



The First Report of the Antimicrobial Resistance of *Acinetobacter baumannii* Isolates from Seafood Samples

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Acinetobacter baumannii is a newly-launched bacterium responsible for rare cases of diarrhea and foodborne diseases. This survey was performed to study the prevalence and antimicrobial resistance of *A. baumannii* isolates recovered from fish, shrimp, and lobster. Five-hundred seafood samples were collected and cultured for isolation of *A. baumannii*. *A. baumannii* isolates were identified by biochemical tests and further confirmed by the PCR. The antimicrobial resistance of isolates was studied using disk diffusion test. Twenty-eight out of 500 (5.6%) seafood samples were contaminated with *A. baumannii*. Fish had the highest prevalence of *A. baumannii* (10%), while lobster had the lowest (2.5%). *A. baumannii* isolates harbored the highest incidence of resistance toward tetracycline (75%), gentamicin (71.4%), ciprofloxacin (64.2%), trimethoprim (57.1%), streptomycin (53.5%), and erythromycin (50%) antimicrobial agents. The lowest incidence of resistance was obtained against chloramphenicol (3.5%) and imipenem (7.1%) antimicrobial agents. This is the first report of isolation and identification and antimicrobial resistance pattern of *A. baumannii* in seafood samples globally. The role of seafood samples as reservoirs of antimicrobial-resistant *A. baumannii* was determined. Complete cooking of seafood and prevention from their consumption in raw form can diminish the risk of *A. baumannii* foodborne diseases.

Keywords: *Acinetobacter baumannii*, Seafoods, Prevalence, Antimicrobial resistance.

Introduction

Seafood, fish, shrimp, and lobster, are significant economic and marketing foodstuffs globally. They are rich sources of proteins, minerals, fatty acids, and vitamins. Their consumption guarantees a healthy life [1]. However, according to the high nutritional parameters, they are severely prone to microbial corruption[2].

Acinetobacter baumannii (*A. baumannii*) is one of the newly launched foodborne bacterium, which acts as an opportunistic pathogen originated from different infection sources, including contaminated water and environment and through human manipulation

of seafood samples [3]. It is an aerobic, Gram-negative, rod and non-fermenting bacterium that colonizes the respiratory tract, skin, urinary and gastrointestinal systems[4]. The bacterium is also responsible for surgical infections, pneumonia, catheter-related blood circulatory, and urinary tract infection, mediastinitis, meningitis, cholangitis, and osteomyelitis [5].

The bacterium is essential from the aspect of antibiotic resistance. It mainly showed intermediate and even high resistance toward diverse classes of antimicrobial groups, particularly penicillins, aminoglycosides, sulfonamides, macrolides, and carbapenems cephalosporins, tetracyclines, and quinolones

[6,7]. However, scarce researches have been performed to assess the role of antibiotic-resistant *A. baumannii* in different food samples, particularly seafood.

According to the importance of *A. baumannii* as a newly-launched foodborne pathogen, the present investigation was addressed to assess the antimicrobial resistance of *Acinetobacter baumannii* isolates from seafood samples.

Materials and Methods

Samples

From April to October 2020, five-hundred seafood samples, including fish (*Scomberomorus commerson*) (n= 150), shrimp (*Penaeus monodon*) (n= 150), and lobster (*Scalloped spiny*) (n= 200) samples, were collected from shopping centers of Shiraz city, Iran. Each sample (100 g from the dorsal muscle) was collected separately in highly hygienic conditions using sterile tissue forceps in laboratory tubes containing a peptone water solution (Merck, Germany). These samples were healthy and fresh, and all were caught from the Persian Gulf, Iran. All samples were transferred to the laboratory using cool boxes at 4°C.

Bacterial isolation and identification

Twenty-five grams of each sample was blended in 225 nutrient broth medium (Merck, Germany) and incubated at 37 °C for 24 h. A loopful of the broth was cultured on both blood agar (BA) and MacConkey agar (MC, Merck, Germany) plates and incubated at 37 °C for 24 h. Suspected colonies which were grown on BA (non-hemolytic, opaque and creamy) and MC agar (non-lactose fermenting) were identified according to various biochemical tests including Gram-staining, motility, catalase, coagulase, oxidase, sugar fermentation, indole, H₂S production, Voges Proskauer, methyl Red, gelatin hydrolysis, nitrate reduction, and urease activity [8].

