AAC Accepts, published online ahead of print on 31 January 2011
Antimicrob. Agents Chemother. doi:10.1128/AAC.01595-10

Revised version

Genomic Analysis of *Acinetobacter baumannii* A118 by Comparison of Optical Maps: Identification of Structures Related to its Susceptibility Phenotype

Maria Soledad Ramirez[1,2], Mark D. Adams[3], Robert A. Bonomo[4], Daniela Centrón[2], and Marcelo E. Tolmasky[1*]

[1]Center for Applied Biotechnology Studies, Department of Biological Science, California State University Fullerton, Fullerton, CA

[2]Departamento de Microbiología, Facultad de Medicina, UBA, Buenos Aires, Argentina

[3]Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH

[4]Departments of Pharmacology and Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, OH, USA and Louis Stokes Cleveland Department of Veteran Affairs Medical Center, Cleveland, OH

Running title: *Acinetobacter baumannii* A118 Optical Mapping

Keywords: *Acinetobacter* spp., optical mapping, nosocomial infection, antibiotic resistance

Correspondence:
Marcelo E. Tolmasky
Center for Applied Biotechnology Studies
Department of Biological Science
California State University Fullerton
800 N State College Boulevard
Fullerton, CA 92831-3599
USA
Phone 657-278-5263
mtolmasky@fullerton.edu

Acinetobacter baumannii A118, a naturally competent clinical isolate, is unusually susceptible to several antibiotics. Comparison of the optical map of strain A118 with *in silico* generated restriction maps of sequenced genomes and sequence analyses showed that the AbaR region, commonly found inserted within the *comM* gene in other isolates, is missing in strain A118, which could in part explain the susceptible phenotype exhibited by this isolate. These comparative studies also showed differences in regions where genes coding for functions that may be involved in drug resistance or susceptibility are located. Further sequencing demonstrated that *cat* and bla_{ADC}, named $bla_{ADC\text{-}55}$, are present but a *tet(A)* gene usually found in other strains is not. In addition *carO* and *pbp2*, which may play a role in susceptibility to carbapenems are present in strain A118. These findings support the idea that *A. baumannii* strains posses multiple mechanisms that contribute to antibiotic resistance and the presence of some of them is not sufficient for a resistant phenotype. The results showed here indicate that optical mapping is a useful tool for preliminary comparative genomic analysis.

Acinetobacter baumannii is an emerging opportunistic human pathogen responsible for a growing number of nosocomial infections mainly affecting patients that are immnosuppresed, that suffer other underlying diseases, or have been treated using certain invasive procedures (20, 26, 30). The incidence of *A. baumannii* is steadily growing and a study indicates that while in 1975 this bacterium was responsible for 1.5% of hospital-acquired pneumonia cases, in 2003 that number had grown to 6.9% (17). The increasing frequency of *A. baumannii* infections may be due to a combination of factors such as its ability to survive for a prolonged length of time in different environments and a rise in the number of susceptible individuals as a result of advancements in medical support of critically ill patients. The ability of *A. baumannii* to form biofilms has also been related to commonly occurring infections associated with medical devices (15, 34). Recent studies identified several other virulence factors and pathogenic islands (6, 38, 40). *A. baumannii* infections have also gained attention due to the high number of soldiers serving in Iraq and Afghanistan and victims of the 2004 Asian tsunami that were infected with this bacterium (9, 16, 18). Treatment of *Acinetobacter* infections is becoming increasingly difficult due to the growing number of multidrug resistant isolates. Compounding the problem, antibiotic drug development to treat this bacterium is almost nonexistent (10, 30, 34, 39). Furthermore, the multiresistant nature of most *A. baumannii* strains makes them difficult to manipulate for genetic studies.

A. baumannii A118, isolated from a blood culture of a patient admitted to an intensive care unit in a hospital in Buenos Aires, Argentina, is rather exceptional for its susceptibility to antibiotics such as ceftazidime, cefepime, piperacillin, minocycline, amikacin, gentamicin, trimethoprim-sulfamethoxazole, kanamycin and ciprofloxacin

(32). This property together with its natural competence led to the suggestion that this strain is a convenient model for genetic studies (33). In this work we analyzed *A. baumannii* A118 genomic regions known for containing potential resistance or susceptibility determinants in previously studied strains using optical mapping, a powerful tool for comparative genomics (37). Optical maps are full genome restriction maps obtained after immobilizing single DNA molecules on a charged substrate and digestion with the restriction endonuclease of interest followed by detection and assembly into a high-resolution ordered full genome restriction map (5). Our results show that the AbaR-type resistance island is missing and suggest that *A. baumannii* resistance to a variety of antibiotics may be due to a combination of mechanisms some of which are present in strain A118 but are not sufficient to confer a resistance phenotype.

MATERIALS AND METHODS

Bacterial strains and genomes. *A. baumannii* A118 is a blood stream isolate recovered from a patient in an intensive care unit (28, 33). The available genomes of *A. baumannii* strains were used for the comparative studies (AYE, NC_010410; AB307-0294, CP001172; AB0057, CP001182; ACICU, CP000863; ATCC17978, CP000521; and SDF, NC_010400). *Escherichia coli* TOP10 (Invitrogen, San Diego, CA) was used as host in recombinant cloning.

General procedures. The *A. baumannii* A118 *Nco*I optical map was generated at OpGen Technologies, Inc. (Madison, WI) as described previously (5). Comparative genomic analysis was carried out by comparing the optical map of *A. baumannii* A118 to *Nco*I restriction maps of *A. baumannii* sequenced genomes using the MapSolver software 2.1.1 (OpGen Technologies, Inc., Madison, WI). PCR reactions were carried out using the QIAGEN *Taq* master mix, and the products were detected by agarose gel electrophoresis. Cloning into pCR2.1 was performed as recommended by the supplier (Invitrogen). DNA sequencing reactions using amplicons as templates were done at the City of Hope sequencing facility. Genomic DNA was prepared for genome sequencing using a Nextera™ kit from Epicentre Biotechnologies. Sequencing was performed on an Illumina Genome Analyzer IIx using paired 76-base reads, resulting in 1,712,408 read pairs. These were assembled using *velvet* resulting in 186 scaffolds that are at least 500 bases long. The scaffold N50 size is 39.3 kbp, meaning that half of the genome is assembled into scaffolds of at least this length. The total assembled length is 3,824 kbp. Genome annotation was performed using ISGA (21). Amino acid sequence comparisons

were performed using the CLUSTAL W program (Pôle Bio-Informatique Lyonnais server [http://npsa-pbil.ibcp.fr/cgi-bin/align_clustalw.pl]) (7).

