



Genetic Diversity of OXA Producing Carbapenem-Resistant *Acinetobacter baumannii* from Environment of Tertiary Hospitals in Central Iran

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Received 2019 June 22; Revised 2019 December 23; Accepted 2019 December 29.

Abstract

Background: The hospital environment usually involves in cross-colonization and/or outbreaks of carbapenem-resistant *Acinetobacter baumannii* (CRAB). This study aims to identify genetic diversity of environmental *Acinetobacter baumannii* (*A. baumannii*) isolates based on OXA-type carbapenems.

Objectives: It was intended to identify the environmental source of *A. baumannii* strains to control future infections and ongoing outbreaks.

Methods: Swab samples were collected from equipment, fluids and surfaces of intensive care units of five hospitals (H1 to H5). The susceptibility of the isolates was defined through disk diffusion method. *bla*_{OXA-51}, *bla*_{OXA-58}, *bla*_{OXA-23}, and *bla*_{OXA-24} genes were detected by multiplex polymerase chain reaction (PCR). Genetic diversity was investigated using ERIC-PCR. Mean values were compared using an Independent samples *t*-test.

Results: The mean number of Gram-negative bacilli colony per sample was significantly higher in moist surfaces (1.9 ± 2.37) than in dry surfaces and medical equipment ($P < 0.05$). Among 32 *A. baumannii* isolates, 17 (53.1%) were classified as CRAB, 13 (40.6%) as multidrug resistant (MDR) and six (18.8%) as extremely drug resistant (XDR) phenotypes. The isolates showed the most susceptibility to tigecycline (81.3%) and doxycycline (81.3%). Totally, 10 isolates (31.3%) carried *bla*_{OXA-23} and one isolate carried *bla*_{OXA-58}, but none contained *bla*_{OXA-24}. Typing of the strains provided seven single types (ST) and nine clusters (A-I). Two isolates in cluster B (different surfaces of H4) and two in cluster I (from ventilators of H3 and H1) were designated as common type (CT). In H4 and H1, different isolates belonging to different clusters were recovered from bed sheet and baby sink bath, respectively.

Conclusions: Clonally-related *bla*_{OXA-23} positive CRABs occurred in the environment, especially on moist surfaces of hospitals. The distribution of the same clones in different hospitals may point to expansion of a specific clone which increases the risk of cross-colonization of vulnerable patients.

Keywords: *Acinetobacter baumannii*, Environment, Cross Infections, Beta-Lactamases

1. Background

It has long been known that Gram-negative bacilli (GNB) are associated with sustaining health-care associated infections (HAIs); among them, *Acinetobacter baumannii* (*A. baumannii*) is an emerging pathogen of human HAIs, with increasing morbidity and mortality worldwide (1). Hospital-acquired *A. baumannii* infections are typically manifested as ventilator-associated pneumonia, meningitis, bacteremia, urinary tract infections, and surgical wound infections (2).

Carbapenems have lost their efficacy against *A. baumannii* nosocomial infections due to increasing administration during the last years (3, 4). Growing prevalence of carbapenem-resistant and multidrug resistant *A. baumannii* is a major risk in care facilities. This resistance is often associated with acquisition of class D β -lactamases including OXA-type carbapenemases (OTC) such as *bla*_{OXA-58}, *bla*_{OXA-24}, *bla*_{OXA-143} and *bla*_{OXA-23} genes (5). Metallo β -lactamases (MBLs) and carbapenemases of Ambler class A also play important roles in this regard (6). Sev-

eral pieces of evidence around the world have shown dissemination of OTC-harboring *A. baumannii* strains within and between hospitals (6, 7).

The two characteristics of multidrug resistance development and survival in the hospital environment have provoked *A. baumannii* to emerge as a successful opportunistic nosocomial pathogen (8). The hospital surface may represent as a reservoir for *A. baumannii* clones which easily circulate in both environmental and clinical settings. Identification of the source or reservoir of *A. baumannii* strains is a key factor to control future infections and ongoing outbreaks.

2. Objectives

In the current report, we evaluated the distribution of carbapenem resistant *A. baumannii* isolates in the environmental surfaces of five hospitals in central Iran.

3. Methods

3.1. Setting and Sampling

During a 9-month period from March to November 2018, swab samples were collected from equipment, fluids and surfaces of intensive care units (ICUs) of five hospitals (H1 to H5) in Qom City, central Iran. H1 is a gynecology hospital established in 1958, with 156 fixed beds and an active neonatal intensive care unit (NICU). H2 is the biggest university hospital in the city, established in 1982, with 500 fixed beds. H3 is a major dialysis center of the city, established in 1964, with 230 fixed beds. H4 is currently the largest trauma center of the city, established in 1945, with 276 fixed beds. Finally, H5 is established in 1990, with 158 fixed beds. Sampling was performed to reach the calculated sample size of 194 Gram negative bacilli. To this end, samples were collected from equipment and medical devices using a moistened sterile swab from a 10 cm² surface area. Similarly, sampling of moist surfaces was carried out by rubbing the swabs on the surface area. Sampling from fluids was also performed from 1 mL of liquids.