PCR confirmation of isolates

A. baumannii isolates were further confirmed by the PCR [9]. The isolates were sub-cultured in nutrient broth and incubated at 37 °C for 24 h. DNA was extracted from *A. baumannii* broth cultures using DNA extraction kit (CinnaGen, Iran). The procedure was performed according to the manufacturer's guidelines. The quality of

extracted DNA was assessed using electrophoresis on the gel. The Nanodrop device (NanoDrop, Thermo Scientific, Waltham, USA) was used to assess the extracted DNA purity. Table 1 indicates primers and PCR conditions. PCR was performed using DNA thermo-cycler (Eppendorf 5330, Germany). PCR products were analyzed using gel electrophoresis (2% agarose gel stained with CYBR Green in 1X TBE buffer) (CinnaGen Co, Iran). Electrophoresis was done at 80 V for 30 min. All PCR materials and ingredients were purchased from CinnaGenCo, Iran [10-12].

Antimicrobial resistance

The disk diffusion examined the antimicrobial resistance pattern of *A. baumannii* isolates. Mueller-Hinton agar (Merck, Germany) medium, Kirby-Bauer disk diffusion method, and guidelines of the Clinical and Laboratory Standard Institute (CLSI) were used for this purpose [13]. A 0.5 McFarland concentration of the bacteria was used for this purpose. Tetracycline (30 µg/disk), ceftazidime (30 µg/disk), ciprofloxacin (5 µg/disk), co-trimoxazole (23.75/1.25 µg/disk), chloramphenicol (30 µg/disk), gentamicin (10 µg/disk), rifampicin (5 µg/disk), streptomycin (10 µg/disk), trimethoprim (5 µg/disk), imipenem (10 µg/disk), nitrofurantoin (300 µg/disk), azithromycin (15 µg/disk), and erythromycin (15 µg/disk) antimicrobial agents were used (Himedia, India). Bacteria were superficially cultured on MHA, and antimicrobial disks were placed on media, and then, media were incubated at 37 °C for 24 h. Then, the bacteria's growth inhibition zone diameter was measured and compared with the standard of the CLSI [14-16].

Statistics

SPSS software and chi-square and Fisher tests were used for statistical examination. Differences in the prevalence and antimicrobial resistance of bacteria between diverse groups were considered. *P*-value < 0.05 was considered as significant level [17,18].

Results

PCR amplification

PCR technique was used to detect the 16S-23S ribosomal DNA gene of the *A. baumannii* isolates from seafood samples. All isolates were confirmed by the PCR (Fig. 1).

TABLE 1. Primers and PCR conditions were used for the detection of *A. baumannii*.

Target gene	Sequence (5'-3')	Size (bp)	PCR cycles	PCR Volume	Reference
<i>16S-23S</i> <i>ribosomal DNA</i>	(F) CATTATCACGGTAAATTAGTG	208	1 cycle: 94 0C ----- 6 min.	5 μ L PCR buffer 10X 2mM MgCl ₂ 150 μ MdNTP 1 μ M of each primers F & R 1 U Taq DNA polymerase 3 μ L DNA template	[9]
	(R) AGAGCACTGTGCACTTAAG		30 cycle: 95 0C ----- 60 s 58 0C ----- 60 s 72 0C ----- 40 s 1 cycle: 72 0C ----- 5 min		