Nucleotide sequence accession number. The nucleotide data are available in the GenBank nucleotide database under accession number AEOW00000000 .

RESULTS AND DISCUSSION

Regions relevant to the antibiotic susceptibility characteristics of *A. baumannii* A118 were studied using optical mapping, a technique that is based on immobilizing single DNA molecules on a charged substrate, digested with a restriction endonuclease, and detected and assembled into a high-resolution ordered restriction map permitting comparison of related genomes (3). The estimated size of the *A. baumannii* A118 chromosome based on the optical mapping is 3.84 Mb and the predicted number of *Nco*I restriction fragments is 465.

The AbaR-type resistance island region. Five of the six *A. baumannii* strains for which the complete genome sequence is known include a region that has transposed or inserted into a specific location within *comM*, a gene that codes for a 495-amino acid protein that includes an ATPase domain (1, 14). In addition, analysis of other *A. baumannii* strains showed that most of them also carry a related insertion (31, 36). These inserted regions are usually characterized by the presence of transposase and antibiotic or heavy metal resistance genes and are known as AbaR-type resistance island regions (1, 31). Comparison of *in silico* generated restriction maps of *A. baumannii* sequenced genomes with the optical map of *A. baumannii* A118 indicated that there was no AbaR-type resistance island in this strain (Fig. 1a). PCR using the primers designed before by Shaik et al. (36), located within the *comM* gene and flanking the location of insertion of AbaR, followed by sequencing of the amplicon showed that the *comM* gene was intact and identical to the gene in *A. baumannii* AB307-0294, a strain known to lack the AbaR-type resistance island (1)(Fig. 1b). This gene has been named *comM* on the basis of the 49.5%

129 homology found between the proteins from *A. baumannii* ADP1 and *Haemophilus*
130 *influenza* (2). Mutagenesis of *comM* in *H. influenza* resulted in a reduced ability to take
131 up DNA (19). These results are in agreement with the susceptible phenotype observed
132 for *A. baumannii* A118 and its natural competency.
133
134 **Other loci related to antibiotic resistance.** The genomes of all sequenced *A. baumannii*
135 from human origin include a *cat* gene. In addition, strains AB0057 and AYE include a
136 second *cat* gene within the AbaR-type resistance island (1, 14). *A. baumannii* A118 lacks
137 this island and therefore this strain must lack at least one of the *cat* genes. Comparative
138 analysis of the *A. baumannii* A118 optical map at the region where the *cat* gene present
139 outside the AbaR-type resistance island is located in the sequenced strains showed some
140 heterogeneity with apparent insertions and deletions. This is best illustrated by the
141 comparison of the optical map of strain A118 with the *in silico* generated *Nco*I restriction
142 maps of strains AB0057 and AYE. While comparing strains A118 and AB0057 suggests
143 that the fragment including *cat* in A118, although not identical, is present, comparison of
144 strains A118 and AYE suggests that there is a deletion in strain A118 that includes the
145 fragment where *cat* should be located. To confirm the presence of *cat* in strain A118 an
146 amplicon of 1261 bp obtained using a pair of primers located within the flanking *greA*
147 and *uspA* genes was sequenced. The results indicated that the genome of *A. baumannii*
148 A118 includes the *cat* gene with near-perfect identity to those present in other *A.*
149 *baumannii* strains (strain AYE, locus tag ABAYE0798; strain AB0057, locus tag
150 AB57_3104; strain ATCC17978, locus tag A1S_2691) (Fig. 2b). These results indicate
151 that there must be some variability at the nucleotide region that resulted in modifications

in the *Nco*I restriction sites patterns that led to the apparent deletion of a DNA fragment in strain A118. We hypothesize that the lower MIC of chloramphenicol exhibited by strain A118 is due to the absence of the *cat* gene located within the AbaR-type resistance island. However, comparison of the MICs of chloramphenicol for *A. baumannii* A118 and ATCC 17978, which also lacks the *cat* present within the AbaR-type resistance island, showed that they were 12 and 48 μg/ml, respectively. Although it is possible that this *cat* gene contributes to resistance to chloramphenicol, it is most likely that other factors may contribute to the overall resistance to chloramphenicol, of which some must be absent in the strain A118.

The genomes of *A. baumannii* AYE, AB0057, AB307-0294, and ATCC17978 genomes include a *tet(A)* gene outside of the AbaR-type resistance island that may be involved in tetracycline resistance (strain AYE, locus tag ABAYE0369; strain AB0057, locus tag AB57_3570; strain AB307-0294, locus tag ABBFA_00039; strain ATCC17978, locus tag A1S_3117). In addition, strains AB0057 and AYE include one, *tet(A)* and two, *tet(A)* and *tet(G)*, genes within the AbaR-type resistance island respectively (1, 14, 24, 31, and http://faculty.washington.edu/marilynr/tetweb1.pdf). A comparison between the optical map of strain A118 and the *in silico* generated *Nco*I restriction maps of *A. baumannii* genomes at the location of *tet(A)* showed that strains ACICU and A118 have a different pattern than the rest of the strains (Fig. 3). To investigate if the differences observed correlated with presence or absence of the *tet(A)* gene the sequences of the ACICU, AYE, AB0057, AB307-0294, and ATCC17978 strains were compared among themselves and to the drafts of the strain A118. The results indicated that while the genes *glyS* and *glyQ* were present and highly homologous in all

strains, ACICU and A118 do not include the *tet(A)* gene, instead there is a short open reading frame with no homology to *tet(A)* upstream of *glyQ* (Fig. 3). Furthermore BLAST analyses comparing the *tet(A)* nucleotide sequence or the coded amino acid sequence against the available sequences of strain A118 showed no homology, confirming that this strain lacks the *tet(A)* gene.

