Identification, Prevalence, and Antibiotic Resistance of Acinetobacter Baumannii Isolated From Foodstuffs

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ABSTRACT

Background: Acinetobacter baumannii is a highly virulent bacterium. It causes opportunistic and nosocomial infections and is a threat to healthcare settings. It has also developed Multidrug Resistance (MDR) capacity. The nosocomial bacteria and antibiotic resistance are primordial and a significant public health concern. These bacteria and their threat can be prevented by reducing infected foodstuffs. Thus, in this study, we investigated A. baumannii isolated from foods in Ardabil City, Iran, and assessed their antibiotic resistance patterns.

Materials and Methods: The identification of bacterium was made by cell morphological and biochemical tests, including sulfide indole motility medium, Simmons citrate agar, the triple sugar iron test, urease, catalase and oxidase test. Also, gene BlaOXA-51 was targeted with the polymerase chain reaction test to select potential MDR strains. The disk diffusion method was used to evaluate the antibiotic susceptibility of the isolates. For the detection of antibiotic resistance genes, β-lactamase was conducted with phenotypic and genotypic assays using the combined disk test and PCR.

Results: Among 100 samples, 27 strains of A. baumannii were isolated. Some antibiotics like imipenem showed 100% sensitivity, and ampicillin-sulbactam showed 100% resistance to isolates. Also, a multidrug resistance profile was assessed and the antibiotic-resistance β-lactamase genes were detected. The prevalence of genes encoding extended-spectrum β-lactamase in the isolates were as follows: SHV, 29.62%; TEM, 18.51%; PER, 14.81%.

Conclusion: A. baumannii isolates showed the highest resistance towards ampicillin-sulbactam (100%) and the lowest resistance to imipenem (0%). These results emphasize the importance of detection and implementation of control measures to prevent the spread of A. baumannii in foodstuffs.

Introduction

Acinetobacter baumannii is an opportunistic human pathogen, highly virulent, and a threat to the healthcare centers [1]. It causes nosocomial infections and is characterized by Multidrug Resistance (MDR) and environmental persistence. Nosocomial infections are a significant public health concern. Since decades, the antibiotic resistance of a wide range of bacteria has increased, and the presence of MDR bacteria has sharply risen in the hospital environment. A. baumannii is highly virulent, with a high mortality rate. It is highly invasive and resists high-stress levels [2].
These bacteria infect human epithelial cells by forming a biofilm and then adhere and colonize the epithelial cells. *A. baumannii* has a high prevalence in Africa and Southeast Asia countries as well as Iran [3]. In Asia and the Middle East, the prevalence of nosocomial infections related to *A. baumannii* is 4% [4]. Although infection rates are lower than other Gram-negative pathogens, the prevalence of MDR is four times higher for *A. baumannii* compared with other Gram-negative pathogens. In South Asia and Iran, *A. baumannii* is a major infection, and its prevention is necessary [5]. This bacterium targets the already sick people and raises their morbidity. Because of the critical pathological condition of the patients targeted, the clinical manifestations of the infection are hard to be characterized. Almost all studies about this infection missed the necessary tests for specific identification of these bacteria [6].

*A. baumannii* species have been isolated from different types of opportunistic infections, including urinary tract infection, meningitis, pneumonia, respiratory tract infection, and so on. Nowadays, their clinical treatment is difficult because of its antibiotic resistance abilities. Treatment by β-lactam antibiotics is the preferred antibacterial choice. However, given the antibiotic adaptability risk, and the increase of β-lactamase positive strains, we need to study other potential options. Some possible probiotics such as Lactobacillus plantarum and Lactobacillus fermentum seem to have an inhibitory effect on *A. baumannii* in vitro [7] and protective effect of symbiotic has been suggested in *A. baumannii*-infection in a murine model [8].

Although potential therapies are under investigation, the highly virulent feature and the capacity of this bacterium to infect humans, animals, environment, and even food is still worrying. Common bacteria are all-pervading in food products such as dairy and meat. Even some bacteria from dairy products like lactic acid bacteria are used as probiotics [9]. However, antibiotic-resistant bacteria can be spread by food and has an essential role in the dissemination of some important human pathogens such as *A. baumannii*.

Some investigations in Switzerland [10], Portugal [11], Iran [5], and Korea [12] has highlighted the prevalence of *A. baumannii* on foodstuffs. Because of the prevalence of MDR bacteria on common foods, we investigated the prevalence and the antibiotic resistance of *A. baumannii* in 100 protein food samples in Ardabil City, Iran.

### Materials and Methods

#### Samples collection

A total of 100 samples of dietary protein food of mutton (n=15), beef (n=15), chicken (n=15), hamburger (n=15), hot dog (n=15), sausage (n=15), and milk (n=10) were purchased from retail stores in Ardabil City.

