JCM
Journals.ASM.org

Single-Locus-Sequence-Based Typing of *bla*$_{\text{OXA-51-like}}$ Genes for Rapid Assignment of *Acinetobacter baumannii* Clinical Isolates to International Clonal Lineages

Spyros Pournaras,[a] Vasiliki Gogou,[b] Maria Giannouli,[c] Evangelia Dimitroulia,[a] Konstantina Dafopoulou,[d] Athanasios Tsakris,[a] Raffaele Zarrilli[c]

Department of Microbiology, Medical School, University of Athens, Athens, Greece[a]; Department of Microbiology[b] and Department of Hygiene and Epidemiology,[d] Faculty of Medicine, University of Thessaly, Larissa, Greece; Department of Public Health, University of Naples Federico II, Naples, Italy[c]

Single-locus *bla*$_{\text{OXA-51-like}}$ sequence-based typing (SBT) was evaluated for its ability to determine correctly sequence types (STs) in *Acinetobacter baumannii* clinical isolates, in comparison with the Pasteur's multilocus sequence typing (MLST) reference method and 3-locus sequence typing (3-LST). The comparative study was performed in 585 multidrug-resistant (MDR) *A. baumannii* clinical isolates recovered from 21 hospitals located throughout Greece, Italy, Lebanon, and Turkey. The isolates belonged to nine clonal complexes (CCs) that correspond to 12 distinct sequence types (STs) and to one singleton ST. These clonal lineages predominate worldwide among nosocomial MDR *A. baumannii* strains. The most common clone was CC2 (ST2 and ST45; *n* = 278 isolates) followed by CC1 (ST1 and ST20; *n* = 155), CC25 (*n* = 65), ST78 (*n* = 62), CC15 (ST15 and ST84; *n* = 9), CC10 (*n* = 4), CC3 (*n* = 4), CC6 (*n* = 3), CC54 (*n* = 3), and CC83 (*n* = 2). Using the *bla*$_{\text{OXA-51-like}}$ SBT method, all 585 isolates of the study were typed and assigned correctly to the nine CCs and the singleton ST78. The 3-LST method was not able to classify isolates belonging to CC6, CC10, CC54, and CC83, which are not yet characterized in its database. The low-cost and convenient *bla*$_{\text{OXA-51-like}}$ SBT method, compared with 3-LST and MLST, discriminated all epidemic and sporadic lineages of our collection and could be effectively applied to type rapidly *A. baumannii* strains.

Outbreaks due to multidrug-resistant (MDR) *Acinetobacter baumannii* clinical strains are increasingly documented worldwide (1). Currently, *A. baumannii* strains causing hospital outbreaks usually belong to a limited number of clonal lineages, among which those applying to the international clones I and II are prevailing (1–3). This development increasingly necessitates monitoring the epidemic evolution of *A. baumannii* strains by grouping them to specific clonal lineages. For this purpose, several genotyping methods exist, such as 3-locus sequence typing (3-LST) (3), ribotyping (4), infrequent-restriction-site analysis (5), repetitive sequence-based PCR (rep-PCR) (6), amplified fragment length polymorphism (7), electrospray ionization mass spectrometry (8), pulsed-field gel electrophoresis (PFGE) (9), and multilocus sequence typing (MLST) (10, 11).

Among the methods most commonly used for tracking the worldwide clonal dissemination of *A. baumannii*, as the reference, the method considered the "gold standard" is MLST, of which two schemes exist, PubMLST (11) and Pasteur MLST (10). Both schemes are based on sequences of seven housekeeping genes and are used for global but also for local epidemiological studies. Concerning MLST, it still remains to be answered whether several loci are required to obtain robust results and if the selection of housekeeping genes is sufficiently reliable to reveal population structures. For instance, the PubMLST *gyrB* and *gpi* genes present recombination, resulting in failure to type some isolates (12). Another method that has been widely applied and produced accurate results for the classification of worldwide clones is the 3-LST scheme (3). It involves amplification and sequencing of the *ompA*, *csuE*, and *bla*$_{\text{OXA-51-like}}$ genes that are under selective pressure and assigns *A. baumannii* strains to seven different sequence groups (SGs), SG1 to SG7, corresponding to international clonal lineages identified by other methods (2, 3, 13, 14). Based on 3-LST, two multiplex PCRs were also designed to selectively amplify the *ompA*, *csuE*, and *bla*$_{\text{OXA-51-like}}$ alleles that correspond to SG1, SG2, SG3, and SG6 (3, 14) without being followed by sequencing, thus serving as a convenient preliminary method to study local epidemiology. The increasing need for molecular surveillance of multidrug-resistant *A. baumannii* in the current era of financial restrictions prompts investigation for reliable typing approaches requiring reduced time, labor, and costs.