Other genes of interest with respect to drug resistance such as $bla_{\text{OXA-51}}$-like, which codes for a β-lactamase that has weak catalytic activity against penicillins and carbapenems but not expanded-spectrum cephalosporins (22); bla_{ADC}, a gene coding for the non-inducible ADC cephalosporinase that has been named $bla_{\text{ADC-55}}$ according to the nomenclature proposed by Hujer et al. (8, 23); *carO*, a gene coding for CarO, an outer membrane protein that participates in the influx of carbapenems (29,); and *pbp2,* a gene coding for the key protein, PBP2, which when expressed at low levels leads to carbapenem resistance (12), were also found in the genome of *A. baumannii* A118.

Comparison between the optical map of strain A118 and the *in silico* generated *Nco*I restriction maps of *A. baumannii* genomes at the region where *carO* is located showed that all six genomes were similar (Fig. 4a). Therefore it was expected that the gene was present in strain A118. The nucleotide sequence confirmed this expectation and the amino acid sequences of all CarO proteins were highly related (Fig. 4b). The results of optical map comparison in the case of the *pbp2* gene were not as straightforward, a first look at the comparison showed an apparent deletion (Fig. 5). However nucleotide sequencing showed that there is a complete copy of the gene in *A. baumannii* A118 but it includes a number of point mutations that are silent and do not result in amino acid changes (data not shown). Two *Nco*I restriction sites are not present

198 in the strain A118 version of the gene due to two of these point mutations, and a third one
199 was not detected by the optical mapping, which resulted in an apparent missing fragment
200 inside the gene sequence. Interestingly, these results are in agreement with a recent
201 analysis of PBP proteins in all the *A. baumannii* genomes deposited in GenBank that
202 showed the presence of several point mutations but >90% of them were silent (4).
203 Carbapenem resistance in *A. baumannii* has been reported to be due to one or a
204 combination of the following factors: enzymatic modification by β-lactamases of
205 different classes, a decrease in permeability as a consequence of alterations in structure
206 and number of porins, the presence of efflux pumps, and changes in structure or
207 expression of PBPs (30). In particular, a recent study of strains isolated from blood
208 samples in a hospital in Spain found that PBP2 was expressed at very low levels in a
209 group *A. baumannii* highly resistant to carbapenems (imipenem and meropenem) as
210 compared to another group that showed significantly higher susceptibility to these
211 antibiotics (12). Our results suggest that PBP2 is present in *A. baumannii* A118 but since
212 the levels of expression are not known the role of this protein in the susceptible
213 phenotype remains undetermined.

214 The comparative analysis at the region where bla_{ADC} is located exhibited similar
215 patterns with minor differences (Fig. 6a). The presence of this gene in strain A118 was
216 confirmed by sequencing. Fig. 6b shows the CLUSTAL W comparison of ADC proteins
217 from complete *A. baumannii* genomes, which are highly homologous. A detailed
218 analysis and discussion on *A. baumannii* ADC proteins has recently been published (35).
219 A factor contributing to the high susceptibility of strain A118 to third-generation
220 cephalosporins and to carbapenems in spite of harboring $bla_{\text{ADC-55}}$ and the $bla_{\text{OXA-51}}$-like

gene *bla*$_{\text{OXA-89}}$ (28, 33) may be the lack of copies of IS*Aba1* or IS*Aba9* (27) upstream of the structural genes to provide a strong promoter necessary for high levels of expression (8, 13).

Concluding remarks. Our work shows that the comparative analysis of optical maps is of help for an initial comparative analysis of a genome but the results must be further confirmed by other means such as amplification and sequencing of the regions in question. *A. baumannii* A118 is susceptible to several antibiotics, a distinguishing characteristic we found in this preliminary study is the lack of the AbaR-type resistance island region and the *tet(A)* gene. In addition two of the genes that may be responsible for resistance to carbapenems and to third-generation of cephalosporins in other strains, a *bla*$_{\text{OXA51}}$-like and *bla*$_{\text{ADC}}$, lack the insertions sequences described to provide a promoter for significant expression. Other genes present in multidrug resistant strains such as *carO, pbp2,* and *cat* are present in strain A118. These results partially explain the susceptible nature of strain A118 but also indicate that drug resistance in *A. baumannii* is a complex process where many factors influence the phenotype including the presence of genes coding for different functions that contribute to resistance or susceptibility to a given antibiotic as well as their level of expression. Further studies including analysis of the complete *A. baumannii* A118 genome sequence when available will permit us to better understand the factors responsible for its susceptibility phenotype. Furthermore, a long term project consisting of systematic gene deletion of multiresistant strains, an approach successfully used in the past with other bacteria (11, 25), could reveal genes involved in resistance that had not been considered as such in the past.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant 2R15AI047115 (to M.E.T.) from the National Institutes of Health and PICT 0354 (to M.S.R.). R.A.B. was supported by Merit Review Award from the Veterans Administration and grants from the National Institutes of Health (NIH/NIAID AI072219 and AI063517). M.S.R. and D.C. are career investigators of CONICET.

REFERENCES

1. **Adams, M. D., K. Goglin, N. Molyneaux, K. M. Hujer, H. Lavender, J. J. Jamison, I. J. MacDonald, K. M. Martin, T. Russo, A. A. Campagnari, A. M. Hujer, R. A. Bonomo, and S. R. Gill.** 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J Bacteriol **190:**8053-8064.

2. **Barbe, V., D. Vallenet, N. Fonknechten, A. Kreimeyer, S. Oztas, L. Labarre, S. Cruveiller, C. Robert, S. Duprat, P. Wincker, L. N. Ornston, J. Weissenbach, P. Marliere, G. N. Cohen, and C. Medigue.** 2004. Unique features revealed by the genome sequence of *Acinetobacter sp.* ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res **32:**5766-5779.

