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REVIEW ARTICLE

Molecular detection of blaIMP and ompA genes in Acinetobacter baumannii isolated from burn wound infections

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Abstract:

Background: antibiotic resistance in Acinetobacter baumannii can be acquired rapidly due mainly to chromosomal mutation, and its antibiotic-resistant metallo-beta-lactamase (MBL) production has been claimed to have a great contribution to nosocomial infection.

Objective: This study aimed to detect the presence of the blaIMP carbapenemase-producing gene and the ompA gene in bacterial isolates obtained from burn patients, using the polymerase chain reaction (PCR) technique. Additionally, the study aimed to evaluate the role of these genes in antibiotic resistance.

Methods: Cross-sectional research was conducted among a total of 150 clinical samples for patients of various ages and both sexes at the Specialist Burns Center inside Al-Diwaniyah, between October 2024 and April 2025. Every single one of these specimens went through cultivation on 5% blood agar and MacConkey agar. They were followed by incubating aerobically for 24 hours at 37°C. Antimicrobial Susceptibility Testing (A.S.T.) was used to isolate organisms that produced carbapenemase; following that, the conventional PCR method had been utilized to identify the blaIMP as well as ompA genes.

Results: The present cross-section study enrolled 150 swab samples from patients with burns, which were investigated for bacterial infection using bacteriological culture of swab samples. The isolation procedure resulted in obtaining 30 (20.0%) isolates of Gram-positive bacteria, 94 (62.7%) of Gram-negative bacteria, and 26 (17.3%) of mixed growth. Thirty-seven of several medical specimens were identified as A. baumannii. Utilizing A.S.T., for A. baumannii (n=37), total resistance to ampicillin and augmentin (100%) was also observed. Colistin (70.3%), meropenem (56.8%), and ceftriaxone (54.1%) demonstrated the best efficacy. Multidrug Resistant (MDR) and Extensively Drug Resistant (XDR) rates among A. baumannii isolates were 43.2% and 56.7%, respectively. Genotypic analysis of A. baumannii revealed high frequencies of ompA (51.4%) and blaIMP (45.9%) genes were detected.

Conclusion: The presence of ompA and blaIMP genes in Acinetobacter baumannii means it can resist antibiotics and cause severe infections. Detecting these genes early helps improve treatment and prevent spread.

Keywords: A. baumannii, *ompA, blaIMP*, conventional PCR technique.

Introduction

Acinetobacter baumannii (A. baumannii) as a serious threat-level pathogen causes several hospital-acquired and healthcare-associated infections worldwide [1]. Among immunocompromised patients, it is responsible for a variety of diseases such as respiratory tract infections (ventilator-associated pneumonia), surgical site infections, burn wound infection, catheter-related blood infection, urinary tract infection, gastrointestinal infection, endocarditis, peritonitis, and meningitis [2]. Several main factors, including virulence potential, biofilm formation, and resistance to a broad range of antimicrobial agents (the emergence of multidrug-resistant isolates), have made it one of the most successful nosocomial pathogens [3]. This pathogen is related to the high rate of mortality
and morbidity throughout the world [4] and intensive care units (ICU), and it is estimated that it causes. Nowadays bacterial resistance to antibiotics is a major issue worldwide, especially in the case of A. baumannii. Carbapenems are currently the primary medications used for the treatment of infections caused by multidrug-resistant A. baumannii. Nonetheless, there is growing concern as carbapenem resistance among A. baumannii isolates may become more widespread [5]. Resistance of

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A. baumannii to carbapenems could be caused by a variety of factors, such as the development of beta-lactamases, changes in proteins that bind to penicillin and outer membrane proteins, and an excess of efflux pumps [6]. Huang and colleagues [7] reported that the synthesis of β-lactamase enzymes is a critical factor contributing to the antibiotic resistance observed in A. baumannii. A number of MBL genes, including Verona integrin metallo-β-lactamase (blaVIM) and imipenemase (blaIMP), have been found in A. baumannii isolates. Globally, Gram-negative bacteria, including Enterobacteriaceae and non-fermenting microorganisms such as Acinetobacter spp., have been identified as producing different blaIMP-type enzymes [8]. A. baumannii's ability to form biofilms enables successful long-term persistence in healthcare facilities and improves its ability to escape the effects of antibiotics by evolving different mechanisms [9]. Many factors contribute to biofilm formation, which is a complex process involving substance aggregation, collagen adhesion, pili expression, and iron acquisition [10].