One of the simplest approaches for the identification (15) and typing (12) of *A. baumannii* involves amplification and sequencing of the *bla*$_{\text{OXA-51-like}}$ gene, which is unique to *A. baumannii*, although the same gene has occasionally been detected in *Acinetobacter nosocomialis* and *Acinetobacter* genomic species "close to 13TU" (16). The potential of the identification of the specific *bla*$_{\text{OXA-51-like}}$ gene to correctly assign *A. baumannii* isolates to international clones as single-locus-based typing (SBT) has been scarcely evaluated previously on international collections, in comparison only with PubMLST (12) and rep-PCR (17). In the present study, the performance of this simple, low-cost, and rapid SBT method was evaluated in a large international collection of *A.*

Received 24 December 2013 **Returned for modification** 28 January 2014
Accepted 27 February 2014
Published ahead of print 12 March 2014
Editor: A. B. Onderdonk
Address correspondence to Spyros Pournaras, spournaras@med.uoa.gr.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.03565-13.

doi:10.1128/JCM.03565-13

TABLE 1 Characteristics of the 585 study isolates[a]

Index strain[b]	Country	$bla_{OXA-51-like}$ allele (no. of isolates)	Pasteur's MLST								3-LST group
			CC	*cpn60*	*fusA*	*gltA*	*pyrG*	*recA*	*rpiB*	*rpoB*	ST (no. of isolates)	
AB3990	Italy	bla_{OXA-66} (278)	CC2	2	2	2	2	2	2	2	ST2 (265)	1
AB3	Greece			2	2	6	2	2	2	2	ST45 (13)	
AB700	Italy	bla_{OXA-69} (155)	CC1	1	1	1	1	5	1	1	ST1 (124)	2
AB2979	Italy			3	1	1	1	5	1	1	ST20 (31)	
AB3237	Lebanon	bla_{OXA-71} (4)	CC3	3	3	2	2	3	1	3	ST3 (4)	3
AB3890	Greece	bla_{OXA-64} (65)	CC25	3	3	2	4	7	2	4	ST25 (65)	4
AB3909	Italy	bla_{OXA-90} (62)	Singleton	25	3	6	2	28	1	29	ST78 (62)	6
AB6	Greece	bla_{OXA-94} (3)	CC6	5	2	4	1	3	3	4	ST85 (3)	NA
AB17	Greece	$bla_{OXA-365}$[c] (3)	CC54	12	3	18	2	17	4	5	ST54 (3)	NA
AB3868	Turkey	bla_{OXA-51} (9)	CC15	6	6	8	2	3	5	4	ST15 (6)	5
AB3871	Turkey			6	6	8	2	3	5	30	ST84 (3)	
AB2977	Italy	$bla_{OXA-128}$ (4)	CC10	28	3	2	1	4	4	4	ST82 (4)	NA
AB3866	Turkey	bla_{OXA-86} (2)	CC83	26	4	2	2	9	1	4	ST83 (2)	NA
OIFC032	Germany	$bla_{OXA-100}$[d]	CC32	1	1	2	2	3	4	4	ST32	NA
AB_TG27343	USA	bla_{OXA-65}[e]	CC79	26	2	2	2	29	4	5	ST79	NA

[a] Abbreviations: CC, clonal complex; ST, sequence type; 3-LST, trilocus sequence-based typing; NA, not available; USA, United States.
[b] Index strains for dendrogram analyses.
[c] GenBank accession number KF885217.
[d] GenBank accession number AM231720.
[e] GenBank accession numbers AY750908 and JQ412185 (23).

baumannii strains in parallel with the widely used Institute Pasteur's MLST and 3-LST schemes.

