Antibiotic Resistance and Genotypes of Nosocomial Strains of Acinetobacter baumannii in Kazakhstan

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Abstract: The aim of this study was to determine the prevalence of A. baumannii antibiotic-resistant strains in Kazakhstan and to characterize genotypes related to epidemic “high-risk” clones. Two hundred and twenty four A. baumannii isolates from four cities of Kazakhstan in 2011–2019 were studied. Antibiotic susceptibility testing was performed by using broth microdilutions method according to EUCAST (v 11.0) recommendations. The presence of blaOXA-23-like, blaOXA-24/40-like, blaOXA-58-like, blaVIM, blaIMP, and blaNDM genes was determined by PCR. Genotyping was performed using high-throughput real-time PCR detection of 21 SNPs at 10 chromosomal loci used in existing MLST schemes. Resistance rates to imipenem, meropenem, amikacin, gentamicin, and ciprofloxacin were 81.3%, 78.6%, 79.9%, 65.2%, and 89.3%, respectively. No colistin resistant isolates were detected. The values of the MIC 50% and the MIC 90% of tigecycline were 0.125 mg/L, only four isolates (1.8%) had the ECOFF value >0.5 mg/L. The presence of acquired carbapenemase genes was found in 82.2% strains, including blaOXA-23-like (78.6%) or blaOXA-58-like (3.6%) genes. The spreading of carbapenem resistant A. baumannii strains in Kazakhstan was associated with epidemic “high-risk” clonal groups, predominantly, CG208(92)OXF/CG2PAS (80.8%) and less often CG231(109)OXF/CG1PAS (1.8%).

Keywords: Acinetobacter baumannii; multidrug-resistant bacteria; carbapenemases; nosocomial infection

1. Introduction

Acinetobacter baumannii is an opportunistic pathogen that often causes diseases in immunocompromised patients [1]. A. baumannii is common in hospitals and causes a variety of nosocomial infections and iatrogenic diseases that include bloodstream infections, urinary tract infections, meningitis, wound infections, and more [1,2]. The World Health Organization categorizes included MDR A. baumannii as the “highest priority pathogen” for which antibiotic development is urgently needed [3]. However, carbapenems are considered as effective antibiotics against many drug-resistant microorganisms [4]. At once, the number of carbapenem-resistant A. baumannii isolates has been increasing recently [5]. The prevalence of carbapenem resistance of A. baumannii in etiological structure of nosocomial infections during last decade was heterogeneous. According to the EARS-Net data from 2013 to 2017, the part of carbapenem resistant A. baumannii in West Europe was 35.6%, at this time the prevalence of carbapenem resistant A. baumannii in Greece and Turkey were 79% and 95% respectively. However, according to longitude study in Austria the level of carbapenem resistant A. baumannii was more than 97% [6]. In alignment, the CHINET surveillance system in China from 2004 to 2015 the level of carbapenem resistant A. baumannii increased from 31 to 66.7% [7]. Russian multicenter surveillance study shown the comparable data when carbapenem resistant A. baumannii were detected in
77% [8]. According to multicenter study nosocomial infections caused by *A. baumannii* in Pakistan the rate of carbapenem resistance in 2017 was 89% [9].

Most isolates are resistant to carbapenems, and also become resistant to other classes of antimicrobial drugs such as aminoglycosides, fluoroquinolones, and to polymyxin in sporadic cases [10,11]. The rapid spread of multidrug-resistant nosocomial strains of *Acinetobacter* is a global concern. The present situation determines the need for regular monitoring of the sensitivity of nosocomial strains of *Acinetobacter* spp. and, if necessary, correction of the therapy strategy for infections caused by them [12,13]. The global spread of multidrug resistant strains, including resistant to carbapenems, among nosocomial *A. baumannii* strains was associated with expansion of two international “high-risk” clonal lineages, called ICL1 and ICL2 [14–17].