3.2. Identification and Culture of the Isolates

The swabs were cultured on sheep blood agar and subcultured on MacConkey agar medium (MicroMedia, Australia). After incubation at 35°C for 48 hours, well-isolated single colonies recovered from agar plates were inoculated into triple sugar iron (TSI) agar (Merck KGaA, Darmstadt, Germany) and the results were used for discriminating nonfermenter bacteria. *Acinetobacter* spp. were identified based on colony characteristics, growth at 44°C, oxidase test, urease, lysine decarboxylase (MicroMedia,

Australia), arginine decarboxylase, DNase, Fluorescence-Lactose-Denitrification and the oxidative fermentative test with maltose, mannitol, fructose and dextrose (Merck KGaA, Darmstadt, Germany) (9). The API 20NE system (bioMerieux, France) was used in some instances. The result of *A. baumannii* detection was confirmed by PCR amplification of *bla*_{OXA-51}-like gene using specific primers.

3.3. Antibiotic Susceptibility Testing

The susceptibility of isolates to antibiotics was determined using the disk diffusion method according to CLSI guidelines (10). The tested antibiotics included imipenem (10 mg), meropenem (10 mg), doripenem (10 mg), cefepime, (30 mg), ceftazidime (30 mg), cefotaxime (30 mg), ceftriaxone (30 mg), amikacin (30 mg), gentamicin (10 mg), doxycycline (30 mg), minocycline (30 mg), tigecycline (15 mg), piperacillin/tazobactam (110 mg), ticarcillin/clavulanic acid (85 mg), gatifloxacin (5 mg), ciprofloxacin (5 mg), levofloxacin (5 mg), and trimethoprim/sulfamethoxazole (25 mg) (Mast Group Ltd., UK). Carbapenem-resistant *A. baumannii* (CRAB) was defined as resistance to at least two carbapenems, and multidrug-resistance (MDR) was defined as isolates showing non-susceptibility to at least one agent in ≥ 3 antimicrobial categories. Isolates that were non-susceptible to at least one agent in all categories except one or two antimicrobial categories regarded as extensively drug resistant (XDR) *A. baumannii*. The CLSI breakpoints and interpretation for zone diameter were referred (10).

3.4. Multiplex PCR for Detection of OXA-Type Carbapenemases Genes

The standard phenol-chloroform extraction procedure was followed for genomic DNA extraction (11). A multiplex PCR was used to detect *bla*_{OXA-58}, *bla*_{OXA-24}, and *bla*_{OXA-23} genes in *A. baumannii* isolates, as described previously (12). *A. baumannii* reference strains NCTC 13304, NCTC 13302, and NCTC 13305 were used as positive control for *bla*_{OXA-23}, *bla*_{OXA-24}, and *bla*_{OXA-58} genes, respectively. The sequencing result was submitted to the GenBank database under the accession number JQ409995.1.

3.5. Enterobacterial Repetitive Intergenic Consensus-PCR

The primer pair ERIC1 (5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC2 (5'-AAGTAAGTACTGGGGTGAGC-3') were used to amplify intervening fragments of ERIC in the genomic DNA. Amplification reactions were performed in a final volume of 25 μ L. Each reaction contained 2.5 μ L of 10 \times PCR ViBuffer, 800 μ M dNTP mixture (CinnaGen, Iran), 2.5 U MaxTaq DNA polymerase enzyme (Vivantis Technologies Sdn. Bhd.,

Malaysia), 0.6 μ M of each primer, 4000 μ M MgCl₂, 3 μ L DNA template, and ddH₂O up to 25 μ L. Amplification reactions were carried out with initial denaturation (5 minutes at 95°C), followed by 35 cycles of denaturation (1 minutes at 94°C), annealing (1 minutes at 37.5°C) and extension (3 minutes at 72°C), with a final extension at 72°C for 5 minutes. Clonal relatedness of the strains was analyzed from scanned images of the agarose gel pictures using the GelCompar II software (Applied Maths, Belgium) with the band matching coefficient of Dice. The isolates were clustered using the unweighted pair-group method with arithmetic mean and displayed in dendrogram forms.

3.6. Statistical Analysis

SPSS version 22 (SPSS Inc., Chicago, IL) was used for statistical analyses of the studied parameters. The mean values between the groups were compared using an Independent sample *t*-test, and $P < 0.05$ were considered statistically significant.

4. Results

Totally, 396 swab samples were collected from surfaces and medical equipment of the five hospitals, and 1205 colonies of bacteria including 194 non-duplicate GNB were isolated (Table 1).

When looking at the data of all the hospitals as a whole, the mean number of GNB colony per sample was 1.9 ± 2.37 (86.6%) in moist surfaces, which was significantly ($P < 0.05$) more than that in dry surfaces (0.51 ± 0.78) and medical equipment (0.43 ± 0.83). Of 151 non-fermentative bacteria, 32 isolates (21.2%) were identified as *A. baumannii* (five from H1, two from H2, six from H3, 12 from H4, and seven from H5). In addition, 25 strains of the genus *Acinetobacter* spp. other than *baumannii* were recorded.

The results also revealed that 17 isolates (53.1%) were CRAB, 15 were CSAB, 13 (40.6%) showed MDR phenotype, six (18.8%) showed XDR phenotype and the remaining were susceptible (Figure 1). Based on the results, six isolates showed intermediate susceptibility to tigecycline (81.3% susceptibility) and six isolates showed resistance to doxycycline (81.3% susceptibility). The rate of resistance was higher for the other antibiotics.