MATERIALS AND METHODS

Bacterial collection. A collection that included 585 *A. baumannii* clinical isolates recovered between 2000 and 2010 from four different countries (Italy, Greece, Turkey, and Lebanon), representing most common worldwide lineages, was tested. The isolates included in this collection were recovered during outbreaks that occurred in 21 unrelated hospitals in the four countries. Representative isolates of this collection were molecularly characterized previously (13, 14, 18, 19). Although not all of the study isolates were selected because of a multidrug resistance (MDR) phenotype, almost all of them actually exhibited MDR and >80% were carbapenem resistant.

Identification of isolates as *A. baumannii* was initially performed by PCR and sequencing of the $bla_{OXA-51-like}$ gene (15) and was confirmed by subsequent assignment of isolates by MLST to clonal lineages specific for *A. baumannii*.

SBT, 3-LST, and MLST. All isolates were tested using the 3-LST protocol developed by the United Kingdom Health Protection Agency (HPA) (3), involving initially two multiplex PCRs identifying only SG1, SG2, SG3, and SG6 and, subsequently, sequencing, confirming the grouping and discriminating SG1, SG2, SG3, SG4, SG5, SG6, and SG7 (available at the Health Protection Agency website [http://www.hpa-bioinformatics.org.uk/AB/]). The sequencing of solely the $bla_{OXA51-like}$ gene using primers encompassing the total gene (forward primer, 5′-ATGAACATTAAAGCACTCTTAC-3′; reverse primer, 5′-CTATAAAATACCTAATTGTTCT-3′ [825-bp amplicon]) was applied as a separate approach for typing the isolates. These primers include the start and stop codons, and a few OXA-51-like variants that differ from the common alleles at the 5′ and 3′ proximities could be misidentified. Nevertheless, the primers were able to amplify $bla_{OXA-51-like}$ alleles of all 585 strains included in our collection. Furthermore, all isolates were tested by the MLST scheme developed by the Pasteur Institute (10), which served as the gold standard method against which the other approaches were evaluated. This scheme involves PCR amplification and sequencing of seven housekeeping genes (*fusA*, *gltA*, *pyrG*, *recA*, *cpn60*, *rpoB*, and *rplB*).

E burst. To determine the clonal complexes (CCs) where isolates belonged, e-BURST analysis was performed using the eBURST software program (http://eburst.mlst.net/).

Phylogeny trees. Neighbor-joining phylogenetic trees based on $bla_{OXA-51-like}$ genes and concatenated alleles of the seven housekeeping genes of Pasteur's MLST scheme were constructed using the phylogeny.fr platform (20) available at http://www.phylogeny.fr/.

RESULTS

STs of the studied isolates using the Pasteur's MLST scheme. Table 1 represents the origin, the typing results, and the classification of the isolates using the three different typing schemes. Overall, by using the reference Pasteur's MLST scheme, 13 STs (ST1, ST2, ST3, ST15, ST20, ST25, ST45, ST54, ST78, ST82, ST83, ST84, and ST85) were identified among the 585 *A. baumannii* isolates of the study.

SBT results. In comparison with the MLST, SBT correctly identified isolates belonging to all major lineages (Fig. 1). In particular, the two major international clonal lineages corresponding to CC1 and CC2 each carried a single $bla_{OXA-51-like}$ variant. All isolates carrying identical bla_{OXA-69} genes belonged to CC1, which included the founder ST of CC1 (ST1) and its single-locus variant (SLV) ST20. CC2 isolates, consisting in our collection of ST2 and the SLV ST45, in all cases carried identical bla_{OXA-66} genes. Isolates belonging to CC3, CC6, CC10, CC15 (ST15 and ST84), CC25, CC54, ST78, and CC83 all carried a unique $bla_{OXA-51-like}$ variant (Table 1). Finally, isolates of CC32 and CC79, although not available in the present collection, could be effectively typed by SBT, as was shown using data obtained from the GenBank; CC32 isolates carry $bla_{OXA-100}$ (GenBank accession number AM231720), and CC79 isolates carry bla_{OXA-65} (accession numbers AY750908 and JQ412185) (Table 1). These data clearly showed that CC1 and CC2 predominated in this international collection.