3. **Cai, W., J. Jing, B. Irvin, L. Ohler, E. Rose, H. Shizuya, U. J. Kim, M. Simon, T. Anantharaman, B. Mishra, and D. C. Schwartz.** 1998. High-resolution restriction maps of bacterial artificial chromosomes constructed by optical mapping. Proc Natl Acad Sci U S A **95:**3390-3395.

4. **Cayo, R., M. Rodriguez, P. Espinal, F. Fernandez-Cuenca, A. Ocampo-Sosa, A. Pascual, J. Vilab, and L. Martinez-Martinez.** 2010. Presented at the 8th International Symposium on the Biology of *Acinetobacter*, Rome, Italy.

5. **Chen, Q., S. Savarino, and M. Venkatesan.** 2006. Substractive hybridization and optical mapping of the enterotoxigenic *Escherichia coli* H10407 chromosome: isolation of unique sequences and demonstration of significant similarity to the chromosome of *E. coli* K-12. Microbiology **152:**1041-1056.

6. **Choi, C. H., S. H. Hyun, J. Y. Lee, J. S. Lee, Y. S. Lee, S. A. Kim, J. P. Chae, S. M. Yoo, and J. C. Lee.** 2008. *Acinetobacter baumannii* outer membrane protein A targets the nucleus and induces cytotoxicity. Cell Microbiol **10:**309-319.

7. **Combet, C., C. Blanchet, C. Geourjon, and G. Deleage.** 2000. NPS@: network protein sequence analysis. Trends Biochem Sci **25:**147-150.

8. **Corvec, S., N. Caroff, E. Espaze, C. Giraudeau, H. Drugeon, and A. Reynaud.** 2003. AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. J Antimicrob Chemother **52:**629-635.

9. **Davis, K. A., K. A. Moran, C. K. McAllister, and P. J. Gray.** 2005. Multidrug-resistant *Acinetobacter* extremity infections in soldiers. Emerg Infect Dis **11:**1218-1224.

10. **Dijkshoorn, L., A. Nemec, and H. Seifert.** 2007. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol **5:**939-951.

11. **Fajardo, A., N. Martinez-Martin, M. Mercadillo, J. C. Galan, B. Ghysels, S. Matthijs, P. Cornelis, L. Wiehlmann, B. Tummler, F. Baquero, and J. L. Martinez.** 2008. The neglected intrinsic resistome of bacterial pathogens. PLoS One **3:**e1619.

12. **Fernandez-Cuenca, F., L. Martinez-Martinez, M. C. Conejo, J. A. Ayala, E. J. Perea, and A. Pascual.** 2003. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of

carbapenems against clinical isolates of *Acinetobacter baumannii*. J Antimicrob Chemother **51:**565-574.

13. **Figueiredo, S., L. Poirel, A. Papa, V. Koulourida, and P. Nordmann.** 2009. Overexpression of the naturally occurring *bla*OXA-51 gene in *Acinetobacter baumannii* mediated by novel insertion sequence IS*Aba9*. Antimicrob Agents Chemother **53:**4045-4047.

14. **Fournier, P. E., D. Vallenet, V. Barbe, S. Audic, H. Ogata, L. Poirel, H. Richet, C. Robert, S. Mangenot, C. Abergel, P. Nordmann, J. Weissenbach, D. Raoult, and J. M. Claverie.** 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS Genet **2:**e7.

15. **Gaddy, J. A., A. P. Tomaras, and L. A. Actis.** 2009. The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infect Immun **77:**3150-3160.

16. **Garzoni, C., S. Emonet, L. Legout, R. Benedict, P. Hoffmeyer, L. Bernard, and J. Garbino.** 2005. Atypical infections in tsunami survivors. Emerg Infect Dis **11:**1591-1593.

17. **Gaynes, R., and J. R. Edwards.** 2005. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis **41:**848-854.

18. **Guerrero, D. M., F. Perez, N. G. Conger, J. S. Solomkin, M. D. Adams, P. N. Rather, and R. A. Bonomo.** 2010. *Acinetobacter baumannii*-associated skin and soft tissue infections: recognizing a broadening spectrum of disease. Surg Infect (Larchmt) **11:**49-57.

19. **Gwinn, M. L., R. Ramanathan, H. O. Smith, and J. F. Tomb.** 1998. A new transformation-deficient mutant of *Haemophilus influenzae* Rd with normal DNA uptake. J Bacteriol **180:**746-748.

20. **Hartstein, A. I., A. L. Rashad, J. M. Liebler, L. A. Actis, J. Freeman, J. W. Rourke, Jr., T. B. Stibolt, M. E. Tolmasky, G. R. Ellis, and J. H. Crosa.** 1988. Multiple intensive care unit outbreak of *Acinetobacter calcoaceticus* subspecies *anitratus* respiratory infection and colonization associated with contaminated, reusable ventilator circuits and resuscitation bags. Am J Med **85:**624-631.

21. **Hemmerich, C., A. Buechlein, R. Podicheti, K. V. Revanna, and Q. Dong.** 2010. An Ergatis-based prokaryotic genome annotation web server. Bioinformatics **26:**1122-1124.

22. **Heritier, C., L. Poirel, P. E. Fournier, J. M. Claverie, D. Raoult, and P. Nordmann.** 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother **49:**4174-4179.

23. **Hujer, K. M., N. S. Hamza, A. M. Hujer, F. Perez, M. S. Helfand, C. R. Bethel, J. M. Thomson, V. E. Anderson, M. Barlow, L. B. Rice, F. C. Tenover, and R. A. Bonomo.** 2005. Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase, ADC-7 beta-lactamase: defining a unique family of class C enzymes. Antimicrob Agents Chemother **49:**2941-2948.

24. **Levy, S. B., L. M. McMurry, T. M. Barbosa, V. Burdett, P. Courvalin, W. Hillen, M. C. Roberts, J. I. Rood, and D. E. Taylor.** 1999. Nomenclature for new tetracycline resistance determinants. Antimicrob Agents Chemother **43:**1523-1524.