Typing of the *A. baumannii* strains by ERIC-PCR provided two to 10 bands of amplification with a range of 160 to 1600 bp sizes in electrophoresis. With similarity level of at least 50%, seven single types (STs) and nine clusters (A - I) were classified. The most prevalent ERIC-PCR genotype was cluster B (15.6%). Isolates with similarity level of 100% were classified as common type (CT). In cluster B, two isolates of 89-7 and 93-2 in H4 were CT, which were recovered

Table 1. Distribution of Isolated Bacteria in the Different Hospitals in Relation to Environmental Specimens

	Recovered Colony Number										Total Mean/ Sample \pm SD		
	H1		H2		H3		H4		H5			Total Bacteria	Total GNB
	All Bacteria	Mean/ Sample	All Bacteria	Mean/ Sample	All Bacteria	Mean/ Sample	All Bacteria	Mean/ Sample	All Bacteria	Mean/ Sample			
Equipment	14	0.2	25	0	63	8	97	9	52	8	251	26	0.43 ± 0.83
Dry surfaces	47	0.54	56	1	178	7	238	12	162	11	681	41	0.51 ± 0.78
Moist surfaces	41	1.47	20	1	72	38	77	30	63	33	273	127	1.9 ± 2.37
Total	102		101	2	313	53	412	51	277	52	1205	194	

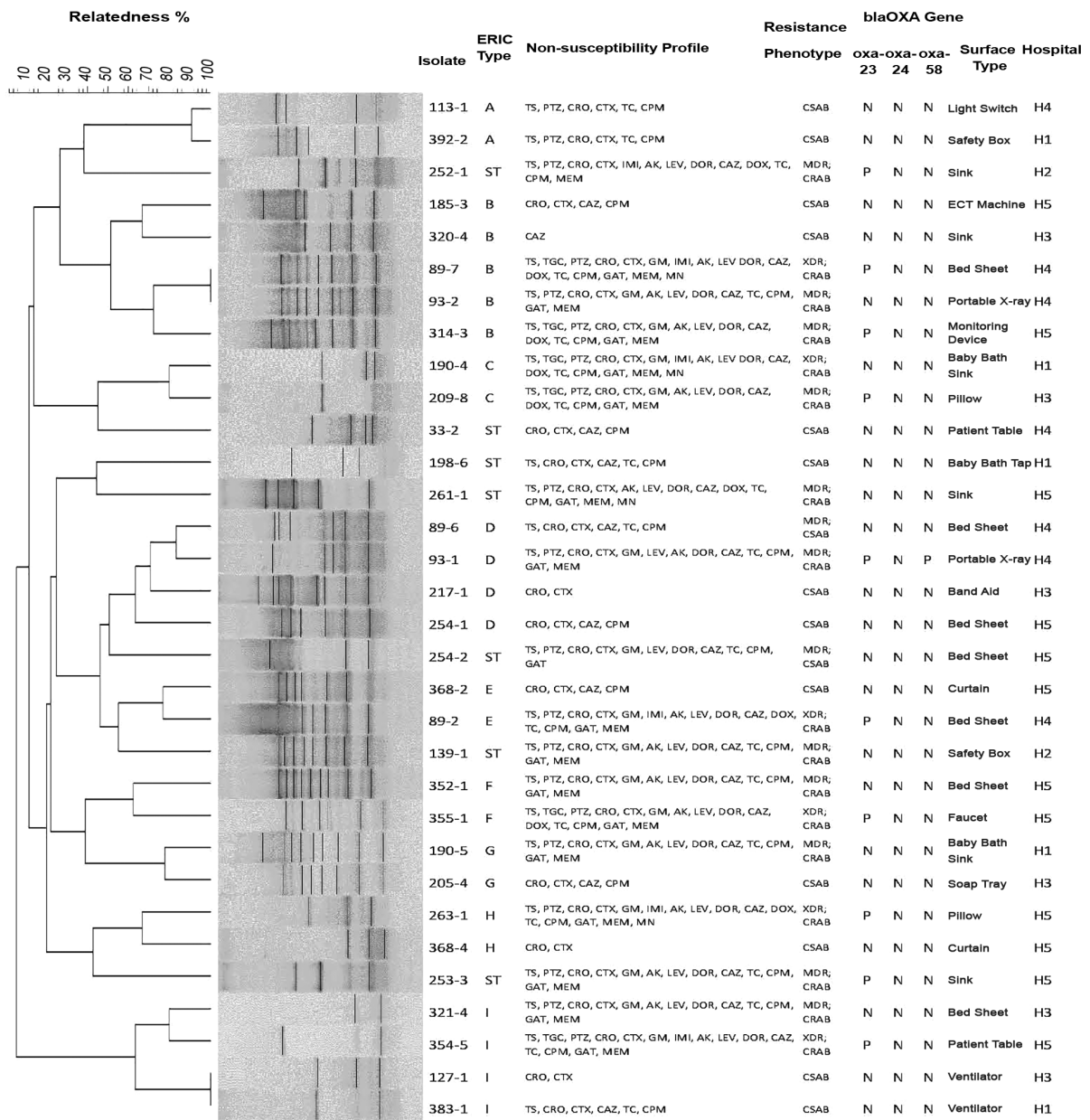


Figure 1. ERIC-PCR profiles and characteristics of the environmental *Acinetobacter baumannii* isolates. N, negative; P, positive; H, hospital; ST, single type; IMI, imipenem; MEM, meropenem; DOR, doripenem; CPM, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; AK, amikacin; GM, gentamicin; MN, minocycline; DOX, doxycycline; TGC, tigecycline; TS, trimethoprim-sulfamethoxazole; CO, colistin sulphate; PTZ, piperacillin-tazobactam; GAT, gatifloxacin; CIP, ciprofloxacin; LEV, levofloxacin; TC, ticarcillin-clavulanic acids.

from bed sheet and portable X-ray, respectively. Moreover, in cluster I, two isolates of 127 - 1 and 383 - 1 from different hospitals (H3 and H1) were designated as CT, and were both recovered from ventilator. Further, in cluster A, two isolates of 113 - 1 and 392 - 2 from different hospitals (H4 and H1) had at least 90% similarities, and were recovered from

light switch and safety box, respectively.