The relatively limited number of OXA-51-like alleles that were identified among the study isolates prompted a GenBank search of all publicly available whole-genome sequences of *A. baumannii*. The results of this search are presented in the Table S1 in the supplemental material. In particular, of the 104 strains available that belonged to ST2, 98 harbored OXA-66 or the single-amino-acid variant OXA-82; the OXA-66 allele had identical nucleotide sequences in all strains, and OXA-82 had identical sequences in all

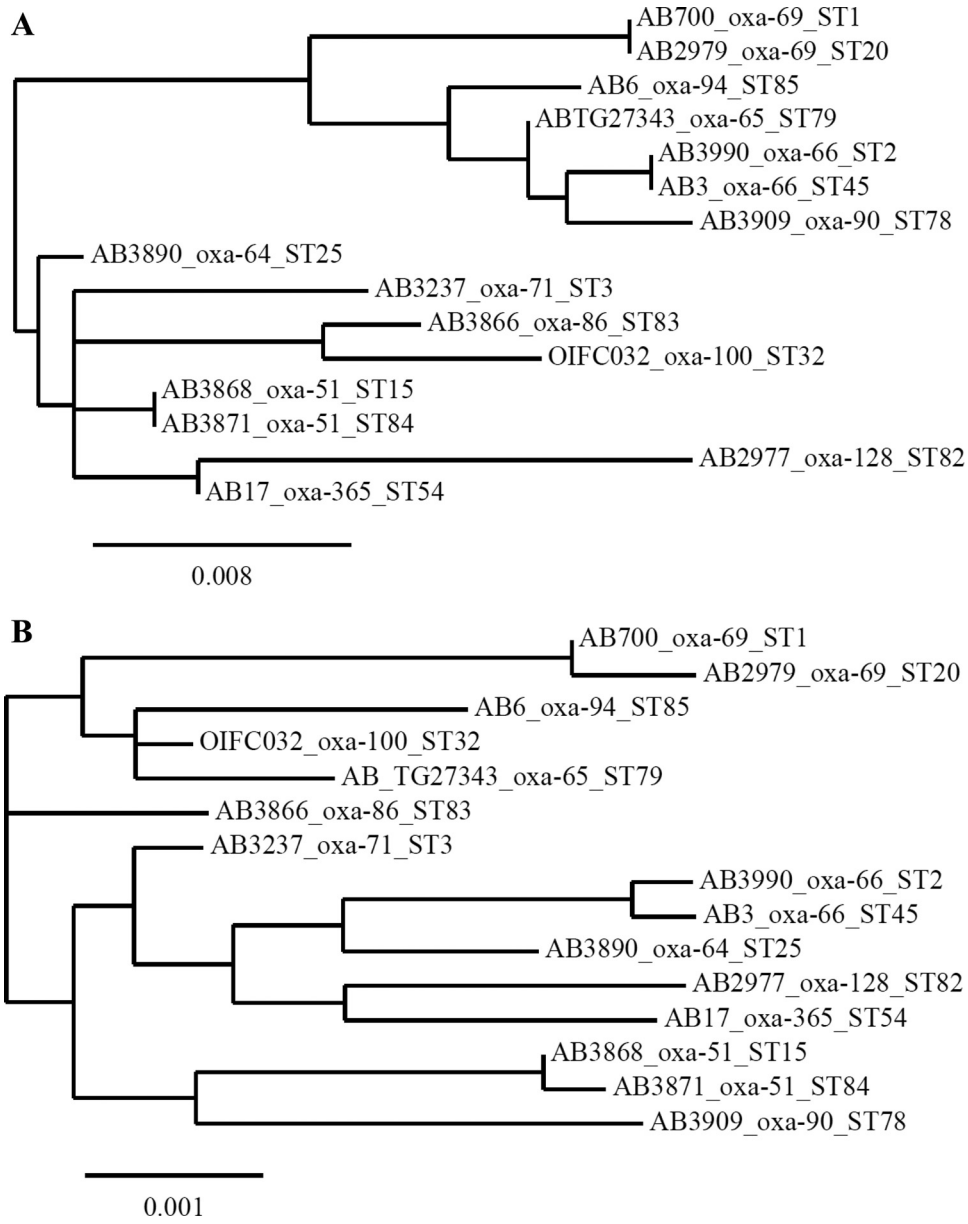

FIG 1 Neighbor-joining phylogenetic trees based on $bla_{OXA-51like}$ genes (A) and concatenated alleles of the seven housekeeping genes of Pasteur's MLST scheme (B). The dendrograms show the amounts of genetic change based on multiple alignments and were generated using phylogeny.fr software (17). The bar at the bottom of each figure shows the amount of genetic change corresponding to the length of each branch.

but 2 strains that harbored alleles differing by 1 nucleotide (nt). Also, 2 strains and 1 strain assigned to ST2 harbored the single-amino-acid variants OXA-109 and OXA-254, respectively, and 3 strains harbored OXA-113 alleles that differed from OXA-66 in six amino acid residues, thus suggesting that limited variability of the $bla_{OXA-51-like}$ gene existed in international clonal linage 2. Despite this variability, SBT was able to correctly type and assign to international clonal lineage 2 all ST2 strains available in GenBank. Furthermore, all 28 ST1 strains harbored identical OXA-69 alleles, 6/8 ST3 strains harbored OXA-71 and 2/8 harbored an allele (OXA-312) with 1-nt difference, 1/2 CC10 strains harbored OXA-68 and the other harbored an allele (OXA-128) with 1-nt difference, 7/7 CC25 strains harbored identical OXA-64 alleles, 3/3 CC32 strains harbored OXA-100, and 3/3 CC79 strains harbored identical OXA-65 alleles.

3-LST results. The 3-LST scheme, although overall it provided data relative to three genes under selective pressure, failed to classify isolates belonging to CC10, CC54, CC83, ST85, CC32, and CC79, as alleles corresponding to these lineages are not yet assigned in its database. Twelve isolates, identified as belonging to ST54, ST82, ST83, and ST85, are considered to belong to micro-epidemic lineages and are not assigned to any SG. As for the simple and rapid 3-LST multiplex PCR, it discriminated only SG1 (CC2), SG2 (CC1), SG3 (CC3), and SG6 (ST78).