25. **Liu, A., L. Tran, E. Becket, K. Lee, L. Chinn, E. Park, K. Tran, and J. H. Miller.** 2010. Antibiotic sensitivity profiles determined with an *Escherichia coli* gene knockout collection: generating an antibiotic bar code. Antimicrob Agents Chemother **54:**1393-1403.

26. **Maragakis, L. L., and T. M. Perl.** 2008. *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis **46:**1254-1263.

27. **Merkier, A. K., M. Catalano, M. S. Ramirez, C. Quiroga, B. Orman, L. Ratier, A. Famiglietti, C. Vay, A. Di Martino, S. Kaufman, and D. Centron.** 2008. Polyclonal spread of *bla*(OXA-23) and *bla*(OXA-58) in *Acinetobacter baumannii* isolates from Argentina. J Infect Dev Ctries **2:**235-240.

28. **Merkier, A. K., and D. Centron.** 2006. *bla*(OXA-51)-type beta-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. Int J Antimicrob Agents **28:**110-113.

29. **Mussi, M. A., A. S. Limansky, and A. M. Viale.** 2005. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. Antimicrob Agents Chemother **49:**1432-1440.

30. **Perez, F., A. M. Hujer, K. M. Hujer, B. K. Decker, P. N. Rather, and R. A. Bonomo.** 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother **51:**3471-3484.

31. **Post, V., P. A. White, and R. M. Hall.** 2010. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. J Antimicrob Chemother **65:**1162-1170.

32. **Predari, S., M. Gutierrez, A. De Paulis, L. Guelfand, and D. Centron Garcia.** 1991. In vitro activity of 16 antibiotics and sulbactam against *Acinetobacter calcoaceticus* phenotype A1. J Chemother **48:**20-25.

33. **Ramirez, M. S., M. Don, A. K. Merkier, A. J. Bistue, A. Zorreguieta, D. Centron, and M. E. Tolmasky.** 2010. Naturally competent *Acinetobacter baumannii* clinical isolate as a convenient model for genetic studies. J Clin Microbiol **48:**1488-1490.

34. **Rodriguez-Bano, J., and R. A. Bonomo.** 2008. Multidrug-resistant *Acinetobacter baumannii*: Eyes Wide Shut? Enferm Infecc Microbiol Clin **26:**185-186.

35. **Rodriguez-Martinez, J., L. Poirel, and P. Nordmann.** 2010. Genetic and functional variability of AmpC-type ß-lactamases from *Acinetobacter baumannii*. Antimicrob Agents Chemother **54:**4930-4933.

36. **Shaikh, F., R. P. Spence, K. Levi, H. Y. Ou, Z. Deng, K. J. Towner, and K. Rajakumar.** 2009. ATPase genes of diverse multidrug-resistant *Acinetobacter baumannii* isolates frequently harbour integrated DNA. J Antimicrob Chemother **63:**260-264.

37. **Shukla, S. K., J. Kislow, A. Briska, J. Henkhaus, and C. Dykes.** 2009. Optical mapping reveals a large genetic inversion between two methicillin-resistant *Staphylococcus aureus* strains. J Bacteriol **191:**5717-5723.

38. **Smith, M. G., T. A. Gianoulis, S. Pukatzki, J. J. Mekalanos, L. N. Ornston, M. Gerstein, and M. Snyder.** 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes Dev **21:**601-614.

39. **Talbot, G. H., J. Bradley, J. E. Edwards, Jr., D. Gilbert, M. Scheld, and J. G. Bartlett.** 2006. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis **42:**657-668.

40. **Zimbler, D. L., W. F. Penwell, J. A. Gaddy, S. M. Menke, A. P. Tomaras, P. L. Connerly, and L. A. Actis.** 2009. Iron acquisition functions expressed by the human pathogen *Acinetobacter baumannii*. Biometals **22:**23-32.

399 Legends to Figures

400 Fig. 1. Genomic comparison. a. *A. baumannii* A118 optical map was compared to the *A.*
401 *baumannii* strains AB0057 and AYE *Nco*I restriction maps obtained *in silico* around the
402 location of the AbaR-type resistance island region using the MapSolver software. The
403 white regions represent DNA fragments missing in strain A118. The AbaR3 and AbaR1
404 regions are the genomic islands present in strains AB0057 and AYE (1, 14). Vertical
405 lines represent *Nco*I restriction sites. b. Diagram showing the point of insertion of AbaR
406 genomic islands within the *comM* gene, which was shown to be intact in *A. baumannii*
407 A118 by sequencing an amplicon generated using total DNA as template and the primers
408 5'-TCCATTTTACCGCCACTTTC and 5'-AATCGATGCGGTCGAGTAAC (36).
409 Nucleotides shown in red are in direct repeats in those strains where AbaR has been
410 inserted.
411
412 Fig. 2. Genomic comparison. a. Comparison of the *A. baumannii* A118 optical map with
413 the *A. baumannii* strains AB0057 and AYE *Nco*I restriction maps obtained *in silico*
414 around the location of the *cat* gene using the MapSolver software. White fragments
415 represent putative missing/inserted fragments. The location of *greA*, *cat*, and *uspA* genes
416 and relevant *Nco*I sites are shown. The numbers indicate the coordinates of *Nco*I sites or
417 the location of specified genes in the GenBank entries for *A. baumannii* AB0057 and
418 AYE genome annotations (accession numbers CP001182 and NC_010410). b.
419 CLUSTALW comparison of chloramphenicol acetyltransferase amino acid sequences.
420 Strain AYE, locus tag ABAYE0798; strain AB0057, locus tag AB57_3104; strain
421 ATCC17978, locus tag A1S_2691.