#### Culture and bacterial identification

We homogenized 25 g of each sample in 25 mL of BHI (Brain Heart Infusion) broth in a stomacher for 1 min and incubated them overnight at 37° C with shaking. Then we cultured the suspension of samples in the MacConkey and blood agar. With the complementing tests, the bacterial samples were inoculated into BHI culture medium, containing 18% glycerol and stored and kept at -70° C. For bacterial identification, the biochemical tests, including urease test, OF (Oxidation/Fermentation) test, SIM (Sulfur,Indol,Motility) test, MRVP (Methyl Red/Voges-Proskauer), TSI (Triple suger Iron), catalase, oxidase, and Simmons citrate tests were done. We also tested the growth rate at 10° C and 48° C to identify different species of Acinetobacter [13].

### Table 1. Reagents used in Polymerase Chain Reaction (PCR)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction mixture</td>
<td>25 μL</td>
</tr>
<tr>
<td>Template DNA</td>
<td>2 μL</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>2 units</td>
</tr>
<tr>
<td>dNTPs</td>
<td>0.2 mM</td>
</tr>
<tr>
<td>DNA buffer</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>Primer</td>
<td>0.2 μM</td>
</tr>
</tbody>
</table>
DNA extraction

The DNA extraction was assessed using the boiling protocol [14]. PCR was carried out in a reaction mixture with the following ingredients mentioned in Table 1. The 100-bp DNA ladder was used.

PCR assessed targeting gene sequences. The primers were used to target BlaOXA-51 gene sequence for confirmation of isolation and identification of A. baumannii isolates (Table 2) [15]:

To identify the resistance against the β-lactam antibiotics, the designed primers targeted β-lactamase genes (Table 3) [15]:

PCR cycle

DNA amplification was done according to standard conditions [16]. Amplification was divided into three cycles (Table 4). Finally, PCR products were placed on a 1.0% agarose gel using 1X Tris–Borate–EDTA (TBE) buffer containing 10 mg/mL ethidium bromide, and the results were visualized under UV light (Table 4).

Antibiotic susceptibility test

We used the disk diffusion method to assess the antibiotic susceptibility of the isolates [17]. The technique was designed according to the Organization for Standardization (ISO) and the quality assurance guidelines of the World Health Organization (WHO). Cultures were swabbed on the agar plates, and antibiotics were used in the form of dodicin. Then all samples were incubated for 24 h at 37° C, and the zones of inhibition were measured. The 0.5 McFarland turbidity of the bacterium was used for antimicrobial testing. The following antibiotics were used: amikacin (30 μg/disk), gentamicin (10 μg/disk), ampicillin-sulbactam (10/10 μg), piperacillin (100 μg),

Table 2. Primers used for amplification of BlaOXA-51 gene

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Designed Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-51 F</td>
<td>5'-TAATGCTTTGATCGGCTTG-3'</td>
</tr>
<tr>
<td>OXA-51 R</td>
<td>5'-TGGATGCACTTCATCTTGG-3'</td>
</tr>
</tbody>
</table>

Table 3. Primers used for amplification of Extended-Spectrum Beta-Lactamase (ESBLs) genes

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Designed Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PER F</td>
<td>-ATGAATGCTATATAAAAGC-3’</td>
</tr>
<tr>
<td>PER R</td>
<td>-AATTGGGGCTAGGGCAGAA-3’</td>
</tr>
<tr>
<td>SHV F</td>
<td>5’-CGGCTTGTATATCTCCCT-3’</td>
</tr>
<tr>
<td>SHV R</td>
<td>5’-CGAGTAGTCCACCAGATCT-3’</td>
</tr>
<tr>
<td>TEM F</td>
<td>5’-TTGCTGTCCGCTCTATCC-3’</td>
</tr>
<tr>
<td>TEM R</td>
<td>5’-ATGTTGCTAGAAGTCTTG-3’</td>
</tr>
</tbody>
</table>

Table 4. Polymerase Chain Reaction (PCR) amplification

<table>
<thead>
<tr>
<th>Process</th>
<th>Temperature</th>
<th>Time Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>95° C</td>
<td>5 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94° C</td>
<td>45 s</td>
</tr>
<tr>
<td>Annealing</td>
<td>At various temperatures</td>
<td>30 s</td>
</tr>
<tr>
<td>Extension</td>
<td>72° C</td>
<td>1 min</td>
</tr>
<tr>
<td>Final extension</td>
<td>72° C</td>
<td>3 min</td>
</tr>
</tbody>
</table>
cefotaxime (30 μg), cefepime (30 μg), ceftazidime (30 μg), imipenem (10 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), tetracycline (30 μg), colistin (10 μg), sulfamethoxazole-trimethoprim (1.25/23.75 μg) and tobramycin (10 μg). Also, *Escherichia coli* ATCC 25922 was used as quality control.