DISCUSSION

Several methods have been applied during the last 2 decades to identify the clonal lineages of *A. baumannii* isolates. Among them, the most common ones have been 3-LST (3) and MLST (10, 11). These methods are appropriately discriminatory but can be considered either time-consuming and labor-intensive or costly. Amplification and sequencing of the $bla_{\text{OXA-51-like}}$ gene have been used successfully for *A. baumannii* identification and within the 3-LST scheme, respectively. It has also been observed during 3-LST analysis (3) that $bla_{\text{OXA-51-like}}$ variants are conserved within most SGs and often correlate well with the MLSTs, thus deserving investigation for the potential of $bla_{\text{OXA-51-like}}$ SBT to be utilized as an independent typing scheme. Until now, this possibility was tested in parallel only with the PubMLST scheme (12), against which the $bla_{\text{OXA-51-like}}$ SBT was shown to identify accurately isolates belonging to the three major epidemic lineages. In another study, the $bla_{\text{OXA-51-like}}$ SBT compared with rep-PCR identified international clonal lineages I to III and worldwide clonal lineages 4 to 8 (17). In the current report, the performance of $bla_{\text{OXA-51-like}}$ SBT was compared with that of the Pasteur's MLST scheme, which does not exhibit recombination in its target loci in contrast with PubMLST, and also with that of 3-LST, including both the initial multiplex PCR and the sequencing analysis. The total $bla_{\text{OXA-51-like}}$ gene was sequenced, as the cost is the same and the nucleotide yield is bigger, enabling the identification of novel $bla_{\text{OXA-51-like}}$ variants, such as the $bla_{\text{OXA-365}}$ allele corresponding to ST54. This comparison was applied to a large, diverse collection of 585 *A. baumannii* strains from several countries, including most common lineages. This collection was also tested by PFGE, but these results were not included in the current presentation because PFGE is not suitable for international population studies.

