An Acinetobacter non-baumannii Population Study: Antimicrobial Resistance Genes (ARGs)

Adam Baraka 1, German M. Traglia 2, Sabrina Montaña 3, Marcelo E. Tolmasky 1 and Maria Soledad Ramirez 1, 2, *

Abstract: Acinetobacter non-baumannii species are becoming common etiologic agents of nosocomial infections. Furthermore, clinical isolates belonging to this group of bacteria are usually resistant to one or more antibiotics. The current information about antibiotic resistance genes in the different A. non-baumannii species has not yet been studied as a whole. Therefore, we did a comparative study of the resistomes of A. non-baumannii pathogens based on information available in published articles and genome sequences. We searched the available literature and sequences deposited in GenBank to identify the resistance gene content of A. calcoaceticus, A. lwoffii, A. junii, A. soli, A. ursingii, A. bereziniae, A. nosocomialis, A. portensis, A. guerree, A. baylyi, A. calcoaceticus, A. disperses, A. johnsonii, A. junii, A. lwoffii, A. nosocomialis, A. oleivorans, A. oryzae, A. pittii, A. radioreisitsens, and A. venetianus. The most common genes were those coding for different β-lactamases, including the carbapenemase genes blaNDM-1 and blaOXA-58. A. pittii was the species with the most β-lactamase resistance genes reported. Other genes that were commonly found include those encoding some aminoglycoside modifying enzymes, the most common being aph(6)-Id, ant(3")-Iia, and aph(3")-Iib, and efflux pumps. All or part of the genes coding for the AdeABC, AdeFGH, and AdeJJK efflux pumps were the most commonly found. This article incorporates all the current information about A. non-baumannii resistance genes. The comparison of the different resistomes shows that there are similarities in the genes present, but there are also significant differences that could impact the efficiency of treatments depending on the etiologic agent. This article is a comprehensive resource about A. non-baumannii resistomes.

Keywords: Acinetobacter non-baumannii; antimicrobial resistance; resistome

1. Introduction

The genus Acinetobacter is a very diverse group of bacteria considered one of the most troublesome opportunistic nosocomial pathogens. It comprises 62 species with assigned names (http://www.bacterio.net/a/acinetobacter.html). A. baumannii is responsible for most nosocomial infections and has an intrinsic ability to acquire resistance to all available antibiotics. The problems caused by this bacterium led World Health Organization (WHO) experts and researchers from the Division of Infectious Diseases at the University of Tübingen (Germany) to include it in the critical priority group. Furthermore, the rise of carbapenem-resistant Acinetobacter led to its placement within a group of five pathogens considered as urgent threats to human health by the Center for Disease Control [1]. Although in the past decades the vast majority of Acinetobacter infections used to be caused by the species baumannii, in the past few years numerous other Acinetobacter species became commonly identified as causative agents of nosocomial infections. A. calcoaceticus, A. lwoffii,
A. junii, A. soli, A. ursingii, A. bereziniae and A. nosocomialis are also becoming common culprits of hospital infections [2,3]. Despite their diversity, a common characteristic of these species is the presence of multiple antibiotic resistance genes. Likewise, multiple antibiotic resistance mechanisms have been documented in other Acinetobacter species (A. lwofii, A. johnsonii, A. junii, A. nosocomialis and A. pittii) mainly recovered from soil and wastewater, as well as vegetables and meats. These species are important environmental reservoirs of resistance genetic determinants, which could later give rise to clinically relevant strains [2–7].

In this work, we conduct a comprehensive analysis of reported antibiotic resistance genes in A. non-baumannii species. Other genes found in a lower number of isolates were those coding for resistance to trimethoprim (dfrA-like), macrolide (ereA2, ermB), colistin (mcr-1, mcr-4), fluoroquinolone (qnrD1, qnrS1), rifampicin (arr-2), chloramphenicol (cat-like), lincosamide (InuF, InuG) and tetracycline (tet-like).

2. Results and Discussion

A summary of all A. non-baumannii species harboring potential antibiotic resistance genes (ARGs) reported in the literature is shown in Table 1. A detailed listing of the individual ARGs identified in each strain is shown in Table S1. The vast majority of ARGs code for β-lactamases of different classes (n = 153 for 31 isolates). The highest represented β-lactamase genes were blaNDM-1 and blaOXA-58 (14 and 12 strains, respectively) (Table S1). NDM-1 is a troublesome metallo-β-lactamase originally identified in a carbapenem-resistant Klebsiella pneumoniae isolate that, except for monobactams, confers resistance to all other β-lactam antibiotics [8–10]. The blaOXA-58 gene was first found in a multiresistant A. baumannii isolated in a hospital in France [11]. OXA-58 belongs to a group of enzymes with a reduced number of variants. A. baumannii strains carrying this enzyme showed different levels of resistance to carbapenems [10]. A. pittii was the species with the most β-lactamase resistance genes reported (n = 28), followed by A. nosocomialis (n = 15) and A. haemolyticus (n = 14) (Figure 1, Figure S1, and Table S1).