Microbial genotyping methods is an important tool for molecular epidemiological studies, particularly, for understanding of the population dynamics and transmission of pathogens. Even the ubiquity of whole-genome data, multilocus sequence typing (MLST) is still a “gold standard” for molecular typing of *A. baumannii* due to standardized landscape about population structure [18,19]. Two available MLST approaches for *A. baumannii* (Oxford [20] and Pasteur [14] schemes) characterized by different resolution ability but provide concordant discrimination at the level of predominant clonal groups [21,22]. In epidemiological studies, the epidemic “high-risk” clones are typically defined using by MLST nomenclature of sequence types (STs) and clonal complexes (CCs) or clonal groups (CGs) [23] and are characterized as CC231(109)\textsuperscript{OXT}/CC1\textsuperscript{PAS} (for ICL1) and CC208(92)\textsuperscript{OXT}/CC2\textsuperscript{PAS} (for ICL2) [16]. However, such approaches is still expensive and laborious especially for analysis of large sample collections, whereas high-throughput approaches allow to study in detail the entire diversity of *A. baumannii* strains. A lot of existing high-throughput approaches have been proposed [24–30] but only single nucleotide typing (SNP)-typing scheme provide direct compare SNP-typing and MLST data due to identify a set of SNPs of MLST loci [30].

In the present study, we aimed to investigate the rates of antibiotic resistance and production of acquired carbapenemase genes and to determine the genotypes and prevalence of “international high-risk clones” among *A. baumannii* isolates in Kazakhstan.

### 2. Results and Discussion

Determining the antimicrobial susceptibility of *A. baumannii* strains results are presented in Table 1. Resistance to carbapenems (imipenem and meropenem) were shown by 81.3% and 78.6% of *A. baumannii* isolates, respectively. Comparable the resistance phenotype was detected in South-East Asia: Korea (87.0%), Singapore (95.2%), Hong Kong (50.0%) and Thailand (59.2%) [31]. Fluoroquinolones have high resistance indicators: 89.3% of *A. baumannii* isolates were resistant to ciprofloxacin. The frequency of resistance to aminoglycosides (amikacin and gentamicin) was 79.9% and 65.2%, respectively. Among non-beta-lactam antibiotics, colistin was highly active in vitro, none resistant isolates were detected. The values of the MIC 50% and the MIC 90% of tigecycline were 0.125 mg/l, only four isolates (1.8%) had the ECOFF value >0.5 mg/L. Combined resistance to imipenem, amikacin, and ciprofloxacin was observed in 73.2% isolates. Extremely profile of resistance make polymyxins (polymyxin B and colistin) as a last-resort treatment option [32].

| Name of Antibiotic | % of Isolates and MIC Value, mg/L | % of Isolates by Category | MIC, mg/L |
|--------------------|----------------------------------|--------------------------|-----------|
|                    | ≤0.06 0.125 0.25 0.5 1 2 4 8 16 32 64 ≥256 | S I R 50% 90%          |           |
| Amikacin           | 3.6 1.8 4.5 6.7 3.6 8.0 20.1 20.1 8.5 23.2 20.1 | 79.9 64 ≥256      |           |
| Gentamicin         | 0.4 8.9 5.4 8.0 12.1 11.6 11.6 17.9 7.1 3.6 13.4 | 34.8 65.2 16 ≥256 |           |
| Imipenem           | 13.4 4.9 0.4 2.2 21.4 29.9 22.8 3.1 1.8 | 18.3 0.5 81.3 32 64 |           |
The presence of genes for acquired molecular class D carbapenemases belonging to the OXA-23 (78.6%) and OXA-58 (3.6%) groups were revealed in 82.2% of A. baumannii isolates. No MBL genes were found in A. baumannii isolates. Actually, number of isolates harboring carbapenemase genes was higher than carbapenem resistant isolates: eight isolates were susceptible to imipenem and meropenem despite the presence of carbapenemase genes. The OXA-23 carbapenemases are major mechanism of resistance A. baumannii to carbapenems in the Central Asia and early the spreading of OXA-23 produced hospitals acquired strains of A. baumannii was described in Russia [8] and South-East Asia countries [17]. The results of assessing the sensitivity of A. baumannii isolates carrying the genes of acquired OXA-carbapenemases are presented in Table 2. The majority of carbapenemase producers were characterized by associated resistance to ciprofloxacin (97.1%), amikacin (89.7%) and gentamicin (69.5%). No resistant to colistin strains were observed.