422

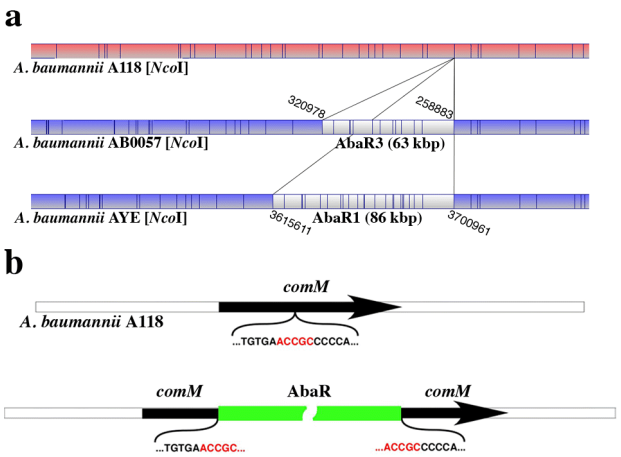
423 Fig. 3. Genomic comparison. Comparison of the *A. baumannii* A118 optical map with
424 the *A. baumannii* strains ATCC 17978, ACICU, AB307-0294, AB0057 and AYE *Nco*I
425 restriction maps obtained *in silico* at the location of the *tet(A)* gene using the MapSolver
426 software. The location of *glyS, glyQ*, and *tet(A)* in the *A. baumannii* ACICU and AYE
427 strains and the position of the relevant *Nco*I sites are shown. The numbers indicate the
428 coordinates of *Nco*I sites or the location of specified genes in the GenBank entries for
429 each strain. Strain AYE, locus tag ABAYE0369; strain AB0057, locus tag AB57_3570;
430 strain AB307-0294, locus tag ABBFA_00039; strain ATCC17978, locus tag A1S_3117.
431

432 Fig. 4. Genomic comparison. a. Comparison of the *A. baumannii* A118 optical map with
433 the *A. baumannii* strains ATCC 17978, ACICU, AB307-0294, AB0057 and AYE *Nco*I
434 restriction maps obtained *in silico* at the location of the *carO* gene using the MapSolver
435 software. The location of *carO* and relevant *Nco*I sites are shown. The numbers indicate
436 the coordinates of *Nco*I sites or the location of specified genes in the GenBank entries for
437 each strain. b. CLUSTALW comparison of CarO amino acid sequences.
438

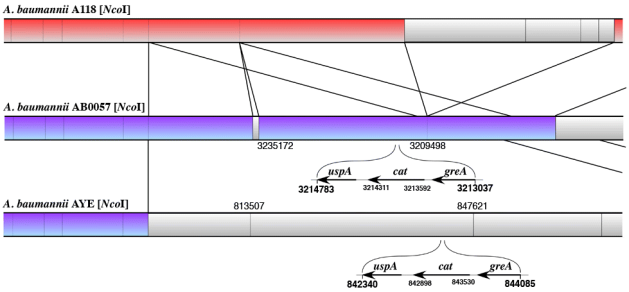
439 Fig. 5. Genomic comparison. Comparison of the *A. baumannii* A118 optical map with
440 the *A. baumannii* strains ATCC 17978, ACICU, AB307-0294, AB0057 and AYE *Nco*I
441 restriction maps obtained *in silico* at a fragment of the *pbp2* gene using the MapSolver
442 software. White fragments represent putative missing/inserted fragments. The location of
443 relevant *Nco*I sites is shown. The numbers indicate the coordinates of *Nco*I sites or the
444 location of specified genes in the GenBank entries for each strain.

445

446 Fig. 6. Genomic comparison. a. Comparison of the *A. baumannii* A118 optical map with
447 the *A. baumannii* strains ATCC 17978, ACICU, AB307-0294, AB0057 and AYE *Nco*I
448 restriction maps obtained *in silico* at the location of the bla_{ADC} gene using the MapSolver
449 software. The location of bla_{ADC} and relevant *Nco*I sites are shown. The numbers indicate
450 the coordinates of *Nco*I sites or the location of specified genes in the GenBank entries for
451 each strain. b. CLUSTALW comparison of amino acid sequences.



a

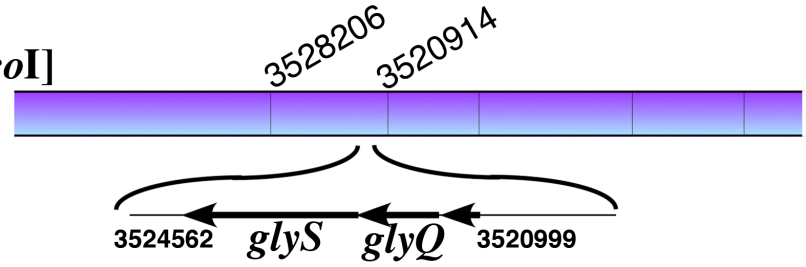


b

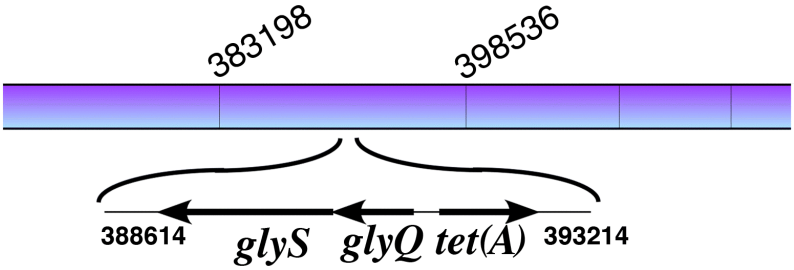
```
A118         MDTLPLINTPVRHWCEFEFISKTVKNPNIHIKGNYSYYSAYWDQGFERCVVRYLHDKPST
AB0057       MDT-PLINTPVRHWCEFEFISKTVKNPNIHIKGNYSYYSAYWDQGFERCVVRYLHDKPST
AYE          MDT-PLINTPVRHWCEFEFISKTVKNPNIHIKGNYSYYSAYWDQGFERCVVRYLHDKPST
ATCC17978    MAE-KLIGSPVRHWCEFEFISKTVKNPNIHIKGNYSYYSAYWDQGFERCVVRYLHDKPST
             *    **.:**********************************************************

A118        PDKPIDQLYIGNFVCFGAECVIMMGGNQLHRPDWISTFPFDTRSFLAAGDTIIADGCWIG
AB0057      PDKPIDQLYIGNFVCFGAECVIMMGGNQLHRPDWISTFPFDTRSFLAAGDTIIADGCWIG
AYE         PDKPIDQLYIGNFVCFGAECVIMMGGNQLHRPDWISTFPFDTRSFLAAGDTIIADGCWIG
ATCC17978   PDKPIDQLYIGNFVCFGAECVIMMGGNQLHRPDWISTFPFDTRSFLAAGDTIIADGCWIG
            ************************************************************

A118        SRAMIMQGVRIGEGAVVATGAVVTKDVPPYTIVGGVPAKIIKYRFPQEQIEKLLALKIYDLDEKQ
AB0057      SRAMIMQGVKIGEGAVVATGAVVTKDVPPYTIVGGVPAKIIKYRFPQEQIEKLLALKIYDLDEKQ
AYE         SRAMIMQGVKIGEGAVVATGAVVTKDVPPYTIVGGVPAKIIKYRFPQEQIEKLLALKIYDLDEKQ
ATCC17978   SRAMIMQGVKIGEGAVVATGAVVTKDVPPYAIVGGVPAKIIKYRFPQEQIEKLLALKIYDLDEKK
            *********:********************:*********************************:

95.14% identity
```