The second most abundant resistance mechanism was that mediated by aminoglycoside modifying enzymes (n = 77 for 20 species, Table 1), aphA6 and strA being the most reported genes. Aminoglycosides are bactericidal antibiotics used to treat a wide range of bacterial infections, including those caused by Acinetobacter [12,13]. The most common mechanism of resistance to aminoglycosides is the enzymatic inactivation of the antibiotic molecule. Aminoglycoside modifying enzymes catalyze the transfer of acetyl, phosphate, or nucleotidyl groups to the –OH or –NH2 groups of the 2-deoxystreptamine or the sugar moieties of the molecule [14].

The third most abundant mechanism was the efflux pumps, i.e., cellular systems that can export compounds usually without specificity. This mechanism specifies resistance through detoxification [15,16]. A total of 38 antibiotic efflux pump genes were identified in 12 isolates (Figure 1, Figure S1, and Table S1). The resistance-nodulation-cell division (RND) family efflux systems AdeABC and AdeIJK were the most common efflux pumps present in A. non-baumannii species. Both efflux systems can expel a broad range of antibiotics [17–19]. In the case of A. baumannii, all isolates studied to date carry AdeIJK, and a majority, but not all, carry AdeABC [19]. A. pittii is the species with the highest variety ARGs (n = 46), followed by A. nosocomialis (n = 35), A. johnsonii (n = 33), A. and A. haemolyticus (n = 28). Thirty-four A. non-baumannii species did not carry any of the tested ARGs (Table S1).
Table 1. Number of antibiotic resistance genes (ARGs) in *Acinetobacter non-baumannii* isolates reported in the literature.

| β-lactamases | Aminoglycosides | Efflux Pump Genes | Sulfonamides | Tetracyclines | Macrolides | ABC-F | Rifampicin | Fluoroquinolones | PBPs | Trimoprim |
|--------------|-----------------|-------------------|--------------|---------------|------------|-------|------------|-----------------|------|-----------|
| *A. baileyi*  | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. baylyi*   | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. apis*     | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. celotinire sistens* | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. cumulans* | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. haemolyticus* | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. johnsonii*| *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. lwoffii*  | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. nosocomialis* | *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. seifertii*| *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |
| *A. johnsonii*| *(n = 1)*        | *(n = 1)*         | *(n = 1)*    | *(n = 1)*     | *(n = 1)*  | *(n = 1)* | *(n = 1)*   | *(n = 1)*       | *(n = 1)* | *(n = 1)*  |

**Number of ARG in *Acinetobacter non-baumannii* isolates reported in the literature.**
Table 1. Cont.

| β-lactamases | Aminoglycosides | Efflux Pump Genes | Sulfonamides | Tetracyclines | Macrolides | ABC-F | Rifampicin | Florfenicol | Bleomycin | Chloramphenicol | Carbapenem (CarO Specific) | OMP | Fluoroquinolone | PBPs | Pmr | Trimhoprim |
|---------------|-----------------|-------------------|--------------|---------------|------------|--------|------------|-------------|-----------|----------------|---------------------------|-----|----------------|-------|------|------------|
| A. gandensis  | A. nosocomialis  |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 2)       | (n = 7)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. seifertii     |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 2)       | (n = 2)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. gauloiseae | A. oleivorans    |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 6)       | (n = 1)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. ursingii      |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 2)       | (n = 3)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. gyllenbergii  |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 1)       |                  |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. haemolylis | A. pittii        |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 3)       | (n = 9)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. indicus       |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 14)      |                  |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. raderesistens |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 6)       |                  |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. johnsonii  | A. radis        |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 3)       | (n = 1)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. keng-sansanis |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 1)       |                  |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. lasenii   | A. soli         |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 9)       | (n = 1)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
|               | A. nosocomialis  |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 15)      |                  |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. olivians  | A. ursingii     |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 2)       | (n = 5)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. parvis    | A. browner      |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 1)       | (n = 5)          |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. pittii    |                |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 28)      |                |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| A. proteolyticus |            |                  |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
| (n = 1)       |                |                   |              |               |            |        |            |             |           |                |                           |     |                |       |      |            |
Table 1. Cont.

