Table S1. Antimicrobial agents tested and interpretative breakpoints

| Antimicrobial agent                  | Concentration range tested (µg/mL) | Enterobacterales | Acinetobacter baumannii complex | Pseudomonas aeruginosa |
|--------------------------------------|------------------------------------|-------------------|--------------------------------|------------------------|
| Amikacin                             | 8-32                               | ≤16/32/>32        | ≤16/32/>32                     | ≤16/32/>32             |
| Amoxicillin-clavulanate\(^2\)        | 8-16                               | ≤8/16/>16         | -                              | -                      |
| Ampicillin                           | 2-16                               | ≤8/16/>16         | -                              | -                      |
| Ampicillin-sulbactam\(^2\)           | 4-16                               | ≤8/16/>16         | ≤8/16/>16                      | -                      |
| Aztreonam                            | 4-16                               | ≤4/8/>8           | ≤8/16/>16                      | ≤8/16/>16             |
| Cefazolin                            | 2-16                               | ≤2/4/>4           | -                              | ≤8/16/>16             |
| Cefepime                             | 2-16                               | ≤2/4-8\(^4\)/>8  | ≤8/16/>16                      | ≤8/16/>16             |
| Cefotaxime                           | 2-32                               | ≤2\(^5\)/-/>4    | -                              | -                      |
| Cefoxitin                            | 4-16                               | ≤8/16/>16         | -                              | -                      |
| Ceftazidime                          | 1-16                               | ≤4/8/>8           | ≤8/16/>16                      | ≤8/16/>16             |
| Ceftriaxone                          | 1-32                               | ≤1/2/>2           | -                              | -                      |
| Cefuroxime                           | 4-16                               | ≤8/16/>16         | -                              | -                      |
| Ciprofloxacin                        | 0.5-2                              | ≤0.5\(^6\)/1/>1  | ≤1/2/>2                        | ≤0.5/1/>1             |
| Ertapenem                            | 0.5-4                              | ≤0.5/1/>1         | -                              | -                      |
| Gentamicin                           | 1-8                                | ≤4/8/>8           | ≤4/8/>8                        | ≤4/8/>8               |
| Imipenem                             | 1-8                                | ≤1/2/>2           | ≤2/4/>4                        | ≤2/4/>4               |
| Levofloxacin                         | 1-4                                | ≤1\(^7\)/-/>1    | ≤1/2/>2                        | ≤2/4/>4               |
| Meropenem                            | 1-8                                | ≤1/2/>2           | ≤2/4/>4                        | ≤2/4/>4               |
| Moxifloxacin                         | 2-4                                | ≤2/4/>4           | -                              | -                      |
| Piperacillin                          | 16-64                              | ≤16/32-64/>64     | ≤16/32-64/>64                  | ≤16/32-64/>64         |
| Piperacillin-tazobactam\(^6\)        | 8-64                               | ≤16/32-64/>64     | ≤16/32-64/>64                  | ≤16/32-64/>64         |
| Tetracycline                         | 2-8                                | ≤4/8/>8           | ≤4/8/>8                        | -                      |
| Tigecycline                          | 1-4                                | ≤2/4/>4           | ≤2/4/>4                        | -                      |
| Tobramycin                           | 2-8                                | ≤4/8/>8           | ≤4/8/>8                        | ≤4/8/>8               |
| Trimethoprim-sulfamethoxazole\(^7\)  | 0.5-2                              | ≤2/-/>2           | ≤2/-/>2                        | -                      |

\(^1\) S, susceptible; I, intermediate; R, resistant
\(^2\) Tested at 2:1 ratios
\(^3\) -, not applicable
\(^4\) Susceptible, dose-dependent
\(^5\) Susceptible range adjusted based on lowest concentration present in panel, with no intermediate value, as susceptible CLSI breakpoints are now one doubling dilution lower
\(^6\) Tested with constant tazobactam concentration of 4 µg/mL
\(^7\) Trimethoprim component of 1:19 ratio of trimethoprim to sulfamethoxazole
Table S2. Categorical susceptibility of testing by reference and direct AST, with differences between the two methods.

