (adeg) gene in bacteria

SN.	Antibiotic	Isolates resistance of adeg N = 60(%)	Resistance of positive adeg gene N=42 (%)
1	Ciprofloxacin	51(85)%	11(26.1)%
2	Tetracycline	39(65)%	18(42.8)%
3	amikacin	57(95)%	13(30.9)%

Several studies have shown that the primary cause of this resistance is efflux pumps (17). In particular, the adeg gene, which encodes proteins that constitute the core components of the AdeABC pump, was identified as the most prevalent gene among the isolates examined, the results of the current study are consistent with previous reports, which demonstrated the prevalence of AdeABC genes in Acinetobacter baumannii strains. [26] Meanwhile, a study by Kor et al. (27) revealed that efflux pump genes were highly prevalent in bacterial isolates from hospitals in Malaysia. Our results are almost identical to those of Daimer-Bioll et al. (28), who found that 100% of multidrug-resistant isolates contained the efflux pump gene. Our results on ciprofloxacin resistance are also consistent with those of Kaviani et al. (4), who revealed the essential role of the RND system genes (AdeI and AdeJ) in increasing bacterial resistance to antibiotics ,the researchers indicated that the expression of the two genes in the isolates reached 90%. At the same time, Ardebeli et al. (29) identified a significant association between the AdeABC efflux system and ciprofloxacin resistance, with 95.6% of the isolates showing resistance to the antibiotic.

4. Conclusion

The wide spread of the antibiotic resistant is the main barriers for the increase in infections due to bacterial such as *Acinetobacter baumannii* (The findings of this study indicated that *adge* gene among efflux pump genes may have crucial role in emergence of resistance against antimicrobials, So far It can be concluded that monitoring would essential studies in order to understand how it really function mechanism and action, which will help to identify critical therapeutic targets and open new challenges responsible for control their dissemination.

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