Number of ARG in *Acinetobacter* non-baumannii Isolates Reported in the Literature

| β-lactamases | Aminoglycosides | Efflux Pump Genes | Sulfonamides | Tetracyclines | Macrolides | ABC-F | Rifampicin | Florfenicol | Bleomycin | Chloramphenicol | Carbapenem (CarO Specific) | OMP | Fluoroquinolone | PBPs | Pmr | Trimhoprim |
|--------------|-----------------|------------------|--------------|---------------|------------|-------|------------|------------|----------|-----------------|------------------------|------|----------------|------|-----|-----------|
| *A. radiore sistens* (*n* = 7) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. schindleri* (*n* = 6) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. seifertii* (*n* = 5) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. soli* (*n* = 4) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. tandaeei* (*n* = 1) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. tanneri* (*n* = 8) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. ursingii* (*n* = 8) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |
| *A. variabilis* (*n* = 2) |                |                  |              |               |            |       |            |            |          |                 |                        |      |                |      |     |           |

Total Number of ARGs

| N = 153 | N = 77 | N = 38 | N = 16 | N = 14 | N = 10 | N = 7 | N = 5 | N = 4 | N = 2 | N = 2 | N = 2 | N = 2 | N = 2 | N = 2 |
|---------|--------|--------|--------|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|

The antibiotic resistance genes were identified searching PubMed. The search was carried out inputting the full name of each species with the All Fields selection, followed by identification of the antibiotic resistance genes in each article. ABC-F: ATP-binding cassette ribosomal protection protein gene resistance; OMP: Outer membrane porin drug resistance genes; PBPs: penicillin-binding proteins; Pmr: phosphoethanolamine transferase.
Figure 1. A. non-baumannii species which carry three or more ARGs as found in the literature. Heatmap showing species that harbor three or more ARGs. Yellow, presence of the gene; red, absence of the gene in the corresponding species. A complete listing including A. non-baumannii species that carry any number of ARGs can be found in Table S1 and Figure S1.

Since the nucleotide sequence data banks include an excess of information when compared to that already published in scientific articles, another set of analyses was carried out using the Basic Local Alignment Search Tool (BLAST) software and the data from the databases CARD-RGI, ARG-ANNOT, and ResFinder. There were 1457 complete A. non-baumannii genomes in GenBank as of June 15, 2020. Each one of them was compared with the databases CARD-RGI, ARG-ANNOT and ResFinder using BLAST. Comparisons to information in the ARG-ANNOT database produced 9676 hits corresponding to efflux pumps genes in 1404 genomes. Genes related to AdeABC, AdeFGH and AdeIJK were the efflux pumps most commonly present, and AdeFGH and AdeIJK were those found more
A total of 245 \textit{A. wuhouensis}, 187 \textit{A. pitti}, and 143 \textit{A. nosocomialis} isolates harbored \(\beta\)-lactamase resistance genes. The \textit{blaOXA-421} and \textit{blaADC-4} genes were the highest represented \(\beta\)-lactamases. The species with the highest ARG content were \textit{A. pitti} (\(n = 3841\)), \textit{A. sp.} (\(n = 2915\)), and \textit{A. nosocomialis} (\(n = 2277\)).

Aminoglycoside modifying enzyme coding genes were the third most abundant group of resistance determinants; 1084 genes being found in 599 genomes in the database. The species with the most aminoglycoside resistance genes were \textit{A. pitti}, \textit{A. sp.}, \textit{A. indicus} and \textit{A. haemolyticus}. The most identified genes were \textit{aph(6)-ld}, \textit{ant(3")-Ia} and \textit{aph(3")-Ib} (Figure S2).

No ARGs were identified in \textit{A. nectaris}, \textit{A. marinus}, \textit{A. equi}, \textit{A. larvae}, \textit{A. qingfengensis} and \textit{A. boissieri} by BLAST comparisons or the literature search. In the cases of \textit{A. albensis}, \textit{A. bouvetii}, \textit{A. brisouii}, \textit{A. celticus}, \textit{A. harbinensis}, \textit{A. juni}, \textit{A. kookii}, \textit{A. pragensis} and \textit{A. pseudolwoffii}, no ARGs were identified in the literature. Still, at least one resistance-nodulation-cell division (RND) antibiotic efflux pump gene was identified by the BLAST comparisons. All adeJK efflux pump genes were identified in \textit{A. defluvii}, \textit{A. junii}, \textit{A. venetianus}, \textit{A. halotolerans}, \textit{A. tjernbergiae}, and \textit{A. wuhouensis}. All adeFGH efflux pump genes were found in \textit{A. courvalinii}. Besides the efflux pump, other ARGs genes were detected in \textit{A. bouvetii}, \textit{A. courvalinii}, \textit{A. defluvii}, \textit{A. junii}, \textit{A. pseudolwoffii}, \textit{A. venetianus} and \textit{A. wuhouensis}.