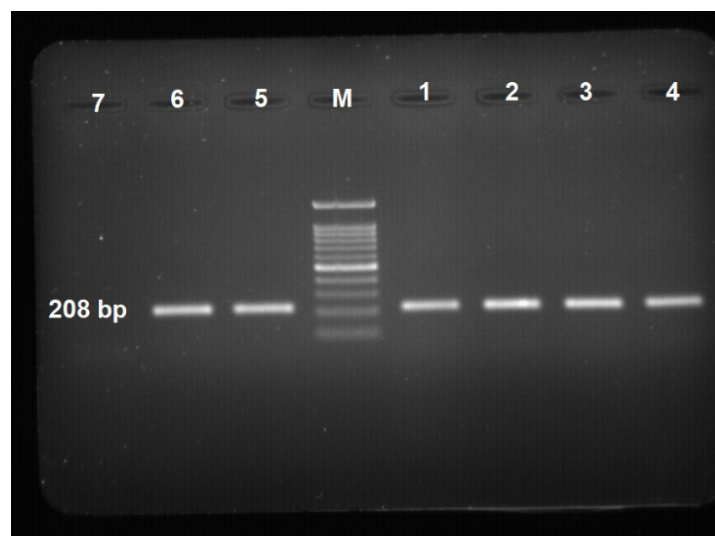


Fig. 1. PCR gel electrophoresis of the 16S-23S ribosomal DNA gene of the *A. baumannii* isolates recovered from seafood samples. M: 100 bp ladder (CinnaGen co, Iran), 1-5: Positive samples for the 16S-23S ribosomal DNA gene of the *A. baumannii* (208 bp), 6: Control positive (*A. baumannii* ATCC 17978), 7: Control negative (PCR-grade water (CinnaGen co, Iran)).

Prevalence of A. baumannii

Table 2 reveals the prevalence of *A. baumannii* in diverse kinds of seafood samples. Twenty-eight out of 500 (5.6%) seafood samples were contaminated with *A. baumannii*. Fish had the highest contamination rate with *A. baumannii* (10%), while lobster had the lowest (2.5%). A significant difference was obtained from the statistical view between the type of samples and prevalence of *A. baumannii* ($P < 0.05$).

Antimicrobial resistance of A. baumannii

Table 3 reveals the antimicrobial resistance pattern of *A. baumannii* isolates recovered from seafood samples. *A. baumannii* isolates showed the maximum rate of resistance against tetracycline (75%), gentamicin (71.4%), ciprofloxacin (64.2%), trimethoprim (57.1%), streptomycin (53.5%), and erythromycin (50%) antimicrobial agents. The lowest rate of resistance was found toward chloramphenicol (3.5%) and imipenem (7.1%) antimicrobial agents. A significant difference was obtained from the statistical view between the type of samples and the prevalence of antibiotic resistance ($P < 0.05$).

Discussion

The growing incidence of foodborne diseases in various world sites has been related to increased raw food consumption [11, 19-25].

Nevertheless, dissimilar to the common pathogens implicated in foodborne diseases, Acinetobacter species are infrequently accompanied by diarrheal disease, possibly due to their hard isolation from food sources. Nonetheless, numerous species of Acinetobacter, particularly *A. baumannii*, harbor some of the features associated with significant pathogens and show an extraordinary aptitude to resist several antimicrobial agents. Some epidemiological information regarding the antibiotic-resistant *A. baumannii* in contaminated dairy products, together with raw fruit and vegetables and meat samples, establish extra-hospital reservoirs of this underrated pathogen, which may signify an augmented risk to immunocompromised individuals, elders, and young children in a hospital setting [26].

The present survey is the first report of the prevalence and antimicrobial resistance of *A. baumannii* isolates from fish, shrimp, and lobster samples globally. Findings revealed the high prevalence of *A. baumannii* strains accompanied with the high prevalence of resistance toward tetracycline, gentamicin, ciprofloxacin, trimethoprim, streptomycin, and erythromycin antimicrobial agents. Transmission of resistant-bacteria from the contaminated staff of the fishing ports and processing companies is the

most probable reason for the findings. However, high, unauthorized, and illegal prescription of antimicrobial agents and using high doses and volumes of disinfectants are probable reasons for antimicrobial resistance in isolated bacteria. The filter-feeding activity of seafood, shrimp, and lobster in particular, maybe the main reason for the high prevalence of *A. baumannii* strains. Accumulation and survival of *A. baumannii* bacteria through the filter-feeding activity has occurred.

Rare researches have been conducted in this field. Aksari et al. [27] reported that the prevalence of *A. baumannii* in animal species' raw meat was 20.10%. They showed that *A. baumannii* isolates harbored the higher prevalence of resistance toward azithromycin (66.6%), gentamicin (87.1%), erythromycin (74.3%), tetracycline (79.4%), trimethoprim (56.4%), ciprofloxacin (58.9%), and rifampin (51.2%), which was relatively similar to our findings. High prevalence of *A. baumannii* in different types of meat samples was also previously reported by Houanget al. [28], Hamouda et al.[29], Lupo et al.[30], and Carvalheira et al.[31]. Kim et al.[32] reported a high prevalence (27.8%) of *A. baumannii* strains in the raw milk samples of naturally infected animal species with higher resistance toward tetracycline (30.8%), followed by ceftriaxone (4.4%), cefotaxime (12.5%), and gentamicin (2.9%) antimicrobial agents. High prevalence of *A. baumannii* in different types of milk samples was also previously reported by Vaz-Moreira et al. (2011) [33], Gurung et al.[34], Saadet al.[35], and Ramos and Nascimento[36]. *A. baumannii* and other *Acinetobacter* species were rarely detected in seafood samples [37-40]. In keeping with this, this pathogen's role in seafood samples