The multiplex PCR assay for OTC genes showed that 10 of the 32 *A. baumannii* isolates (31.3%) carried *bla*_{OXA-23}, one isolate carried *bla*_{OXA-58}, and none contained *bla*_{OXA-24}. All *bla*_{OXA-23} gene positive isolates showed CRAB MDR/XDR phenotype.

Two *bla*_{OXA-23} gene positive CRAB isolates in cluster B (89 - 7, 314 - 3) were recovered from bed sheet and monitoring device in H4. From the same hospital, two other *bla*_{OXA-23} positive CRAB isolates (93 - 1 and 89 - 2) from clusters D and E were recovered from portable X-ray and bed sheet, respectively. One *bla*_{OXA-23} positive CRAB isolate was obtained from isolates of H2 (252 - 1) and H3 (209 - 8) as different genotypes. In H5, four *bla*_{OXA-23} positive CRAB isolates (253 - 3, 355 - 1, 263 - 1, and 354 - 5) with different genotypes were isolated from sink, faucet, pillow and patient table, respectively. The co-occurrence of *bla*_{OXA-58} and *bla*_{OXA-23} was found in the CRAB MDR isolate (93 - 1) of cluster D genotype from H4.

5. Discussion

There are limited reports about the investigation of *A. baumannii* in the hospital environment from Iran and other countries of the region (13-16). We screened surfaces and equipment of five hospitals and observed a high frequency (53.1%) of CRAB isolates which were simultaneously resistant to some tested antimicrobial agents. Previously, the high distribution of XDR *A. baumannii* in clinical isolates was reported in hospitals of Tehran, central Iran (7, 17). Based on the present results, tigecycline exhibited promising *in vitro* activity against *A. baumannii* isolates. This finding is in agreement with previous works, showing the highest susceptibility of *A. baumannii* clinical isolates to tigecyclin (18). Therefore, this antibiotic might be regarded as a treatment option for infections due to MDR/XDR *A. baumannii* (19, 20).

CRAB isolates were also detected on devices often used for patient care such as portable X-ray and also on surfaces touched by staff and patients. *A. baumannii* might first spread from infected patients or colonized personnel/visitors and then contaminate surfaces, thereby acting as prolonged sources for HAIs. Several studies suggested that *A. baumannii* could colonize different medical equipment and surfaces (11, 21, 22) and also could persist for years in patients (23) with the ability of biofilm formation (11). In the present study, we observed a significant MDR (21.9%) and XDR (18.8%) frequency. The XDR rate in this study was lower than the usual reported rates of clinical *A. baumannii* isolates, as it reached more than 90% in one report from Tehran (7). However, the MDR rate in this study was higher than reported rates of clinical isolates.

The ERIC-PCR result revealed at least four distinct clusters of *A. baumannii* in ICUs of all the hospitals except for H2 which showed two STs. In this study, some *A. baumannii* isolates with similar typing pattern were recovered from the different hospitals, indicating that clonal expansion

of certain strains might take place in some of the hospitals probably through shared visitors or patients. Despite the clonal relatedness of these strains, the presence of the *bla*_{OXA-23} gene was not demonstrated for these isolates, and the resistance profiles of 127 - 1 and 383 - 1 were not identical. This discrepancy might be explained to some extent by the potential of *A. baumannii* to acquire foreign resistance elements as a result of selective pressure forced with antibiotics in patients and/or with antiseptics in the healthcare environment (24, 25).

A notable issue is the detection of two isolates in one clone from two geographically distinct hospitals, which were both obtained from ventilator. This suggests the possible potential of some specific genotypes of *A. baumannii* to resist and overcome hospital conditions, leading to inter-hospital dissemination of the same clones. Ventilator associated pneumonia due to MDR *A. baumannii* is one of the most common nosocomial infections, which accounts for high mortality rate in ICU hospitalized patients (26).

Detection of three different clones from baby bath sink of the H1 NICU and three different clones from bed sheet of the H4 ICU, which were all recovered in the same month, may reflect a heavy contamination source of endogenous *A. baumannii* and probably failure to conform to control measures and guidelines in these hospitals (27).

In different regions of Iran, OXA type β -lactamases have been widely studied in clinical isolates of *A. baumannii* (17, 28, 29) and it has been shown that *bla*_{OXA-23} is the most frequent OTC gene among nosocomial *A. baumannii* isolates (17, 30-33). However, data on the distribution of *bla*_{OXA-23} in the environmental isolates of *A. baumannii* is uncommon. While in this study, 31.3% of the isolates had *bla*_{OXA-23}, a recent study from Iran reported that 77.5% of 40 strains isolated from air, water and surfaces carried *bla*_{OXA-23} (34). Similarly, other studies suggested higher frequency of 68.7% (15), 58% (20) and 43.3% (27) for *bla*_{OXA-23} gene positive isolates in the hospital environment, as compared to the present results. The stability and circulation of *bla*_{OXA-23} producing isolates may finally result in acquisition and horizontal transferring of genes to other strains.