### Table 2. Antibiotic sensitivity of A. baumannii isolates (n = 184) producing acquired OXA-carbapenemases.

| Name of Antibiotic | % of Isolates and MIC Value, mg/L | % of Isolates by Category MIC, mg/L |
|--------------------|-----------------------------------|-----------------------------------|
|                    | ≤0.06 0.125 0.25 0.5 1 2 4 8 16 32 64 128 ≥256 | S I R 50% 90% |
| Amikacin           | 1.1 0.5 2.2 3.8 2.7 8.7 23.9 22.8 10.3 23.9 10.3 | 90.7 64 ≥256 |
| Gentamicin         | 0.5 4.3 3.3 9.2 13.0 13.6 13.6 19.0 7.1 2.7 13.6 | 30.4 69.6 16 ≥256 |
| Imipenem           | 2.7 2.2 | 0.5 26.1 36.4 26.6 3.3 2.2 | 4.9 0.0 95.1 32 64 |
| Meropenem          | 0.5 3.3 0.5 | 2.2 39.1 34.2 16.3 1.6 2.2 | 4.4 2.2 93.5 32 64 |
| Netilmicin         | 4.9 8.2 20.1 15.2 8.7 16.3 11.4 6.5 1.6 7.1 | ND ND ND 8 64 |
| Ciprofloxacin      | 1.6 1.1 1.1 | 0.5 3.3 2.2 89.7 | 0.0 2.7 97.3 ≥256 256 |
| Tigecycline        | 39.7 54.3 1.1 3.3 1.6 | ND ND ND ≤0.06 0.125 |
| Colistin           | 13.6 84.8 1.6 | 100.0 0.0 1 1 |

ND—no data, according to EUCAST criteria [33] for netilmicin and tigecycline interpretation criteria are absent.
Figure 1. Genetic diversity and production of carbapenemases in *A. baumannii* strains in Kazakhstan. UPGMA algorithm was used for hierarchical cluster analysis by PHYLOViZ software [35]. Horizontal rectangles correspond to different genotypes (SNP-types). Length of the rectangles is proportional to the number of isolates. MLST nomenclature was used to characterize prevalent clonal groups. Types of acquired carbapenemase genes are highlighted.

Among other clonal groups, isolates harboring *bla*<sub>OXA-23-like</sub> carbapenemase genes have been also found in SNP-type 7 (3 of 4 isolates, 75.0%) and SNP-type 47 (3 of 3 isolates, 100.0%). Through *in silico* analysis, nucleotide profiles of SNP-type 7 corresponded to a set of STs that combined into a CG231<sup>OXF</sup> (formerly known as CC109<sup>OXF</sup>) and CG1<sup>PAS</sup>. CG231(109)<sup>OXF</sup>/CG1<sup>PAS</sup> was corresponded to another international clonal lineage ICL1 [16,34], however, an incidence of CG231(109)<sup>OXF</sup>/CG1<sup>PAS</sup> was sporadic in Kazakhstan—four isolates were collected from Nur-Sultan in 2014 and 2018. Three isolates of SNP-type 47 harboring *bla*<sub>OXA-23-like</sub> genes were also collected from Nur-Sultan in 2015. Interestingly enough, the epidemic “high-risk clones” CG231(109)<sup>OXF</sup>/CG1<sup>PAS</sup> and CG208(92)<sup>OXF</sup>/CG2<sup>PAS</sup> harboring *bla*<sub>OXA-23-like</sub> carbapenemases genes were concomitant with spreading of carbapenem resistant *A. baumannii* strains in Pakistan [9].

Isolates harboring *bla*<sub>OXA-58-like</sub> carbapenemase genes (*n* = 8) have been found in same genotype (SNP-type 83). All isolates of this genotype were carriers of *bla*<sub>OXA-58-like</sub> genes and were isolated in 2016 and 2017 from Nur-Sultan. One isolate of SNP-type 83 was investigated by MLST to clarify phylogenetic traits and was assigned to ST184<sup>OXF</sup> and ST218<sup>PAS</sup>. Strains of this clonal group CG184<sup>OXF</sup>/CG218<sup>PAS</sup> were previously found in South Korea in 2008 and in China in 2009-2010, according to the pubMLST database. Strains of CG184<sup>OXF</sup>/CG218<sup>PAS</sup> have not been previously detected in neighboring countries.