In the tested collection, $bla_{\text{OXA-51-like}}$ SBT identified successfully the common lineages CC1, CC2, CC3, CC10, CC15, and CC25. It was also shown in the publicly available databases that each of the remaining rather common clones CC32 and CC79 contains a specific $bla_{\text{OXA-51-like}}$ allele ($bla_{\text{OXA-100}}$ and $bla_{\text{OXA-65}}$, respectively) and can be efficiently identified by the $bla_{\text{OXA-51-like}}$ SBT. A single $bla_{\text{OXA-51-like}}$ allele was found to correspond to two different STs ($bla_{\text{OXA-69}}$ to ST1 and ST20 and $bla_{\text{OXA-66}}$ to ST2 and ST45) that, however, were included in the same CC, not affecting prompt identification of the respective international lineages. In all cases, the same $bla_{\text{OXA-51-like}}$ allele was detected in strains belonging to the same CC and no case was observed where different $bla_{\text{OXA-51-like}}$ alleles were detected in the same ST. In contrast, the application of $bla_{\text{OXA-51-like}}$ SBT in comparison with PubMLST (12) resulted in the detection of the same $bla_{\text{OXA-51-like}}$ allele(s) in unrelated STs ($bla_{\text{OXA-51}}$ in CC4 and ST20) and of distinct alleles in a single lineage ($bla_{\text{OXA-69}}$ and $bla_{\text{OXA-112}}$ in international clone I; $bla_{\text{OXA-82}}$ and $bla_{\text{OXA-83}}$ in ST22; bla_{OXA}-107 and bla_{OXA}-110 in ST49; and bla_{OXA}-51 and bla_{OXA}-68 in ST20). The correlation of the $bla_{\text{OXA-51-like}}$ gene with PubMLST was better when alleles exhibiting recombination (*gpi* and *gyrB*) were excluded from the analysis (12).

In this study, testing 585 multidrug-resistant clinical isolates, only 13 STs were identified, while at least 450 STs were found to be currently distinguishable by the Pasteur's MLST. However, it is well known that the high diversity of the general population of *A. baumannii* at the strain level especially involves susceptible strains (10). Also, although this large collection includes also epidemiologically related isolates, these isolates came from numerous unrelated hospitals in four different countries, indicating the circulation of limited clones in the hospital epidemiological niche and supporting the observed bottleneck effect for MDR *A. baumannii* (10). Furthermore, the present analysis, which detected identical alleles in multiple *A. baumannii* isolates from each lineage, underlines the intrastrain stability of typing characteristics using the $bla_{\text{OXA-51-like}}$ SBT method.

The reliability of the $bla_{\text{OXA-51-like}}$ SBT method is further validated by the analysis of *A. baumannii* whole-genome sequences (WGS) available in GenBank. Despite a slight variability of the $bla_{\text{OXA-51-like}}$ gene found in *A. baumannii* strains belonging to international clone 2 and also within the predominant international clones 1 and 3 (see Table S1 in the supplemental material), this SBT is able to correctly type and assign to the major international clonal lineages *A. baumannii* WGS available in GenBank. These data indicate that the vast majority of the predominant lineages of *A. baumannii* could be effectively identified by this SBT and clearly support our view of the clinical applicability of $bla_{\text{OXA-51-like}}$ SBT as a simple first-line typing approach.

As for 3-LST, although this scheme analyzes three loci and provides more comprehensive data from an evolutionary point of view than the $bla_{\text{OXA-51-like}}$ SBT scheme, it has not yet been applied in collections large enough to identify all common lineages. Its database currently includes only SG2 (CC1 according to Pasteur's scheme), SG1 (CC2), SG3 (CC3), SG4 (CC25), SG5 (CC15), SG6 (ST78), and SG7, which were almost all identified in our collection. In that respect, $bla_{\text{OXA-51-like}}$ SBT identified more STs defined by MLST than 3-LST. However, it is evident that a future application of 3-LST in larger collections representing all lineages might further discriminate strains. We should note here for 3-LST that two unrelated strains were previously shown to carry the $bla_{\text{OXA-51-like}}$ gene that corresponds to CC1 but also a *csuE* allele that was characteristic of CC2 (21). However, both of these strains actually belonged to CC1 as shown by MLST and it was concluded that they had acquired the *csuE* gene from other *A. baumannii* strains. In fact, one of these strains, A388, carried OXA-92 (12), which differs from OXA-69 by only one nucleotide (22), indicating that it would be correctly grouped by OXA-51-like SBT as CC1. It is thus indicated that we should treat with caution results that include the *csuE* allele, such as those from 3-LST, and that OXA-51-like alleles are possibly more conserved and may correlate better with MLST than does correlate the 3-LST scheme. Finally, as could be anticipated, simple multiplex PCR of the 3-LST scheme, when applied as an independent method, identified fewer SGs than the other typing approaches tested.