We detected one *bla*_{OXA-58} positive isolate. The first known *bla*_{OXA-58} producing *Acinetobacter* isolate was recovered in France in 2003. The co-occurrence of *bla*_{OXA-23} and *bla*_{OXA-58} in clinical isolates has been reported occasionally (30, 35-38). There is evidence supporting the presence of *bla*_{OXA-58} carbapenemase in outbreak strains of *A. baumannii* and its contribution in CRAB outbreaks occurring in hospitals (38-41).

5.1. Conclusions

This study provides evidence of the presence of clonally related *bla*_{OXA-23} producing CRAB in the hospital environment, particularly on moist surfaces in ICUs of tertiary hospitals. The clinical significance of multi resistant *A. baumannii* is of great concern since the distribution of the same clones in different hospitals on equipment such as ventilator may increase the risk of cross-colonization of vulnerable patients. Moreover, a combination of multi-drug resistance and *bla*_{OXA-23} and/or *bla*_{OXA-58} may result in sustained surveillance of such isolates in the healthcare environment and their involvement in outbreaks.

Acknowledgments

We would like to thank the staff of the Cellular and Molecular Research Center, Qom University of Medical Sciences, for their kind help during the experiments.

Footnotes

Authors' Contribution: Study concept and design: Mahmoud Nateghi Rostami and Masoumeh Douraghi; acquisition of data: Mahmoud Nateghi Rostami, Farzaneh Mehrban, and Sedigheh Ghourchian ; analysis and interpretation of data: Mahmoud Nateghi Rostami, Farzaneh Mehrban, and Masoumeh Douraghi; drafting of manuscript: Mahmoud Nateghi Rostami and Masoumeh Douraghi; statistical analysis: Mahmoud Nateghi Rostami.

Conflict of Interests: The authors declare there is no conflict of interest.

Funding/Support: This research has been supported by a grant from Qom University of Medical Sciences (QUMS), Qom, Iran.