| Antimicrobial agent                  | n   | S (%) | I (%) | R (%) | AST category by reference method (%) | AST category by direct method (%) | Difference (%) |
|-------------------------------------|-----|-------|-------|-------|--------------------------------------|----------------------------------|----------------|
| Amikacin                            | 86  | 83.7  | 5.8   | 10.5  | 74.4                                | 8.1                              | 17.4           |
| Amoxicillin-clavulanate             | 72  | 50    | 11.1  | 38.9  | 47.2                                | 5.6                              | 47.2           |
| Ampicillin                          | 72  | 22.2  | 4.2   | 73.6  | 22.2                                | 2.8                              | 75.0           |
| Ampicillin-sulbactam                | 82  | 43.9  | 12.2  | 43.9  | 41.5                                | 9.8                              | 48.8           |
| Aztreonam                           | 76  | 48.7  | 1.3   | 50    | 46.1                                | 1.3                              | 52.6           |
| Cefazolin                           | 72  | 38.9  | 2.8   | 58.3  | 33.3                                | 4.2                              | 62.5           |
| Cefepime                            | 86  | 57    | 7.0   | 36    | 48.2                                | 1.2                              | 50.6           |
| Cefotaxime                          | 72  | 48.6  | 0     | 51.4  | 47.2                                | 0                               | 52.8           |
| Cefoxitin                           | 72  | 55.6  | 16.7  | 27.8  | 55.6                                | 9.7                              | 34.7           |
| Ceftazidime                         | 86  | 53.5  | 4.7   | 41.9  | 49.4                                | 7.1                              | 43.5           |
| Ceftriaxone                         | 72  | 45.8  | 0     | 54.2  | 45.8                                | 1.4                              | 52.8           |
| Cefuroxime                          | 72  | 40.3  | 4.2   | 55.6  | 44.8                                | 2.8                              | 52.8           |
| Ciprofloxacin                       | 86  | 44.2  | 1.2   | 54.7  | 44.2                                | 1.2                              | 54.7           |
| Ertapenem                           | 72  | 69.4  | 4.2   | 26.4  | 65.3                                | 4.2                              | 30.6           |
| Gentamicin                          | 86  | 67.4  | 2.3   | 30.2  | 66.3                                | 3.5                              | 30.2           |
| Imipenem                            | 86  | 70.9  | 7.0   | 22.1  | 66.3                                | 1.2                              | 32.6           |
| Levofoxacin                         | 86  | 46.5  | 1.2   | 52.3  | 46.5                                | 1.2                              | 52.3           |
| Meropenem                           | 86  | 73.3  | 3.5   | 23.3  | 68.6                                | 1.2                              | 30.2           |
| Moxifloxacin                        | 72  | 45.8  | 0.0   | 54.2  | 45.8                                | 0                               | 54.2           |
| Piperacillin                        | 86  | 34.9  | 4.7   | 60.5  | 27.9                                | 9.3                              | 62.8           |
| Piperacillin-tazobactam             | 86  | 64    | 8.1   | 27.9  | 58.1                                | 4.7                              | 37.2           |
| Tetracycline                        | 82  | 58.5  | 7.3   | 34.1  | 50.0                                | 13.4                             | 36.6           |
| Tigecycline                         | 69  | 92.8  | 5.8   | 1.5   | 89.9                                | 7.3                              | 2.9            |
| Tobramycin                          | 86  | 61.6  | 2.3   | 36    | 60.5                                | 4.7                              | 34.9           |
| Trimethoprim-sulfamethoxazole       | 82  | 50    | 0.0   | 50    | 46.3                                | 0                               | 53.7           |
| All agents                          | 1985| 55.0  | 4.7   | 40.4  | 52.7                                | 4.2                              | 44.0           |

S, susceptible; I, intermediate; R, resistant

1 Difference between reference and direct methods
Table S3. Categorical agreement of direct and reference AST for non-β-lactams

| Antimicrobial agent                  | Number of result pairs | CA    | mE  | ME  | VME |
|--------------------------------------|------------------------|-------|-----|-----|-----|
| Amikacin                             | 86                     | 76    | 6   | 4   | 0   |
| Ciprofloxacin                        | 86                     | 84    | 0   | 0   | 2   |
| Gentamicin                           | 86                     | 84    | 1   | 0   | 1   |
| Levofloxacin                         | 86                     | 86    | 0   | 0   | 0   |
| Moxifloxacin                         | 72                     | 72    | 0   | 0   | 0   |
| Tetracycline                         | 82                     | 73    | 9   | 0   | 0   |
| Tigecycline                          | 69                     | 66    | 3   | 0   | 0   |
| Tobramycin                           | 86                     | 84    | 2   | 0   | 0   |
| Trimethoprim-sulfamethoxazole        | 82                     | 79    | 0   | 3   | 0   |
| All non-β-lactam agents              | 735                    | 632   | 21  | 7   | 3   |
|                                      |                        | (95.8%) | (2.9%) | (1.0%) | (0.4%) |

CA, categorical agreement; mE, minor error; ME, major error; VME, very major error
Table S4. Categorical agreement of direct and reference AST for β-lactams with and without expert rules