Despite the presence of *bla*<sub>OXA-58-like</sub> genes, all but one isolates of CG184<sup>OXF</sup>/CG218<sup>PAS</sup> were susceptible for imipenem and meropenem. For susceptible isolates, distribution of MIC ranged from 0.5 to 1 mg/L for imipenem and 0.25 to 1 mg/L for meropenem. Expression of *bla*<sub>OXA-58-like</sub> gene depends on the presence of insertion sequence, most often ISAba3, in association with carbapenemase gene [36,37], so, presence of *bla*<sub>OXA-58-like</sub> gene does not always lead to resistance to carbapenems. Furthermore, another isolate harbored *bla*<sub>OXA-23-like</sub>...
like gene was found in CG208(92)OXF/CG2PAS and had a low MIC values (0.5 mg/L) for imipenem and meropenem.

*A. baumannii* strains harboring *bla*OXA-58-like* genes were appeared quite recently in Kazakhstan although that strains were a serious problem since the dawn of the worldwide spreading of carbapenem resistant strains of *A. baumannii* [16]. For the first mention, OXA-58 carbapenemase was found in *A. baumannii* isolate in France in 2003 [38]. Afterwards, *A. baumannii* strains harboring *bla*OXA-58-like* genes became widespread in European (Greece, France, Belgium, Italy and Turkey [39–42]), American (Argentina and USA [43–45]), and Asian regions (China, Taiwan and Singapore [46–48]). Patient transportation appears to be one of decisive factors in the spread of OXA-58-positive strains in global population of *A. baumannii* [40,44]. In neighboring countries, OXA-58-positive strains were found in Russia and that strains were noticed in cities bordering Kazakhstan, including Novosibirsk and Yekaterinburg (https://amrmap.net/, last accessed on 12 March 2021 [49]) [8]. According some epidemiological studies, several countries (China, Austria and Iran) remains the endemic regions of *A. baumannii* strains harboring *bla*OXA-58-like* genes [6,50,51].

Comparison of resistance among isolates of different clonal groups is presented in Table 3. CG208(92)OXF/CG2PAS isolates were significantly more resistant to multiple drugs compared to minor genotypes: to imipenem (93.9% vs 22.6%, *p* < 0.0001), meropenem (93.4% vs 12.9%, *p* < 0.0001), amikacin (91.7% vs 25.8%, *p* < 0.0001), gentamicin (68.5% vs 32.3%, *p* = 0.0002), and ciprofloxacin (100.0% vs 38.7%, *p* < 0.0001). It was impossible to compare resistance for CG231(109)OXF/CG1PAS or CG184OXF/CG218PAS due to small number of strains. The values of MIC 90% for tigecycline were 0.125 mg/L for each clonal groups while isolates with ECOFF value >0.5 mg/L were found in CG208(92)OXF/CG2PAS (*n* = 3) and SNP-type 37 (*n* = 1). Combined resistance to imipenem, amikacin, and ciprofloxacin among CG208(92)OXF/CG2PAS isolates was also significantly (*p* > 0.0001) higher compared to minor genotypes: 87.3% (95% CI: 81.7.91.4%) vs 12.9% (95% CI: 5.1–28.9%). Two of four isolates of CG231(109)OXF/CG1PAS and none of CG184OXF/CG218PAS also had combined resistance to imipenem, amikacin and ciprofloxacin.