References

- Doi Y, Bonomo RA, Hooper DC, Kaye KS, Johnson JR, Clancy CJ, et al. Gram-negative bacterial infections: Research priorities, accomplishments, and future directions of the antibacterial resistance leadership group. *Clin Infect Dis*. 2017;**64**(suppl_1):S30-5. doi: [10.1093/cid/ciw829](https://doi.org/10.1093/cid/ciw829). [PubMed: [28350901](https://pubmed.ncbi.nlm.nih.gov/28350901/)]. [PubMed Central: [PMC5848311](https://pubmed.ncbi.nlm.nih.gov/PMC5848311/)].
- Abbott I, Cerqueira GM, Bhuiyan S, Peleg AY. Carbapenem resistance in *Acinetobacter baumannii*: Laboratory challenges, mechanistic insights and therapeutic strategies. *Expert Rev Anti Infect Ther*. 2013;**11**(4):395-409. doi: [10.1586/eri.13.21](https://doi.org/10.1586/eri.13.21). [PubMed: [23566149](https://pubmed.ncbi.nlm.nih.gov/23566149/)].
- Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. *Int J Antimicrob Agents*. 2015;**45**(6):568-85. doi: [10.1016/j.ijantimicag.2015.03.001](https://doi.org/10.1016/j.ijantimicag.2015.03.001). [PubMed: [25857949](https://pubmed.ncbi.nlm.nih.gov/25857949/)].
- Mathlouthi N, Al-Bayssari C, Bakour S, Rolain JM, Chouchani C. Prevalence and emergence of carbapenemases-producing Gram-negative bacteria in Mediterranean basin. *Crit Rev Microbiol*. 2017;**43**(1):43-61. doi: [10.3109/1040841X.2016.1160867](https://doi.org/10.3109/1040841X.2016.1160867). [PubMed: [27387224](https://pubmed.ncbi.nlm.nih.gov/27387224/)].
- Bialvaei AZ, Kouhsari E, Salehi-Abargouei A, Amirmozafari N, Ramazanzadeh R, Ghadimi-Daresajini A, et al. Epidemiology of multidrug-resistant *Acinetobacter baumannii* strains in Iran: A systematic review and meta-analysis. *J Chemother*. 2017;**29**(6):327-37. doi: [10.1080/1120009X.2017.1338377](https://doi.org/10.1080/1120009X.2017.1338377). [PubMed: [28622734](https://pubmed.ncbi.nlm.nih.gov/28622734/)].
- Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int J Antimicrob Agents*. 2013;**41**(1):1-9. doi: [10.1016/j.ijantimicag.2012.09.008](https://doi.org/10.1016/j.ijantimicag.2012.09.008). [PubMed: [23127486](https://pubmed.ncbi.nlm.nih.gov/23127486/)].
- Jasemi S, Douraghi M, Adibhesami H, Zeraati H, Rahbar M, Boroumand MA, et al. Trend of extensively drug-resistant *Acinetobacter baumannii* and the remaining therapeutic options: A multicenter study in Tehran, Iran over a 3-year period. *Lett Appl Microbiol*. 2016;**63**(6):466-72. doi: [10.1111/lam.12669](https://doi.org/10.1111/lam.12669). [PubMed: [27626896](https://pubmed.ncbi.nlm.nih.gov/27626896/)].
- Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat Rev Microbiol*. 2018;**16**(2):91-102. doi: [10.1038/nrmicro.2017.148](https://doi.org/10.1038/nrmicro.2017.148). [PubMed: [29249812](https://pubmed.ncbi.nlm.nih.gov/29249812/)]. [PubMed Central: [PMC6571207](https://pubmed.ncbi.nlm.nih.gov/PMC6571207/)].
- Koneman EW. *Color atlas & textbook of diagnostic microbiology*. 6th ed. Philadelphia: LWW; 2005.
- The Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing 27th; CLSI supplement M100-S27*. CLSI; 2017.
- Aliramezani A, Douraghi M, Hajhasani A, Mohammadzadeh M, Rahbar M. Clonal relatedness and biofilm formation of OXA-23-producing carbapenem resistant *Acinetobacter baumannii* isolates from hospital environment. *Microb Pathog*. 2016;**99**:204-8. doi: [10.1016/j.micpath.2016.08.034](https://doi.org/10.1016/j.micpath.2016.08.034). [PubMed: [27569533](https://pubmed.ncbi.nlm.nih.gov/27569533/)].
- Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents*. 2006;**27**(4):351-3. doi: [10.1016/j.ijantimicag.2006.01.004](https://doi.org/10.1016/j.ijantimicag.2006.01.004). [PubMed: [16564159](https://pubmed.ncbi.nlm.nih.gov/16564159/)].
- Al-Kadmy IMS, Ali ANM, Salman IMA, Khazaal SS. Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment. *New Microbes New Infect*. 2018;**21**:51-7. doi: [10.1016/j.nmni.2017.10.010](https://doi.org/10.1016/j.nmni.2017.10.010). [PubMed: [29204285](https://pubmed.ncbi.nlm.nih.gov/29204285/)]. [PubMed Central: [PMC5705800](https://pubmed.ncbi.nlm.nih.gov/PMC5705800/)].
- Erturk A, Cicek AC, Gumus A, Cure E, Sen A, Kurt A, et al. Molecular characterisation and control of *Acinetobacter baumannii* isolates resistant to multi-drugs emerging in inter-intensive care units. *Ann Clin Microbiol Antimicrob*. 2014;**13**:36. doi: [10.1186/s12941-014-0036-2](https://doi.org/10.1186/s12941-014-0036-2). [PubMed: [25048577](https://pubmed.ncbi.nlm.nih.gov/25048577/)]. [PubMed Central: [PMC4378696](https://pubmed.ncbi.nlm.nih.gov/PMC4378696/)].
- Bardbari AM, Arabestani MR, Karami M, Keramat F, Alikhani MY, Bagheri KP. Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental *Acinetobacter baumannii* isolates. *Microb Pathog*. 2017;**108**:122-8. doi: [10.1016/j.micpath.2017.04.039](https://doi.org/10.1016/j.micpath.2017.04.039). [PubMed: [28457900](https://pubmed.ncbi.nlm.nih.gov/28457900/)].
- Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, Asgharzadeh M, Aghazadeh M. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. *Iran J Microbiol*. 2015;**7**(5):226-46. [PubMed: [26719779](https://pubmed.ncbi.nlm.nih.gov/26719779/)]. [PubMed Central: [PMC4695504](https://pubmed.ncbi.nlm.nih.gov/PMC4695504/)].
- Rezaei A, Fazeli H, Moghadampour M, Halaji M, Faghri J. Determination of antibiotic resistance pattern and prevalence of OXA-type carbapenemases among *Acinetobacter baumannii* clinical isolates from inpatients in Isfahan, central Iran. *Infez Med*. 2018;**26**(1):61-6. [PubMed: [29525799](https://pubmed.ncbi.nlm.nih.gov/29525799/)].
- Anane AY, Apalata T, Vasaikar S, Okuthe GE, Songca S. Prevalence and molecular analysis of multidrug-resistant *Acinetobacter baumannii* in the extra-hospital environment in Mthatha, South Africa. *Braz J Infect Dis*. 2019;**23**(6):371-80. doi: [10.1016/j.bjid.2019.09.004](https://doi.org/10.1016/j.bjid.2019.09.004). [PubMed: [31706742](https://pubmed.ncbi.nlm.nih.gov/31706742/)].