Concluding remarks. Overall, $bla_{\text{OXA-51-like}}$ SBT, compared with Pasteur's MSLT and 3-LST, was shown to identify accurately isolates belonging to all major *A. baumannii* lineages that were available in our collection. It can be assumed that this SBT, being evidently easier, faster, and cheaper than MLST, could be applied efficiently for the provisional molecular typing of *A. baumannii*.

REFERENCES

1. **Peleg AY, Seifert H, Paterson DL.** 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. **21**:538–582. http://dx.doi.org/10.1128/CMR.00058-07.
2. **Zarrilli R, Pournaras S, Giannouli M, Tsakris A.** 2013. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. Int. J. Antimicrob. Agents **41**:11–19. http://dx.doi.org/10.1016/j.ijantimicag.2012.09.008.

3. **Turton JF, Gabriel SN, Valderrey S, Kaufmann ME, Pitt TL.** 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. Clin. Microbiol. Infect. **13:** 807–815. http://dx.doi.org/10.1111/j.1469-0691.2007.01759.x.
4. **Brisse S, Milatovic D, Fluit AC, Kusters K, Toelstra A, Verhoef J, Schmitz FJ.** 2000. Molecular surveillance of European quinolone-resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. using automated ribotyping. J. Clin. Microbiol. **38:**3636–3645.
5. **Yoo JH, Choi JH, Shin WS, Huh DH, Cho YK, Kim KM, Kim MY, Kang MW.** 1999. Application of infrequent-restriction-site PCR to clinical isolates of *Acinetobacter baumannii* and *Serratia marcescens*. J. Clin. Microbiol. **37:**3108–3112.
6. **Huys G, Cnockaert M, Vaneechoutte M, Woodford N, Nemec A, Dijkshoorn L, Swings J.** 2005. Distribution of tetracycline resistance genes in genotypically related and unrelated multiresistant *Acinetobacter baumannii* strains from different European hospitals. Res. Microbiol. **156:** 348–355. http://dx.doi.org/10.1016/j.resmic.2004.10.008.
7. **Spence RP, van der Reijden TJ, Dijkshoorn L, Towner KJ.** 2004. Comparison of *Acinetobacter baumannii* isolates from United Kingdom hospitals with predominant northern European genotypes by amplified-fragment length polymorphism analysis. J. Clin. Microbiol. **42:**832–834. http://dx.doi.org/10.1128/JCM.42.2.832-834.2004.
8. **Ecker JA, Massire C, Hall TA, Ranken R, Pennella TT, Agasino Ivy C, Blyn LB, Hofstadler SA, Endy TP, Scott PT, Lindler L, Hamilton T, Gaddy C, Snow K, Pe M, Fishbain J, Craft D, Deye G, Riddell S, Milstrey E, Petruccelli B, Brisse S, Harpin V, Schink A, Ecker DJ, Sampath R, Eshoo MW.** 2006. Identification of *Acinetobacter* species and genotyping of *Acinetobacter baumannii* by multilocus PCR and mass spectrometry. J. Clin. Microbiol. **44:**2921–2932. http://dx.doi.org/10.1128/JCM.00619-06.
9. **Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, Heersma H, Dijkshoorn LJ.** 2005. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. J. Clin. Microbiol. **43:**4328–4335. http://dx.doi.org/10.1128/JCM.43.9.4328-4335.2005.
10. **Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S.** 2010. The population structure of *Acinetobacter baumannii:* expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One **5:**e10034. http://dx.doi.org/10.1371/journal.pone.0010034.
11. **Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez-Valera F.** 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J. Clin. Microbiol. **43:**4382–4390. http://dx.doi.org/10.1128/JCM.43.9.4382-4390.2005.