**Combined disk test**

To detect Extended-Spectrum Beta-Lactamase (ESBLs) (β-lactamase genes), we used the Combined Disk Test (CDT). Bacteria were cultured on the Mueller-Hinton Agar (MHA) medium with a disk of ceftazidime (30 μg), ceftazidime+clavulanic acid (30 μg/10 μg), cefotaxime (30 μg) and cefotaxime+clavulanic acid (30 μg/10 μg) placed at an appropriate distance on a MHA plate inoculated with the bacterial suspension and incubated at 37°C overnight. An increase in the inhibition zone diameter of more than 5 mm for a combination disk versus ceftazidime or cefotaxime disk alone was confirmed as ESBLs production [3, 18].

**Results**

**Identification of *A. baumannii***

Hundred samples from different foodstuffs were collected: mutton (n=15), beef (n=15), chicken (n=15), hamburger (n=15), hot dog (n=15), sausage (n=15) and milk (n=10). Isolation of possible *A. baumannii* was assessed, and then, characterization was made according to morphological and biochemical features. Also, gene *BlaOXA-51* was targeted with PCR to select potential MDR to *A. baumannii*. Among 100 isolates, we identified 27 samples that belonged to *A. baumannii* species. Prevalence of *A. baumannii* was highlighted in each foodstuff. Samples that had prevalence rates of up to 26.67% were 4 mutton samples. Also, 3 beef samples with 20.00% prevalence, 9 chicken samples with 60.00% prevalence, 4 hamburgers with 26.67% prevalence, 2 hot dogs with 13.33 prevalence, 1 sausage with 6.67% prevalence, and 4 milk samples with 40.00% prevalence were identified as *A. baumannii* infected samples (Figure 1).

**Antibiotic susceptibility**

An antibiotic susceptibility test was carried out on 27 *A. baumannii* colonies against 15 antibiotics. The resistant, sensitive, and intermediate resistant colonies according to each antibiotic were reported on a graph (Figure 2). Thus, colonies were significantly resistant to piperacillin/tazobactam (92.6%), amikacin (88.9%), cefotaxime (70.4%), ampicillin-sulbactam (100%), and piperacillin (92.6%). The isolates were sensitive to cefepime (63.0%), imipenem (100%), ciprofloxacin (88.9%), gentamicin (85.2%) and sulfamethoxazole-trimethoprim (59.3%). For other antibiotics, non-significant results were observed. Thus, the results demonstrated the MDR capacity of the selected bacteria. Imipenem showed 100% sensitivity to isolates and ampicillin/sulbactam showed 100% resistance to isolates.

The β-lactam compound is used as an antibiotic for a wide range of bacteria. Since several years ago, some bacteria develop resistance against this broad-spectrum antibiotic. One of the main resistance mechanisms is inhibition of β-lactam by β-lactamase. β-Lactamase is an enzyme category with over 4300 enzymes divided on

![Figure 1. Prevalence of *A. baumannii* in food samples](#)

+samples means the percentage of samples where *A. baumannii* was detected; -samples means the percentage of samples where *A. baumannii* was absent.
groups A, B, C, and D that are subdivided in families. The family key enzymes on group A responsible for the broad-spectrum resistance are SHV, TEM, and PER.

Nowadays, the β-lactam antibiotic is the preferred treatment against *A. baumannii*. To determine isolates with resistance ability against β-lactam, a detection test of β-lactamase was used with phenotypic and genotypic assays using CDT and PCR. Using the CDT, an inhibition raise from more than 5 mm was observed on 92.52% disks containing bacteria, β-lactam, and clavulanic acid in comparison with disks without clavulanic acid. With PCR, the presence of 3 β-lactamase family genes was investigated in all isolates. Thus, 29.62% of the isolates expressed SHV (Figure 3 A), 18.51% expressed TEM, and 14.81% expressed PER (Figure 3 B). The presence of β-lactamase enzymes on bacteria from food is related to health and lead to resistance against β-lactam antibiotics.

**Discussion**

By excluding environmental risks such as food contamination, we could prevent *A. baumannii* infections in hospital environments. Some of these bacteria are seen in various foodstuffs such as meat or dairy products, over several countries such as Switzerland, Iran, and Korea [5, 10, 12]. No study has evaluated the prevalence of *A. baumannii* in foodstuffs in Ardabil City, Iran. Thus, we investigated the characterization, prevalence, and antibiotic resistance of *A. baumannii* infect-

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**Figure 2.** Antibiotic susceptibility test

CFT, Ceftazidime; CFE, Cefepime; I, Imipenem; CIP, Ciprofloxacin; G, Gentamicin; ST, Sulfamethoxazole-trimethoprim; PT, Piperacillin/Tazobactam; AM, Amikacin; COL, Colistin; CFO, Cefotaxime; E, Enrofloxacin; O, Ofloxacin; TO, Tobramycin; AMP, Ampicillin-sulbactam; PIP, Piperacillin.