19. Razavi Nikoo H, Ardebili A, Mardaneh J. Systematic review of antimicrobial resistance of clinical *Acinetobacter baumannii* isolates in Iran: An update. *Microb Drug Resist*. 2017;**23**(6):744–56. doi: [10.1089/mdr.2016.0118](https://doi.org/10.1089/mdr.2016.0118). [PubMed: [28085571](https://pubmed.ncbi.nlm.nih.gov/28085571/)].
20. Obeidat N, Jawdat F, Al-Bakri AG, Shehabi AA. Major biologic characteristics of *Acinetobacter baumannii* isolates from hospital environmental and patients' respiratory tract sources. *Am J Infect Control*. 2014;**42**(4):401–4. doi: [10.1016/j.ajic.2013.10.010](https://doi.org/10.1016/j.ajic.2013.10.010). [PubMed: [24679567](https://pubmed.ncbi.nlm.nih.gov/24679567/)].
21. Lerner AO, Abu-Hanna J, Carmeli Y, Schechner V. Environmental contamination by carbapenem-resistant *Acinetobacter baumannii*: The effects of room type and cleaning methods. *Infect Control Hosp Epidemiol*. 2019;1–6. doi: [10.1017/ice.2019.307](https://doi.org/10.1017/ice.2019.307). [PubMed: [31722777](https://pubmed.ncbi.nlm.nih.gov/31722777/)].
22. Tajeddin E, Rashidan M, Razaghi M, Javadi SS, Sherafat SJ, Alebouyeh M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. *J Infect Public Health*. 2016;**9**(1):13–23. doi: [10.1016/j.jiph.2015.05.010](https://doi.org/10.1016/j.jiph.2015.05.010). [PubMed: [26117707](https://pubmed.ncbi.nlm.nih.gov/26117707/)].
23. Sung JY, Koo SH, Kim S, Kwon GC. Persistence of multidrug-resistant *Acinetobacter baumannii* isolates harboring blaOXA-23 and bap for 5 years. *J Microbiol Biotechnol*. 2016;**26**(8):1481–9. doi: [10.4014/jmb.1604.04049](https://doi.org/10.4014/jmb.1604.04049). [PubMed: [27221112](https://pubmed.ncbi.nlm.nih.gov/27221112/)].
24. Katchanov J, Asar L, Klupp EM, Both A, Rothe C, Konig C, et al. Carbapenem-resistant Gram-negative pathogens in a German university medical center: Prevalence, clinical implications and the role of novel beta-lactam/beta-lactamase inhibitor combinations. *PLoS One*. 2018;**13**(4). e0195757. doi: [10.1371/journal.pone.0195757](https://doi.org/10.1371/journal.pone.0195757). [PubMed: [29649276](https://pubmed.ncbi.nlm.nih.gov/29649276/)]. [PubMed Central: [PMC5896976](https://pubmed.ncbi.nlm.nih.gov/PMC5896976/)].
25. Raible KM, Sen B, Law N, Bias TE, Emery CL, Ehrlich GD, et al. Molecular characterization of beta-lactamase genes in clinical isolates of carbapenem-resistant *Acinetobacter baumannii*. *Ann Clin Microbiol Antimicrob*. 2017;**16**(1):75. doi: [10.1186/s12941-017-0248-3](https://doi.org/10.1186/s12941-017-0248-3). [PubMed: [29145853](https://pubmed.ncbi.nlm.nih.gov/29145853/)]. [PubMed Central: [PMC5691885](https://pubmed.ncbi.nlm.nih.gov/PMC5691885/)].
26. Bozorgmehr R, Bahrani V, Fatemi A. Ventilator-associated pneumonia and its responsible germs; an epidemiological study. *Emerg (Tehran)*. 2017;**5**(1). e26. [PubMed: [28286833](https://pubmed.ncbi.nlm.nih.gov/28286833/)]. [PubMed Central: [PMC5325895](https://pubmed.ncbi.nlm.nih.gov/PMC5325895/)].
27. Zenati K, Touati A, Bakour S, Sahli F, Rolain JM. Characterization of NDM-1- and OXA-23-producing *Acinetobacter baumannii* isolates from inanimate surfaces in a hospital environment in Algeria. *J Hosp Infect*. 2016;**92**(1):19–26. doi: [10.1016/j.jhin.2015.09.020](https://doi.org/10.1016/j.jhin.2015.09.020). [PubMed: [26615460](https://pubmed.ncbi.nlm.nih.gov/26615460/)].
28. Oliveira EA, Paula GR, Mondino PJJ, Chagas TPG, Mondino SSB, Mendonca-Souza CRV. High rate of detection of OXA-23-producing *Acinetobacter* from two general hospitals in Brazil. *Rev Soc Bras Med Trop*. 2019;**52**. e20190243. doi: [10.1590/0037-8682-0243-2019](https://doi.org/10.1590/0037-8682-0243-2019). [PubMed: [31508786](https://pubmed.ncbi.nlm.nih.gov/31508786/)].
29. Sarikhani Z, Nazari R, Nateghi Rostami M. First report of OXA-143-lactamase producing *Acinetobacter baumannii* in Qom, Iran. *Iran J Basic Med Sci*. 2017;**20**(11):1282–6. doi: [10.22038/IJBMS.2017.9490](https://doi.org/10.22038/IJBMS.2017.9490). [PubMed: [29299207](https://pubmed.ncbi.nlm.nih.gov/29299207/)]. [PubMed Central: [PMC5749364](https://pubmed.ncbi.nlm.nih.gov/PMC5749364/)].
30. Bagheri Josheghani S, Moniri R, Firoozeh F, Sehat M, Dasteh Goli Y. Susceptibility pattern and distribution of oxacillinases and bla PER-1 genes among multidrug resistant *Acinetobacter baumannii* in a teaching hospital in Iran. *J Pathog*. 2015;**2015**:957259. doi: [10.1155/2015/957259](https://doi.org/10.1155/2015/957259). [PubMed: [26881082](https://pubmed.ncbi.nlm.nih.gov/26881082/)]. [PubMed Central: [PMC4736200](https://pubmed.ncbi.nlm.nih.gov/PMC4736200/)].