***A. baumannii* A118 [*Nco*I]**

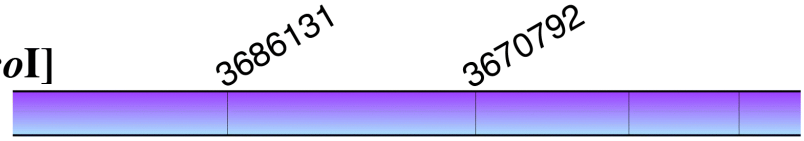
***A. baumannii* ACICU [*Nco*I]**



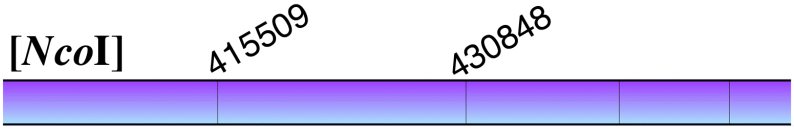
***A. baumannii* AYE [*Nco*I]**



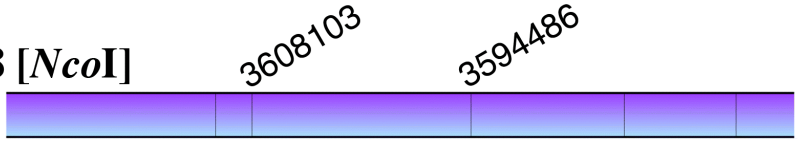
***A. baumannii* AB0057 [*Nco*I]**



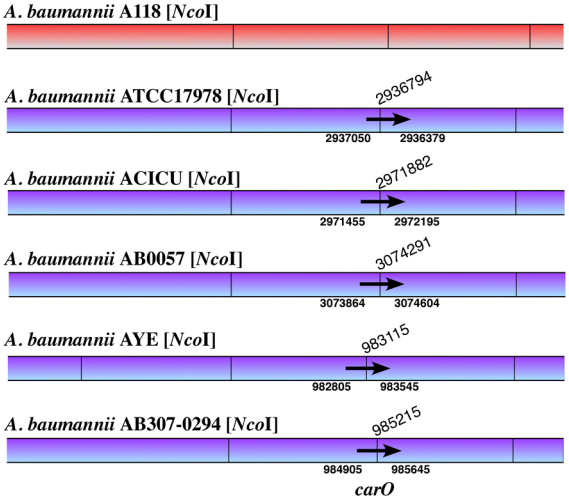
***A. baumannii* AB307-0294 [*Nco*I]**



***A. baumannii* ATCC17978 [*Nco*I]**

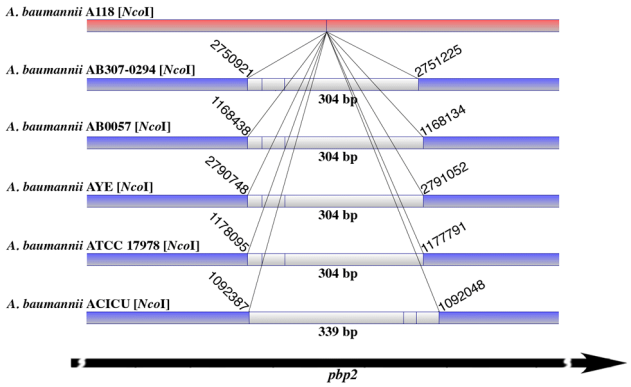


a

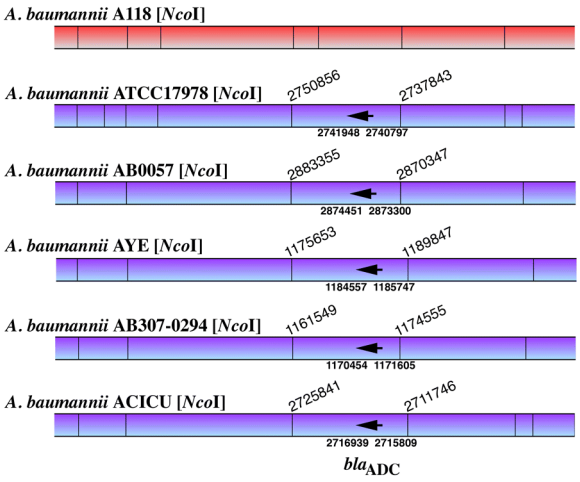


b

```
                    10        20        30        40        50        60
                     |         |         |         |         |         |
A118                MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
ATCC17978           MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
AB0057              MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
AB307-0294          MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
AYE                 MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
ACICU               MKVLRVLVTTTALLAAGAAMADEAVVHDSYAFDKNQLIPVGARAEVGTTGYGGALLWQAN
                    ************************************************************

                            70        80        90       100       110       120
                             |         |         |         |         |         |
A118                PYVGLALGYNGGDISWRDDLSINGTKYDVDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
ATCC17978           PYVGLALGYNGGDISWRDDLSINGTKYDVDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
AB0057              PYVGLALGYNGGDISWSDDVKVNGSTYDLDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
AB307-0294          PYVGLALGYNGGDISWSDDVKVNGSTYDLDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
AYE                 PYVGLALGYNGGDISWSDDVKVNGSTYDLDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
ACICU               PYVGLALGYNGGDISWSDDVKVNGSTYDLDMDNNNVYLNAEIRPWGASTNRWAQGLYVAA
                    *************** **:.:**:.**:*********************************

                           130       140       150       160       170       180
                             |         |         |         |         |         |
A118                GAAYLDNDYDLTK-RSQDGTIKVNGNNYNFNGS---VDGKLSYKNDIAPYLGFGFAPKIN
ATCC17978           GAAYLDNDYDLTK-RSSDGTIKINGTNYSYNGS---VNGQLSYKNDIAPYLGFGFAPKIN
AB0057              GAAYLDNDYDLTRNVDATRSFRVNNQDFIAGADGVKINGQMSYKNDIAPYLGFGFAPKIN
AB307-0294          GAAYLDNDYDLTRNVDATRSFRVNNQDFIAGADGVKINGQMSYKNDIAPYLGFGFAPKIN
AYE                 GAAYLDNDYDLTRNVDATRSFRVNNQDFIAGADGVKINGQMSYKNDIAPYLGFGFAPKIN
ACICU               GAAYLDNDYDLTRNVDATRSFRVNNQDFIAGADGVKINGQMSYKNDIAPYLGFGFAPKIN
                    ************:  .   ::::*. ::  ...   ::*::*********************

                           190       200       210       220       230       240
                             |         |         |         |         |         |
A118                KNWGVFGEVGAYYTGNPTVNLKSNGTFVNVNGADFDKDLRAEENKIRNDDKYQWLPVGKVGVNFFW
ATCC17978           KNWGVFGEVGAYYTGNPTVELDKQGTFVNAAGGNADADLRAEENKIRNDDKYKWFPVGKVGVNFYW
AB0057              KNWGVFGEVGAYYTGNPTVKLVSSGSAVTTGDQTLEEAVNAEARKIANDDKYKWLPVGKVGVNFFW
AB307-0294          KNWGVFGEVGAYYTGNPTVKLVSSGSAVTTGDQTLEEAVNAEARKIANDDKYKWLPVGKVGVNFFW
AYE                 KNWGVFGEVGAYYTGNPTVKLVSSGSAVTTGDQTLEEAVNAEARKIANDDKYKWLPVGKVGVNFFW
ACICU               KNWGVFGEVGAYYTGNPTVKLVSSGSAVTTGDQSLEEAVNAEARKIANDDKYKWLPVGKVGVNFFW
                    ********************:* ..*: *.. .   :  :.** .** *****:*:*********:*
```



a



b

```
                    10        20        30        40        50        60        70        80        90
                    |         |         |         |         |         |         |         |         |
A118                MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNSSTIFELGSVS
ATCC17978           MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNSSTIFELGSVS
AB0057              MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNRSTIFELGSVS
AYE                 MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNRSTIFELGSVS
AB307-0294          MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNRSTIFELGSVS
ACICU               -------MSTFIPAFYFSTSIYAGNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNRSTIFELGSVS
                           : * * ************************************************************** **********

                   100       110       120       130       140       150       160       170       180
                    |         |         |         |         |         |         |         |         |
A118                KLFTATAGGYAKNKGKISFDDKPGKYWKELKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWKPKNPIGEYRQYSNPSIG
ATCC17978           KLFTATAGGYAKNKGKISFDDTPGKYWKVLKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWKPKNPIGEYRQYSNPSIG
AB0057              KLFTATAGGYAKNKGKISFDDTPGKYWKELKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWQPKNPIGEYRQYSNPSIG
AYE                 KLFTATAGGYAKNKGKISFDDTPGKYWKELKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWQPKNPIGEYRQYSNPSIG
AB307-0294          KLFTATAGGYAKNKGKISFDDTPGKYWKELKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWQPKNPIGEYRQYSNPSIG
ACICU               KLFTATAGGYAKNKGKISFDDTPGKYWKELKNTPIDQVNLLQLATYTSGNLALQFPDEVQTDQQVLTFFKDWQPKNPIGEYRQYSNPSIG
                    ********************.****** ****************************************:*****************

                   190       200       210       220       230       240       250       260       270
                    |         |         |         |         |         |         |         |         |
A118                LFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIYANLNPQKYPA
ATCC17978           LFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKYPA
AB0057              LFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKYPA
AYE                 LFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKYPA
AB307-0294          LFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKYPA
ACICU               LFRKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKYPA
                    ** ************************************************************************:**********

                   280       290       300       310       320       330       340       350       360
                    |         |         |         |         |         |         |         |         |
A118                DIQRAINETHQGRYQVNTMYQALGWEEFAYPATLQTLLDSNSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
ATCC17978           DIQRAINETHQGFYQVNTMYQALGWEEFSYPATLQTLLDSNSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
AB0057              DIQRAINETHQGFYQVNTMYQALGWEEFSYPATLQTLLDSNSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
AYE                 DIQRAINETHQGFYQVNTMYQALGWEEFSYPATLQTLLDSNSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
AB307-0294          DIQRAINETHQGFYQVNTMYQALGWEEFSYPATLQTLLDSNSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
ACICU               DIQRAINETHQGFYQVNTMYQALGWEEFSYPATLQTLLDSSSEQIVMKPNKVTAISKEPSVKMYHKTGSTNGFGTYVVFIPKENIGLVML
                    *********** ***************:***********.************************************************

                   370       380
                    |         |
A118                TNKRIPNEERIKAAYAVLNAIKK
ATCC17978           TNKRIPNEERIKAAYAVLNAIKK
AB0057              TNKRIPNEERIKAAYAVLNAIKK
AYE                 TNKRIPNEERIKAAYAVLDAIKK
AB307-0294          TNKRIPNEERIKAAYAVLDAIKK
ACICU               TNKRIPNEERIKAAYAVLDAIKK
                    ******************:****
```