12. **Hamouda A, Evans BA, Towner KJ, Amyes SG.** 2010. Characterization of epidemiologically unrelated *Acinetobacter baumannii* isolates from four continents by use of multilocus sequence typing, pulsed-field gel electrophoresis, and sequence-based typing of $bla_{OXA-51-like}$ genes. J. Clin. Microbiol. **48:**2476–2483. http://dx.doi.org/10.1128/JCM.02431-09.
13. **Giannouli M, Tomasone F, Agodi A, Vahaboglu H, Daoud Z, Triassi M, Tsakris A, Zarrilli R.** 2009. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* strains in intensive care units of multiple Mediterranean hospitals. J. Antimicrob. Chemother. **63:**828–830. http://dx.doi.org/10.1093/jac/dkp032.
14. **Giannouli M, Cuccurullo S, Crivaro V, Di Popolo A, Bernardo M, Tomasone F, Amato G, Brisse S, Triassi M, Utili R, Zarrilli R.** 2010. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a tertiary care hospital in Naples, Italy, shows the emergence of a novel epidemic clone. J. Clin. Microbiol. **48:**1223–1230. http://dx.doi.org/10.1128/JCM.02263-09.
15. **Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL.** 2006. Identification of *Acinetobacter baumannii* by detection of the $bla_{OXA-51-like}$ carbapenemase gene intrinsic to this species. J. Clin. Microbiol. **44:**2974–2976. http://dx.doi.org/10.1128/JCM.01021-06.
16. **Lee YT, Kuo SC, Chiang MC, Yang SP, Chen CP, Chen TL, Fung CP.** 2012. Emergence of carbapenem-resistant non-baumannii species of *Acinetobacter* harboring a $bla_{OXA-51-like}$ gene that is intrinsic to *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **56:**1124–1127. http://dx.doi.org/10.1128/AAC.00622-11.
17. **Zander E, Nemec A, Seifert H, Higgins PG.** 2012. Association between β-lactamase-encoding $bla_{(OXA-51)}$ variants and DiversiLab rep-PCR-based typing of *Acinetobacter baumannii* isolates. J. Clin. Microbiol. **50:** 1900–1904. http://dx.doi.org/10.1128/JCM.06462-11.
18. **Di Popolo A, Giannouli M, Triassi M, Brisse S, Zarrilli R.** 2011. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries with a multilocus sequence typing scheme. Clin. Microbiol. Infect. **17:**197–201. http://dx.doi.org/10.1111/j.1469-0691.2010.03254.x.
19. **Gogou V, Pournaras S, Giannouli M, Voulgari E, Piperaki E-T, Zarrilli R, Tsakris A.** 2011. Evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages: a 10 years study in Greece (2000–2009). J. Antimicrob. Chemother. **66:**2767–2772. http://dx.doi.org/10.1093/jac/dkr390.
20. **Post V, Hamidian M, Hall RM.** 2012. Antibiotic-resistant Acinetobacter baumannii variants belonging to global clone 1. J. Antimicrob. Chemother. **67:**1039–1040. http://dx.doi.org/10.1093/jac/dkr586.
21. **Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O.** 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. **36**(Web Server Issue)**:**W465–W469. http://dx.doi.org/10.1093/nar/gkn180.
22. **Tsakris A, Ikonomidis A, Spanakis N, Pournaras S, Bethimouti K.** 2007. Identification of a novel $bla_{(OXA-51)}$ variant, $bla_{(OXA-92)}$, from a clinical isolate of *Acinetobacter baumannii*. Clin. Microbiol. Infect. **13:**348–349. http://dx.doi.org/10.1111/j.1469-0691.2006.01598.x.
23. **Sahl JW, Gillece JD, Schupp JM, Waddell VG, Driebe EM, Engelthaler DM, Keim P.** 2013. Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in Acinetobacter. PLoS One **8:**e54287. http://dx.doi.org/10.1371/journal.pone.0054287.