is potentially unknown, and further research is required to understand this fact. High prevalence of resistance of *A. baumannii* strains toward commonly-used antimicrobials, particularly tetracycline, gentamicin, ciprofloxacin, trimethoprim, streptomycin, and erythromycin was reported from Brazil [41], Iran [42], United States [43], Jordan [44], Africa [45], and Korea [46]. As antibiotic-resistant *A. baumannii* was detected in large number of seafood samples. Proper monitoring of the seafood microbial quality should perform at the site of fishing and also after their transport.

The present investigation was limited to the low numbers of isolated bacteria in seafood samples and the lack of additional research on other seafood samples presented in Iran. Additionally, the absence of significant literature-based documents is another important limitation of the current survey. However, this is a preliminary and first report of the presence of antibiotic-resistant *A. baumannii* among seafood samples globally.

Conclusion

The present research findings revealed the role of seafood samples, mainly fish, shrimp, and lobster, as important reservoirs of the *A. baumannii* strains in the environment. The high prevalence of *A. baumannii* was accompanied by the high prevalence of resistance toward routinely applied antimicrobials. Well-cooking of fish, shrimp, and lobster before consumption can diminish the risk of diarrheal illnesses due to the *A. baumannii* strains. Complete cooking of seafood samples before consumption can reduce the risk of antibiotic-resistant *A. baumannii* related infections.

TABLE 2. Prevalence of *A. baumannii* in diverse seafood samples.

Types of samples	N. samples collected	N. (%) samples positive for <i>A. baumannii</i>
Shrimp	150	8 (5.3)
Lobster	200	5 (2.5)
Fish	150	15 (10)
Total	500	28 (5.6)

TABLE 3. Antimicrobial resistance pattern of *A. baumannii* isolates from seafood samples

Samples (N, <i>A. baumannii</i>)	N (%) isolates harbored resistance to each antibiotic agent												
	tet*	cef	cip	cot	c30	gen	rif	s10	trp	imp	nit	az	er
Shrimp (8)	6 (75)	3 (37.5)	5 (62.5)	3 (37.5)	-	5 (62.5)	2 (25)	4 (50)	4 (50)	-	2 (25)	3 (37.5)	4 (50)
Lobster (5)	3 (60)	1 (20)	3 (60)	2 (40)	-	3 (60)	1 (20)	1 (20)	2 (40)	1 (20)	1 (20)	1 (20)	1 (20)
Fish (15)	12 (80)	7 (46.6)	10 (66.6)	6 (40)	1 (6.6)	12 (80)	7 (46.6)	10 (66.6)	10 (66.6)	1 (6.6)	6 (40)	7 (46.6)	9 (60)
Total (28)	21 (75)	11 (39.2)	18 (64.2)	11 (39.2)	1 (3.5)	20 (71.4)	10 (35.7)	15 (53.5)	16 (57.1)	2 (7.1)	9 (32.1)	11 (39.2)	14 (50)

*tet: tetracycline (30 µg/disk), cef: ceftazidime (30 µg/disk), cip: ciprofloxacin (5 µg/disk), cot: co-trimoxazole (23.75/1.25 µg/disk), c30: chloramphenicol (30 µg/disk), gen: gentamicin (10 µg/disk), rif: rifampicin (5 µg/disk), s10: streptomycin (10 µg/disk), trp: trimethoprim (5 µg/disk), imp: imipenem (10 µg/disk), nit: nitrofurantoin (300 µg/disk), az: azithromycin (15 µg/disk), er: erythromycin (15 µg/disk).

Acknowledgments

Many thanks from the staff of the Researches Center of Medicinal Plants and Ethno-veterinary, Islamic Azad University, Shahrekord Branch, Shahrekord, and Research & Development (R & D) Team, Gol Daru Company, Isfahan, Iran for their laboratory supports.

Conflict of interest

The authors declared no conflict of interest.

Funding statement

Self-funding.

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