31. Al-Hamad A, Pal T, Leskafi H, Abbas H, Hejles H, Alsubikhy F, et al. Molecular characterization of clinical and environmental carbapenem resistant *Acinetobacter baumannii* isolates in a hospital of the Eastern Region of Saudi Arabia. *J Infect Public Health*. 2019. doi: [10.1016/j.jiph.2019.08.013](https://doi.org/10.1016/j.jiph.2019.08.013). [PubMed: [31551188](https://pubmed.ncbi.nlm.nih.gov/31551188/)].
32. Mohajeri P, Farahani A, Mehrabzadeh RS. Molecular characterization of multidrug resistant strains of *Acinetobacter baumannii* isolated from intensive care units in West of Iran. *J Clin Diagn Res*. 2017;**11**(2):DC20–2. doi: [10.7860/JCDR/2017/21156.9397](https://doi.org/10.7860/JCDR/2017/21156.9397). [PubMed: [28384861](https://pubmed.ncbi.nlm.nih.gov/28384861/)]. [PubMed Central: [PMC5376806](https://pubmed.ncbi.nlm.nih.gov/PMC5376806/)].
33. Sohrabi N, Farajnia S, Akhi MT, Nahaei MR, Naghili B, Peymani A, et al. Prevalence of OXA-type beta-lactamases among *Acinetobacter baumannii* isolates from Northwest of Iran. *Microb Drug Resist*. 2012;**18**(4):385–9. doi: [10.1089/mdr.2011.0077](https://doi.org/10.1089/mdr.2011.0077). [PubMed: [22352411](https://pubmed.ncbi.nlm.nih.gov/22352411/)].
34. Shamsizadeh Z, Nikaeen M, Nasr Esfahani B, Mirhoseini SH, Hatamzadeh M, Hassanzadeh A. Detection of antibiotic resistant *Acinetobacter baumannii* in various hospital environments: potential sources for transmission of *Acinetobacter* infections. *Environ Health Prev Med*. 2017;**22**(1):44. doi: [10.1186/s12199-017-0653-4](https://doi.org/10.1186/s12199-017-0653-4). [PubMed: [29165152](https://pubmed.ncbi.nlm.nih.gov/29165152/)]. [PubMed Central: [PMC5664838](https://pubmed.ncbi.nlm.nih.gov/PMC5664838/)].
35. Davandeh I, Erac B, Aydemir SS. Investigation of class-d beta-lactamases causing carbapenem resistance in clinical *Acinetobacter baumannii* isolates. *Turk J Med Sci*. 2017;**47**(5):1661–6. doi: [10.3906/sag-1607-91](https://doi.org/10.3906/sag-1607-91). [PubMed: [29152950](https://pubmed.ncbi.nlm.nih.gov/29152950/)].
36. Ramoul A, Loucif L, Bakour S, Amiri S, Dekhil M, Rolain JM. Co-occurrence of blaNDM-1 with blaOXA-23 or blaOXA-58 in clinical multidrug-resistant *Acinetobacter baumannii* isolates in Algeria. *J Glob Antimicrob Resist*. 2016;**6**:136–41. doi: [10.1016/j.jgar.2016.05.003](https://doi.org/10.1016/j.jgar.2016.05.003). [PubMed: [27530856](https://pubmed.ncbi.nlm.nih.gov/27530856/)].
37. Mathlouthi N, Ben Lamine Y, Somai R, Bouhalila-Besbes S, Bakour S, Rolain JM, et al. Incidence of OXA-23 and OXA-58 carbapenemases co-expressed in clinical isolates of *Acinetobacter baumannii* in Tunisia. *Microb Drug Resist*. 2018;**24**(2):136–41. doi: [10.1089/mdr.2016.0306](https://doi.org/10.1089/mdr.2016.0306). [PubMed: [28691891](https://pubmed.ncbi.nlm.nih.gov/28691891/)].
38. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N, et al. Characterisation of carbapenem-resistant *Acinetobacter baumannii* outbreak strains producing OXA-58 in Turkey. *Int J Antimicrob Agents*. 2010;**36**(2):114–8. doi: [10.1016/j.ijantimicag.2010.03.017](https://doi.org/10.1016/j.ijantimicag.2010.03.017). [PubMed: [20510587](https://pubmed.ncbi.nlm.nih.gov/20510587/)].
39. Moro M, Nizzero P, Biancardi A, Baldan R, Scarpellini P, Curti C, et al. An outbreak caused by multidrug-resistant OXA-58-positive *Acinetobacter baumannii* in an intensive care unit in Italy. *J Hosp Infect*. 2008;**68**(1):97–9. doi: [10.1016/j.jhin.2007.10.007](https://doi.org/10.1016/j.jhin.2007.10.007). [PubMed: [18063199](https://pubmed.ncbi.nlm.nih.gov/18063199/)].
40. Bogaerts P, Naas T, Wybo I, Bauraing C, Soetens O, Pierard D, et al. Outbreak of infection by carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-58 in Belgium. *J Clin Microbiol*. 2006;**44**(11):4189–92. doi: [10.1128/JCM.00796-06](https://doi.org/10.1128/JCM.00796-06). [PubMed: [16957031](https://pubmed.ncbi.nlm.nih.gov/16957031/)]. [PubMed Central: [PMC1698292](https://pubmed.ncbi.nlm.nih.gov/PMC1698292/)].
41. Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. *Antimicrob Resist Infect Control*. 2019;**8**:185. doi: [10.1186/s13756-019-0611-6](https://doi.org/10.1186/s13756-019-0611-6). [PubMed: [31832185](https://pubmed.ncbi.nlm.nih.gov/31832185/)]. [PubMed Central: [PMC6868752](https://pubmed.ncbi.nlm.nih.gov/PMC6868752/)].