| Antimicrobial agent                  | Number of result pairs | Categorical agreement without rules | Categorical agreement with rules | CA difference |
|--------------------------------------|------------------------|------------------------------------|---------------------------------|---------------|
|                                      |                        | CA  | mE  | ME  | VME | CA  | mE  | ME  | VME |               |
| Amoxicillin-clavulanate              | 72                     | 65  | 6   | 1   | 0   | 65  | 6   | 1   | 0   | 0             |
| Ampicillin                           | 72                     | 68  | 3   | 1   | 0   | 68  | 3   | 1   | 0   | 0             |
| Ampicillin-sulbactam                 | 82                     | 72  | 10  | 0   | 0   | 72  | 10  | 0   | 0   | 0             |
| Aztreonam                            | 76                     | 70  | 2   | 3   | 1   | 76  | 0   | 0   | 0   | 6 (7.9%)      |
| Cefazolin                            | 72                     | 67  | 3   | 2   | 0   | 68  | 3   | 1   | 0   | 1 (1.4%)      |
| Cefepime                             | 86                     | 73  | 7   | 6   | 0   | 84  | 2   | 0   | 0   | 11 (12.8%)    |
| Cefotaxime                           | 72                     | 69  | 0   | 2   | 1   | 72  | 0   | 0   | 0   | 3 (4.2%)      |
| Cefoxitin                            | 72                     | 59  | 11  | 1   | 1   | 61  | 9   | 1   | 1   | 2 (2.8%)      |
| Ceftazidime                          | 86                     | 78  | 4   | 3   | 1   | 86  | 0   | 0   | 0   | 8 (9.3%)      |
| Ceftriaxone                          | 72                     | 70  | 1   | 0   | 1   | 72  | 0   | 0   | 0   | 2 (2.8%)      |
| Cefuroxime                           | 72                     | 67  | 5   | 0   | 0   | 69  | 3   | 0   | 0   | 2 (2.8%)      |
| Ertapenem                            | 72                     | 66  | 6   | 0   | 0   | 69  | 3   | 0   | 0   | 3 (4.2%)      |
| Imipenem                             | 86                     | 76  | 7   | 3   | 0   | 85  | 1   | 0   | 0   | 11 (12.8%)    |
| Meropenem                            | 86                     | 80  | 2   | 4   | 0   | 86  | 0   | 0   | 0   | 6 (7.0%)      |
| Piperacillin                          | 86                     | 75  | 9   | 2   | 0   | 75  | 9   | 2   | 0   | 0             |
| Piperacillin-tazobactam              | 86                     | 74  | 9   | 3   | 0   | 74  | 9   | 3   | 0   | 0             |
| All β-lactam agents                  | 1250                   | 1129(90.3%) | 85 (6.8%) | 31 (2.5%) | 5 (0.4%) | 1182 (94.6%) | 58 (4.7%) | 9 (0.7%) | 1 (0.1%) | 53 (4.3%) |

CA, categorical agreement; mE, minor error; ME, major error; VME, very major error

1 $P<0.001$ vs without rules
Table S5. Phenotypic ESBL testing of Enterobacterales

| Resistance mechanism | ESBL detected with cefotaxime-clavulanate | ESBL detected with ceftazidime-clavulanate |
|----------------------|------------------------------------------|--------------------------------------------|
|                      | N | Reference | Direct | Reference | Direct |
| Carbapenemase        | 22 | 13 | 12 | 13 | 13 |
| ESBL                 | 15 | 15 | 15 | 14 | 15 |
| Other\(^1\)          | 35 | 0 | 0 | 0 | 0 |
| All                  | 72 | 25 | 27 | 27 | 28 |

\(^1\) Isolates without carbapenemases or ESBLs
Table S6. Essential agreement (EA) of AST by reference and direct methods

| Antimicrobial agent                | Number of result pairs | EA | EA%  |
|-----------------------------------|------------------------|----|------|
| Amikacin                          | 5                      | 5  | 100  |
| Amoxicillin-clavulanate           | 3                      | 3  | 100  |
| Ampicillin                        | 7                      | 7  | 100  |
| Ampicillin-sulbactam              | 11                     | 11 | 100  |
| Aztreonam                         | 4                      | 4  | 100  |
| Cefazolin                         | 3                      | 3  | 100  |
| Cefepime                          | 1                      | 1  | 100  |
| Cefotaxime                        | 1                      | 0  | 0    |
| Cefoxitin                         | 6                      | 6  | 100  |
| Ceftazidime                       | 21                     | 17 | 81.0 |
| Ceftriaxone                       | 1                      | 1  | 100  |
| Cefuroxime                        | 4                      | 4  | 100  |
| Ciprofloxacin                     | 3                      | 3  | 100  |
| Ertapenem                         | 0                      | 0  | -    |
| Gentamicin                        | 11                     | 11 | 100  |
| Imipenem                          | 0                      | 0  | -    |
| Levofloxacin                      | 3                      | 3  | 100  |
| Meropenem                         | 1                      | 1  | 100  |
| Piperacillin                      | 2                      | 2  | 100  |
| Piperacillin-tazobactam           | 4                      | 3  | 75.0 |
| Tetracycline                      | 10                     | 10 | 100  |
| Tigecycline                       | 6                      | 6  | 100  |
| Tobramycin                        | 3                      | 3  | 100  |
| Trimethoprim-sulfamethoxazole     | 1                      | 1  | 100  |
| **Total**                         | **111**                | **105** | **94.6** |