The BLAST comparison permitted us to detect genes and the number of allelic variants (\(n\)) that are not yet reported in the literature. All known \textit{Acinetobacter} species, as listed in the List of Prokaryotic names with Standing in Nomenclature [20], were used in a PubMed literature search. Two species, (\textit{A. portensis} and \textit{A. guerrea}), recently discovered by Carvalheira et. al. [21] were also included. The search was conducted by inputting the full name of each species with the All Fields selection in the PubMed database and then identifying the antibiotic resistance genes described in each article. CARD-RGI software [22] was used to verify the antibiotic resistance nature of genes that were not well defined in the publications.

3. Materials and Methods

3.1. Literature Search

All known \textit{Acinetobacter} species, as listed in the List of Prokaryotic names with Standing in Nomenclature [20], were used in a PubMed literature search. Two species, (\textit{A. portensis} and \textit{A. guerrea}), recently discovered by Carvalheira et. al. [21] were also included. The search was conducted by inputting the full name of each species with the All Fields selection in the PubMed database and then identifying the antibiotic resistance genes described in each article. CARD-RGI software [22] was used to verify the antibiotic resistance nature of genes that were not well defined in the publications.
3.2. Genome Sequences Collection and Antibiotic Resistance Genes Prediction

All complete *A. non-baumannii* genome nucleotide sequences were downloaded from GenBank NCBI. Predictive identification of antibiotic resistance genes (ARGs) was performed using the BLASTp, the CARD-RGI [22], ARG-ANNOT [23] and ResFinder [24] software databases. For ARGs prediction, 70% coverage, 95% amino acid identity and $<10^{-6}$ e-value were used as the BLASTp parameters. Data prediction using the three databases were integrated for clustering analysis. Clustering analysis was carried out using the *hclust* and *dist* R package [25]. The Euclidean method was used as distance method. The agglomerative method was used as Hierarchical clustering method. Within the agglomerative methods, the complete-linkage algorithm was used for hierarchical clustering analysis. Agglomerative methods were reported as the most adequate to analyze the presence and absence of genes over genomes [26].

4. Conclusions

The importance of *A. non-baumannii* as pathogens, and the volume of information about their resistance to antimicrobials, is rapidly increasing. This article describes an initial study of the resistome of *A. non-baumannii* pathogens. The impact of this group of bacteria as causative agents of nosocomial infections is growing. As a consequence, while *Acinetobacter* infections are still mainly caused by *A. baumannii* [1,27], it is quite probable that other species will become as important in the future. It is already evident that multiple *A. non-baumannii* clinical isolates are resistant to one or more antibiotics [2,3]. The present and future efforts to design effective therapies against these pathogens require understanding their resistance profiles and genes. The comparison of existing data (literature and GenBank) about resistance genetic determinants showed similarities and significant differences that could impact the efficiency of treatments depending on the species that originate each infection.

Supplementary Materials: The following are available online at https://www.mdpi.com/2079-382/10/1/16/s1, Figure S1. Heatmap showing absence and presence of ARGs in *A. non-baumannii* reported in the literature. Red color indicates the absence of genes and blue color indicates the presence of gene. The clustering analysis was done using *hclust* R package. The heatmap was done using ggplot2 R package. Figure S2. Heatmap showing absence and presence of ARGs in *A. non-baumannii* genomes available in the GenBank database. Red color indicates the absence of a gene and blue color indicates the presence of a gene. The GenBank Assembly Accession of the genomes used is indicating in Table S3. The clustering analysis was done using the *hclust* R package. The heatmap was done using the ggplot2 R package. Table S1. List of ARGs in *Acinetobacter*, excluding *A. baumannii*, reported in the literature. The presence of ARGs is indicated with color cell and the absence of ARGs is indicated with white cell. Table S2. List of ARGs in *Acinetobacter*, excluding *A. baumannii*, predicted in available genomes in GenBank Database. The presence of ARGs is indicated with 1 and the absence of ARGs is indicated with 0. Table S3. List of Code used in Figure S2.

Author Contributions: Conceptualization, A.B., M.E.T., M.S.R.; methodology, A.B., G.M.T., M.E.T., M.S.R.; software, A.B., G.M.T.; formal analysis, A.B., G.M.T., S.M., M.E.T., M.S.R.; investigation, A.B., G.M.T., M.S.R.; resources, M.S.R.; data curation, A.B., G.M.T., S.M., M.E.T., M.S.R.; writing—original draft preparation, S.M., M.E.T., M.S.R.; writing—review & editing, A.B., S.M., M.E.T., M.S.R.; supervision, M.S.R.; project A.B. ministration, M.E.T., M.S.R.; funding acquisition, M.E.T. and M.S.R. All authors have read and agreed to the published version of the manuscript.

Funding: The authors’ work was supported by NIH SC3GM125556 to MSR and 2R15AI047115 to MET. AB was supported by grant MHRP 2T37MD001368 from the National Institute on Minority Health and Health Disparities, National Institute of Health.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

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