Materials and methods:

Methods:

Sample collection: 150 medical specimens of patients of every age as well as sex who had been brought to the Specialist Burns Center inside AL Diwaniyah Province for treatment as well as underwent screening for the purpose of the research between October 2024 and April 2025 were the subject of a cross sectional investigation. All of the patients had a Burns swab collected. Discarded gloves as well as a protective gown had been employed when there was immediate contact with the patient. The samples had been sent to the research center using sterilized leakproof containers.

Bacterial Isolate Identification: By applying standard microbiological techniques, isolates of Acinetobacter baumannii have been determined as well as verified. The materials were grown on MacConkey agars, certified agars, and blood agars in order to examine the morphologies of A. baumannii populations. Additionally, specimens of A. baumannii were identified utilizing Gram stain [11]. Oxidation enzyme, IMVIC, and catalase tests are explained through other research findings [12]. Utilizing movement media, a mobility experiment was conducted (Collee et al., 1996). Nutritional agar dishes were used for the bacterial growth test at 42°C, and the results demonstrate that the bacterium grew at this temperature [12]. Antibiotic-resistant bacterium was identified as well as studied using the VITEK-2 system. Utilizing professionally made antibacterial discs on Mueller Hinton agar plates as well as the disk diffusion technique, the Antibacterial Resistant Test (A.S.T) was performed on the identified A. baumannii isolations.

DNA Extraction: Following the supplier's instructions, the bacterial DNA was obtained through A. baumannii strains that were growing in the incubator (ZR Fungal/Bacterial/ Yeast DNA Mini-Prep TM D.6005). The primer solutions were made by melting these substances in deionized distilled water (d.d.H_2O) to an ending level of 100 pmol/µl as a stock solution, in accordance with the directions provided by the supplier (Integrated DNA Technologies business; I.D.T. Canada). Prior to being utilized, the primer's stock solutions were stored at 20°C. To make 10pmol/µl concentrations as work primer suspension, 10µl of stock solution had been combined thoroughly with 90µl of d.d.H2O using a vortex mixer for homogenizing it prior to utilization. It then remained at -20°C when it came time to utilize it. Generally two genes were utilized during this study, including the blaIMP and ompA genes. The blaIMP primer pairs forward 5'-GGTAACCAGCTCAGCCACAT-3', blaIMP reverse 5'-GAAGGCGTTTATGTTCATAC-3' with molecular weight 587 b.p. [13], and ompA primer pairs forward 5'-GGTTAGGTCGTATGCCGTACAAAGG-3',

ompA reverse 5'. AGTGTAGATGTCCAGGTCGTCAGTG .3' with molecular weight 1071 b.p. [14]

P.C.R. for gene detection: In accordance with Nakayama et al. (2016), a high level as well as quality for extracting bacterium DNA from A. baumannii isolates was assessed. [15]. Next, in accordance with the directions provided by the company (iNtRON), a 20 l Maximum P.C.R. pre-Mix-kit (i-Taq) was used to produce the reaction mixture. Initial denaturation at 95°C, 5 min., 1 cycle; denaturation at 95°C, 45 sec., 35 cycles; annealing at 60°C, 45 sec., 35 cycles; extension at 72°C, 45 sec., 35 cycles; and final extension at 72°C, 7 min., 1 cycle were the ideal circumstances for the identification of ompA and blaIMP genes. Detecting DNA with amplification bands: The DNA amplification bands' existence was examined. [16].

Statistical Analysis: Frequencies & percentages have been utilized to provide descriptive statistics for categorical data. To determine the relationship between categorical data, the chi-square test is utilized. Throughout this investigation, p-values below 0.05 were regarded as statistically significant.

Results:

1.Isolation & the identification of bacterial isolates associated with burns:

The present cross-section study enrolled 150 swab samples from patients with burns, which were investigated for bacterial infection using bacteriological culture of swab samples. The isolation procedure resulted in obtaining 30 (20.0%) isolates of Gram-positive bacteria, 94 (62.7%) of Gram negative bacteria, and-negative 26 (17.3%) of mixed growth, as shown in figure (1).

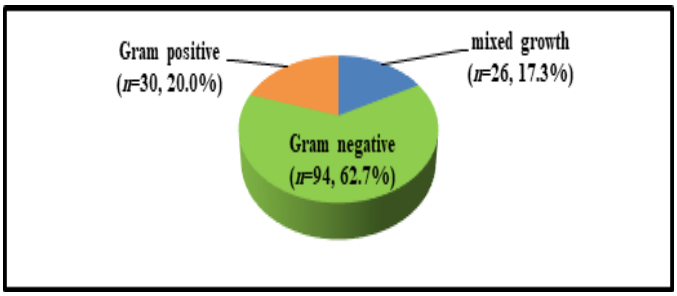

Figure (1): Prevalence of bacterial infection among patients with burns

2.Antibiogram Testing.