| Antibiotic | CG208(92)OXF/CG2PAS | CG231(109)OXF/CG1PAS | CG184OXF/CG218PAS | Minor Genotypes |
|------------|---------------------|---------------------|-------------------|----------------|
| % of R     | 95% CI              | % of R              | 95% CI            | % of R         | 95% CI |
| Imipenem   | 93.9                | 89.5–96.6           | 100.0             | 51.0–100.0      | 12.5    | 2.2–47.1 |
| Meropenem  | 93.4                | 88.8–96.2           | 75.0              | 30.1–95.4       | 0.0     | 0.0–32.4 |
| Amikacin   | 91.7                | 86.8–94.9           | 50.0              | 15.0–85.0       | 37.5    | 13.7–69.4|
| Gentamicin | 68.5                | 61.4–74.8           | 100.0             | 51.0–100.0      | 100.0   | 67.6–100.0|
| Ciprofloxacin | 100.0           | 97.9–100.0          | 100.0             | 51.0–100.0      | 37.5    | 13.7–69.4 |

Thus, the results of this study indicate the advisability in the routine microbiological diagnostic the detection of carbapenemases genes and genetic profiles for phylogenetic and epidemiological surveillance.

3. Materials and Methods

3.1. Sources of Bacterial Isolates

*Acinetobacter baumannii* (*n* = 224) were collected from inpatients of four cities of Kazakhstan (Karaganda, Nur-Sultan, Almaty, and Jezkazgan) in 2011–2019. Isolation and primary identification of bacterial isolates were carried out in local clinical microbiological laboratories by using standard microbiological methods. The final identification of all bac-
Material obtained from patients after 48 h of admission to the dispensary for confirmation of nosocomial infection [54,55]. Collected isolates were recovered from respiratory tract of intensive care unit patients (n = 126, 56.7%), surgical intra-abdominal infection (n = 35, 15.6%), blood of intensive care patients (n = 18, 8.0%), urinary tract infections (n = 17, 7.6%), surgical soft tissue infections (n = 17, 7.6%), bones and joints infections (n = 3, 1.3%), central nervous system lesions (n = 3, 1.3%), and other sources (n = 4, 1.8%).

3.2. Species Identification and Storage of Isolates

The isolates were identified by matrix-assisted laser desorption/ionization—time-of-flight mass spectrometry (MALDI-TOF MS) using the Microflex LT system and the MALDI Biotyper Compass v.4.1.80 software (Bruker Daltonics, Hamburg, Germany). The values known for Acinetobacter representatives were used as a criterion for reliable species identification score ≥ 2.0 according to manual. The species identification of A. baumannii isolates was confirmed by detection of species-specific blaOXA-51-like genes using real-time PCR with commercial kits “AmpliSensR MDR Ab-OXA-FL” (Central Research Institute of Epidemiology, Moscow, Russia) and the DTPrime 5X1 system (DNA-Technology, Moscow, Russia). Prior to analysis, the isolates were stored at −70 °C in trypticase-soy broth (BD, Sparks, MD, USA) supplemented with 30% glycerol. The obtained strains from local laboratories were revived on Columbia blood agar (BD, USA) aerobically at 36 ± 1 °C.

3.3. Determination of Sensitivity to Antibiotics

Determination of sensitivity to antimicrobial drugs (amikacin, gentamicin, imipenem, meropenem, netilmicin, ciprofloxacin, tigecycline, colistin) was carried out by the microdilution method in Mueller-Hinton broth. Interpretation of susceptibility testing results was performed according to recommendation of EUCAST v 11.0 [33]. To control the quality of the sensitivity determination, we used Escherichia coli ATCC®25922, Escherichia coli ATCC®35218, Pseudomonas aeruginosa ATCC®27853.

3.4. Identification of Carbapenemase Genes

The presence of acquired class D carbapenemases genes common for Acinetobacter spp. (groups OXA-23, OXA-24/40, and OXA-58), as well as class B carbapenemases (metallo-beta-lactamases (MBL) of VIM, IMP, and NDM groups) were determined by real-time PCR using commercial kits “AmpliSensR MDR Acinetobacter-OXA-FL” and “AmpliSensR MDR MBL-FL” (Central Research Institute of Epidemiology, Moscow, Russia). For amplification, a DTPrime 5X1 real-time PCR system (DNA Technology, Moscow, Russia) was used. Strains A. baumannii, A. pittii, and P. aeruginosa, carrying the known carbapenemases genes of the listed groups were used as positive controls. DNA extraction was performed by express method using InstaGeneTM matrix (Bio-Rad, Hercules, CA, USA). Samples of extracted DNA were stored at −20 °C before testing. The results of assessing the sensitivity to antibiotics and determining the genes of various types of carbapenemases have been deposited to the AMRmap website database [49].