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**Figure 3.** Expression of genes by Polymerase Chain Reaction (PCR)

A. PCR gel of the expression of SHV. Lane M: DNA size marker (100-bp DNA ladder); Lane 1: negative control; Lane 2-3: PCR products of the isolates containing SHV (293 bp); Lane 4: positive control.

B. Percentage of strains expressing SHV, TEM, and PER.
ed foodstuff in Ardabil. After characterization by morphological biochemical as well as genotypic methods, 27 strains of *A. baumannii* were identified. All these tests were necessary to differentiate these bacteria from other bacteria [19, 6].

According to the evaluations, the prevalence of *A. baumannii* was high in products such as chicken (60%) and milk (40%); low in products like hot dog (13.33%) and sausage (6.67%); intermediate in mutton (26.67%), beef (20.00%), and hamburger (26.67%). These results suggest the difference in prevalence according to the kind of food product and among various kinds of meat. Yet, to our knowledge, it is the first study showing the prevalence of *A. baumannii* with so many different products.

The biggest issue of pathogen bacteria, such as *A. baumannii*, is its multidrug resistance [20]. To assess the antibiotic resistance of the isolates, the antibiotic susceptibility test was carried out with the disk diffusion method. About 15 antibiotics were used against isolates, including amikacin, gentamicin, ampicillin-sulbactam, piperacillin, cefotaxime, cefepime, ceftazidime, imipenem, ofloxacin, ciprofloxacin, enrofloxacin, tetracycline, colistin, sulfamethoxazole-trimethoprim, and tobramycin. Results vary from high sensitivity to high resistance of these strains against antibiotics. These results showed a high rate of *A. baumannii* resistance against drugs and should alert authorities on the presence of MDR bacteria among regular foodstuffs. However, some antibiotics displayed high inhibitory performance on the isolates (100%). These kinds of antibiotics could be a new perspective on treatment.

β-Lactam antibiotics are among the most commonly used antimicrobial agents with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative pathogens [21]. These antibiotics act by inhibiting a set of transpeptidase enzymes that are essential for the synthesis of the peptidoglycan layer of the bacterial cell wall, resulting in the death of growing bacteria [22]. In response, bacteria have evolved defense mechanisms to resist the lethal effects of these drugs, e.g., efflux, reduced penetrability, altered transpeptidases, and inactivation by β-lactamases [20]. The outcome is a high resistance among Gram-negative bacteria, including *A. baumannii*.

Three primary genes of SHV, TEM, and PER are responsible for the broad-spectrum resistance in bacteria [22]. To assess the presence of these β-lactamase genes inside the isolates, we investigated with phenotypic and genotypic assays using ESBLs CDT and PCR. Results of PCR showed the presence of β-lactamase genes. According to the family group, 29.62% of bacteria express the gene of the SHV family, 18.51% TEM family and 14.81% PER family. The presence of β-lactamase enzymes on bacteria from food is a health concern and leads to resistance against β-lactam antibiotics. Inhibition of β-lactamase by ETX leads to the sensitivity against β-lactam antibiotics. Thus, through CDT, inhibition of bacteria by β-lactam antibiotics was assessed. Strains of *A. baumannii* were mostly β-lactamase positive. This result could explain the high resistance to β-lactam antibiotics.

Finally, the main goal of this study was to isolate *A. baumannii* from infected foodstuff and evaluate the MDR potential of the isolated strains. We showed the resistance of the pathogen against β-lactam antibiotics, highlighted some potential antibiotics that could be useful for the treatment of this infection, and reported on the prevalence of *A. baumannii* depending on the foodstuff. Some likely antibiotics such as cefepime, imipenem, ciprofloxacin, gentamicin, and sulfamethoxazole-trimethoprim were detected thanks to their sensitivity. Because of the adaptability of *A. baumannii*, monotherapies using these antibiotics should be avoided, and the study of multiple therapies involving various antibiotics with sensitive potentials should be investigated.

These results should be considered to prevent nosocomial infections. As it is speculated that the foodstuffs have an essential role in the dissemination of this organism, further work should be done to limit the food contamination by *A. baumannii*. Strict hygiene and critical control point principles should be implemented along the entire food production chain to prevent potentially pathogenic bacteria reaching to consumers. In other words, this could be a preventive step to avoid contamination of these bacteria into hospitals. Even if *A. baumannii* is less sensitive than other bacteria, these kinds of protocols should be done in the best way to avoid other contaminations produced by bacteria and to limit the spread of *A. baumannii* in the health care center and to save heavy disinfection regimes and prolonged periods of dryness.

**Ethical Considerations**

**Compliance with ethical guidelines**

All ethical principles were considered in this article.
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Conflict of interest

The author declared no conflict of interest.

References