Antibiogram testing was performed with selected antibiotics that are commonly used and recommended by AST-N222\cards were used in the automated-VITEK-2 compact-system to evaluate CLSI antimicrobial resistance testing and by the disc diffusion method for in vitro antibiotic susceptibility tests (Appendixes II – III – V – IV – VI). The results are shown in figure (2) and represent the antibiogram profile of Acinetobacter baumannii, indicating that isolates varied in their susceptibility to the antibiotics.

3. Acinetobacter baumannii Antimicrobial Resistance Testing:

The rise of multidrug-resistant (M.D.R.) bacterium has caused a surprising rise in sepsis as well as deaths arising through burn-wound-related illnesses both locally and globally. One of the more prevalent bacterial pathogens associated with wound infections that are resistant to several drugs is A. baumannii. So, during present research, antimicrobial resistance was performed on every 37 Acinetobacter baumannii isolates toward 16 antibiotics represented by amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazidime, cefotaxime, ceftriaxone, ampicillin, augmentin, piperacillin, Aztreonam, clindamycin, ciprofloxacin,aztreonam, levofloxacin, and colistin by using the Kirby-Bauer technique, a disc-based diffusing technique on Muller-Hinton agar according to CLSI guidelines (2024).

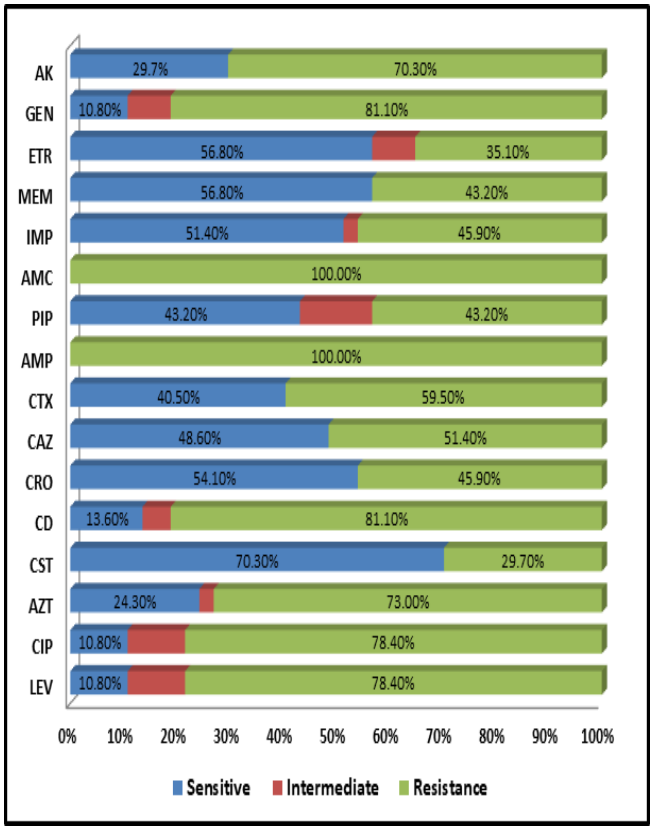

Figure (2): Sensitive Testing against different species of Acinetobacter baumannii

4. Multi-drug Resistant Acinetobacter baumannii

Interestingly, the present finding demonstrated that such P. aeruginosa isolation demonstrated high drug resistance. However, non-susceptibility up to ≥1 factors within 3 antimicrobial categories was observed in about 16 (43.2%) of isolates, which were characterized as MDR A. baumannii. While the XDR ratio showed non-susceptibility to up to ≥1 factor within every isolation, ≤2 antimicrobial categories were seen in 21 (56.7%).

5. Molecular detection for antimicrobial resistant & virulence genes of Acinetobacter baumannii.

A specific PCR primer was used for the molecular finding of two antibiotic resistance and virulence genes (blaIMP and ompA) in Acinetobacter baumannii isolated from burn infections; that is illustrated in table (3) & figure (2), & the present finding of gel electrophoresis is shown in table (1). This study found that (blaIMP) genes were observed in 17 (45.9%) of A. baumannii isolates and the ompA gene was observed in 19 (51.4%) genes for A. baumannii isolation; also, the difference was significant.