3.5. Molecular Genotyping of A. baumannii

Genetic diversity of A. baumannii was explored by single nucleotide polymorphism (SNP)-typing method based on the analysis of 21 informative SNPs at 10 chromosomal loci (gltA, recA, cpn60, gyrB, gdbB, rpoD, fusA, pyrG, rplB, and rpoB) used in the University of Oxford and the Institute Pasteur multilocus sequencing-typing (MLST) schemes [30]. Detection of each SNP was performed by allele-specific real-time PCR according to the high-throughput approach proposed by Myakishev et al. [56]. Obtained sequences of 21 nucleotide bases for each A. baumannii isolate were used to assign a certain genotype (SNP-
type) and to assess genetic relatedness between genotypes. The QIAgility system (QIAGEN, Hilden, Germany) and DTPrime 5X1 (DNA-Technology, Moscow, Russia) were used to prepare and conduct PCR in 384-well format that ensured high-throughput genotyping of isolates. A. baumannii strains of known STs submitted to pubMLST database (https://pubmlst.org/organisms/acinetobacter-baumannii, last accessed on 12 March 2021): id4785, id4790, id4793, id4798, id4816, id4825, id4841, id4932, id4985, id5036 were used as controls.

The selected set of 21 SNPs from MLST loci provided a comparison between obtained SNP-types with known STs and CCs according to the MLST nomenclature, including the so-called “high-risk international clones”. That correspondence between SNP-typing and MLST data was provided by SQL database and software platform (called SNPTAb, http://snpt.antibiotic.ru:9002/, last accessed on 12 March 2021) [57]. Furthermore, the SNPTAb database was used to store of SNP-typing data with individual isolates data (e.g., source, geographical origin, data of isolation, resistance to carbapenems, and production of carbapenemases). Cluster analysis of obtained SNP profiles and was carried out using the PHYLOViZ 2.0 software [35].

MLST was performed for one isolate using both the University of Oxford and the Institute Pasteur schemes as described previously [14,20]. Obtained MLST sequences were uploaded to PubMLST database (https://pubmlst.org/organisms/acinetobacter-baumannii, last accessed on 12 March 2021) to identify alleles and STs.

4. Conclusions

Nosocomial infections by carbapenem resistant A. baumannii strains emerge sharply in Kazakhstan since 2011. The results of this study indicate a high prevalence of resistance to most antimicrobials, including all carbapenems, aminoglycosides, and fluoroquinolones used to treat infections caused by this pathogen. Colistin was highly active on all A. baumannii isolates. The main mechanism of resistance to carbapenems of A. baumannii was the production of acquired carbapenemases belonging to the OXA-23 group. The spreading of carbapenem resistant A. baumannii strains in Kazakhstan was associated with epidemic “high-risk” clonal groups, predominantly, CG208(92)OXF/CG2PAS (80.8% isolates) and less often CG231(109)OXF/CG1PAS (1.8% isolates). Isolates of these clonal groups were significantly more resistant to aminoglycosides and fluoroquinolones compared to other genetic lines. Furthermore, several isolates were carrying \( \text{bla}_\text{OXA-58} \)-like carbapenemase genes and combined into one clonal group CG184OXF/CG218PAS. Given the high probability of A. baumannii strains resistance to the main antibacterial drugs for the treatment of nosocomial infections, the choice of antibiotics for empiric therapy is extremely difficult and requires regular local monitoring of sensitivity in each hospital.

Author Contributions: Conceptualization, A.L. and A.T.; Data curation, I.A.; Formal analysis, E.S. and I.A.; Investigation, A.L. and S.K.; Methodology, A.L. and I.A.; Resources, A.L.; Software, E.S.; Supervision, A.T.; Writing—original draft, A.L. and S.K.; Writing—review & editing, A.L. and A.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare no conflict of interest.
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