Table(1): Prevalence of antimicrobial resistance and virulence genes among Acinetobacter baumannii isolates.

Antibiotics resistance and virulence genes	Positive samples	%
bla IMP	17	45.9%
omp A	19	51.4%

Note: Statistical analysis using Chi-square test (χ^2=0.030), showed no significant association (p = 0.862). Among 37 Acinetobacter

baumannii isolates, PCR analysis revealed that 17 isolates (45.9%) isolates carried the blaIMP gene (587 bp), as shown in Figure (3). Additionally, 19 isolates (51.4%) from the same group harbored the ompA gene (1071 bp), as illustrated in Figure(4).

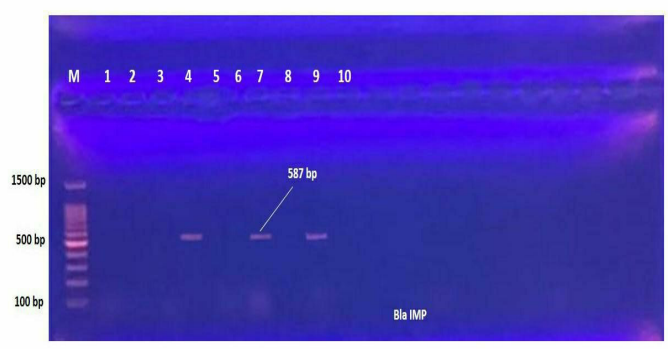

Figure (3): Electrophoresis of blaIMP (587 bp). Utilizing 1.5% agaros gel at 90.V for 60 min. in 1x TBE buffer, and visualized under transilluminator UV after staining by Ethidium bromide. Lane M: 100-1500 bp DNA ladder.

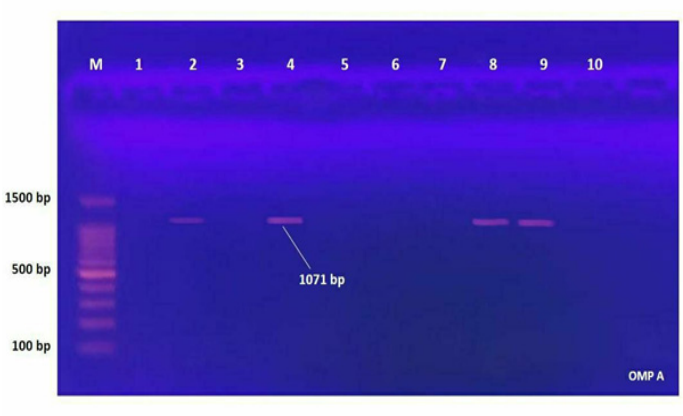

Figure (4): Electrophoresis of ompA (587 bp). Utilizing 1.5% agarose gel at 90.V for 60 min. in 1x TBE buffer, and visualized under transilluminator UV after staining by Ethidium bromide. Lane M: 100-1500 bp DNA ladder.

Discussion:

Burn wound infection has been regarded as one of the significant obstacles in the therapy of burn wounds, and it is morbid and mortal globally. Many researchers who have conducted domestic and international investigations that confirmed that gram-negative strains of bacteria are one of the more common causes of burn infections were in parity with the present finding [17], reporting 95%, 92.47%, 85.4%, 83%, and 80%, respectively, of diagnosed burn infections as gram-negative. Findings in this regard were also near [19], who registered 54.9 percent, respectively. Antibiotics are commonly used in curbing the growth of wound infection, but with the rise in the frequency of MDR bacteria, the availability of antibiotics as a wound infection control method is restricted. Moreover, the ability of the systemic antibiotic treatment to prevent wound infection might be precluded by various factors such as necrosis, granulation tissue, inadequate peripheral blood flow, and even fibrosis, which behave as obstacles to the intrusion of antibiotics into the burned tissues. Hence, topical administration of an antibacterial agent may prove beneficial in the management of burn wound infection. [20]. One of the significant tests to be identified is the antibiotic susceptibility test to detect sensitivity and resistance of the bacteria to antibiotics, which is necessary for clinical purposes. [21]. The percentage of resistance of the A. baumannii isolates taken out of burn infection patients was high towards the antibiotics; resistance was amoxicillin-clavulanic acid (AMC) at 100%. The resistance to penicillins (ampicillin) and Augmentin is high, which shows that they can no longer be effective in the treatment of A. baumannii. The extreme pressure due to overuse The extreme pressure of overuse Most β-lactams have been reported to be intrinsically susceptible to A. baumannii. The most common mechanism of resistance to β-lactam drugs is A. baumanni. Inactivation of β-lactam drugs by either chromosomal β-lactamase or plasmid β-lactamases [22]. Local studies also recorded a high resistance percentage to Augmentin, with 91.7% (Chelkeba et al., 2021), 91.3% (Raheem & Al-Hasnawy, 2020), and 89.57% (Al-Tamimi et al., 2022). [17, 23, 24]. In addition, Al-Kadmy et al. (2018) and Al-Tamimi et al. (2022) showed a resistance percentage of A. baumannii isolates of 100% for ampicillin. [25, 24]. The resistance percentage of A. baumannii isolates to aminoglycosides in the current study was 81.1% to gentamicin and 70.3% to amikacin. Other studies, such as Al-Kadmy et al. (2018), showed a resistance percentage of 14.28% to gentamicin. [25]. The resistance percentage to carbapenems in the current study was 45.9% for imipenem-resistant A. baumannii and 43.2% for meropenem-resistant A. baumannii. A local study by Ahmed et al. (2019) showed the percentage of imipenem-resistant A. baumannii was 100%, while the Al-Kadmy et al. (2018) study showed a resistance percentage of 44.70% for imipenem and 36% for meropenem. A study by Mshachal et al. (2017) showed a resistance percentage of 50% for imipenem. A study in Egypt by Abd El-Baky et al. (2020) showed a 20% resistance percentage to imipenem. [26, 25, 27, 28]. The ability of A. baumannii to rapidly acquire antibiotic-resistance genes and induce multidrug-resistance (MDR) or extensive drug-resistance (XDR) is considered another threat from this opportunistic pathogen [29]. The mechanisms of antibiotic resistance associated with clinical XDR A. baumannii isolates are complex. The widely accepted theory holds that multidrug resistance is often the result of the acquisition of external resistance genes through horizontal gene transfer and/or through mutational resistance [30]. The rate of XDR A. baumannii found in this study could be attributed to the continuous and uncontrolled consumption of antibiotics in Iraq. It is troublesome that the rate of XDR A. baumannii has been reported to be high recently in Iraqi studies (Narjis and Mahdi, 2023) [31]. The sporadic investigation, testing, and checking of antibiotic resistance of A. baumannii is very essential in order to provide updated information about the recent activity of commonly used antibacterial agents in Al-Diwaniyah hospitals and would assist the physicians to detect points in the resistance pattern to the frequently recommended antibiotics against the nosocomial pathogenic A. baumannii. The results of the amplification of the blaIMP gene (gene detection) indicated that 17 (45.9%) clinical isolates were identified as A. baumannii. In another study, several local studies demonstrated parallel results, such as Nocera et al. (2020), Gautam (2023), and a study done by Khalid et al. (2024) [32, 33, 34]. These studies detected blaIMP in 66%, 40%, and 29.9% of isolates. On the other hand, a study

done by Kanaan and Khashan (2022) detected this gene in 76% [35]. In addition to many global studies such as Nikibakhsh et al. (2021) from Iran, Sharma et al. (2019) from India, and Al-Rashed et al. (2024) from the Kingdom, who recorded that the blaIMP gene was also detected in 58.5%, 70.03%, and 94% of A. baumannii isolates, respectively. [36, 37, 38] Regarding the ompA gene, this study showed that 51.4% of A. baumannii clinical isolates carried the ompA gene. A study in Iraq by Al-Kadmy et al. (2018) found the gene in 97% of isolates. [25]. The ompA gene is a virulence factor with a complex profile in A. baumannii and involves pathogenesis, antibiotic resistance, and biofilm formation. Its widespread occurrence in clinical isolates and functional complexities place it as an important biomarker and a novel target of emerging antimicrobial measures. Ongoing efforts of OmpA-based therapy could provide a new light in the fight against multiple drug-resistant A. baumannii [39].

Conclusion:

The study demonstrated a high prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Acinetobacter baumannii isolates in burn wound infections, with reduced antibiotic treatment effectiveness. Molecular detection of blaIMP and ompA genes highlighted the significant roles of antibiotic resistance and virulence in these isolates. Specifically, 16 isolates (43.2%) were classified as MDR, while 21 isolates (56.7%) were recognized as XDR. The blaIMP gene was detected in 17 isolates (45.9%), and the ompA gene in 19 isolates (51.4%). These findings underscore the critical need for molecular surveillance and stringent infection control measures in burn care settings.

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