Supplementary Material

Figure S1. Distribution of meropenem and ceftazidime-avibactam MIC values using the panel of *P. aeruginosa* isolates collected by the 2017 SENTRY surveillance program (N=2910) and the representative panel used in this study (N=500).

Avibactam was tested at fixed 4 µg/ml.
Table S1. Correlation of *mexAB-oprM* expression with aztreonam/avibactam MIC

| Mpel Name | Aztreonam alone | Aztreonam w/avibactam at 4 µg/ml | Relative *mexB* expression* | Amino acid substitution(s) in: | MexR | NaIC | NaID |
|-----------|-----------------|----------------------------------|----------------------------|-------------------------------|------|------|------|
| PAM1020   | 4               | 4                                | 1.0                        |                               | Same as PAO1 | Same as PAO1 | Same as PAO1 |
| PA5323    | 8               | 4                                | 0.7                        | V126E                         | G71E E153Q S209R | Same as PAO1 |
| PA5317    | 4               | 4                                | 0.7                        | V126E                         | G71E S209R | Same as PAO1 |
| PA5297    | 4               | 4                                | 0.9                        |                               | same as PAO1 | G71E | Same as PAO1 |
| PA5300    | 4               | 4                                | 0.9                        |                               | same as PAO1 | G71E | Same as PAO1 |
| PAM3353   | 32              | 2                                | 0.9                        | V126E                         | G71E E153Q S209R | Same as PAO1 |
| PA5407    | 4               | 2                                | 1.0                        |                               | same as PAO1 | G71E S209R | Same as PAO1 |
| PAM3253   | 2               | 4                                | 1.4                        |                               | same as PAO1 | G71E S209R | Same as PAO1 |
| PAM3218   | 8               | 4                                | 1.2                        |                               | Same as PAO1 | G71E | Same as PAO1 |
| PAM3224   | 32              | 4                                | 1.3                        |                               | same as PAO1 | G71E S209R | Same as PAO1 |
| PAM3235   | 8               | 8                                | 1.5                        | V126E                         | G71E A145V S209R | Same as PAO1 |
| PAM3377   | 8               | 8                                | 0.8                        |                               | same as PAO1 | G71E S209R | Same as PAO1 |
| PAM3312   | 16              | 8                                | 1.3                        | R83C                          | G71E S209R | Same as PAO1 |
| PAM1032   | 16              | 16                               | 5.4                        | L75R                          | Same as PAO1 | Same as PAO1 |
| PAM3244   | 32              | 32                               | 2.2                        |                               | R59G V126E, V132A | G71E S209R | Same as PAO1 |
| PAM3231   | 16              | 16                               | 4.3                        |                               | S26N, D29Y, D34H, E109D, V115I, E145V | K58Q, G71E S209R | Same as PAO1 |
| PA5498    | 32              | 32                               | 4.8                        |                               | R91C V126E | G71E S209R | Same as PAO1 |
| PA5456    | 64              | 64                               | 3.1                        |                               | FS at aa#38 | G71E | Same as PAO1 |
| PA5397    | 64              | 64                               | 4.3                        |                               | FS at Q49 | G71E | Same as PAO1 |
| PA5369    | 64              | 64                               | 6.8                        |                               | FS at aa#17 | G71E A186T | Same as PAO1 |
| PA5426    | 64              | 64                               | 10.5                       |                               | FS at L123 | G71E S209R | Same as PAO1 |
| PA5459    | 64              | 64                               | 2.1                        |                               | E109stop | G71E S209R | Same as PAO1 |
| PA5352    | 65              | 64                               | 2.3                        |                               | same as PAO1 | G71E A145V S209R | Same as PAO1 |
| PA5338    | >64             | >64                              | 3.0                        |                               | V40stop | G71E S209R | Same as PAO1 |
| PA5465    | >64             | >64                              | 6.1                        |                               | L57P V126E | G71E A145V S209R | Same as PAO1 |

* Relative to the expression of PAM1020 (PAO1), which is assigned a value of 1. MexR, NaIC and NaID are the regulators of the *mexAB-oprM* operon expression. Amino acid substitutions (1-6), frame-shift (FS) mutations and premature terminations that are known to be associated with the increased expression of *mexAB-oprM* are in bold letters.
Table S2. In vitro potency of various beta-lactams combined with QPX7728 at 8 µg/ml against the MBL-negative strains from the challenge panel of *Pseudomonas aeruginosa* according to OprD and Efflux due to MexAB-OprM

|                  | MEM  | MEM+ QPX 8 µg/ml | ATM  | ATM + QPX 8 µg/ml | PIP  | PIP + QPX 8 µg/ml | TOL  | TOL+ QPX 8 µg/ml | FEP  | FEP+ QPX 8 µg/ml |
|------------------|------|-----------------|------|------------------|------|------------------|------|-----------------|------|-----------------|
| **No MBL (N=229)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 16   | 8               | 32   | 16               | >64  | 16               | 2    | 1               | 32   | 8               |
| MIC<sub>90</sub> | 64   | 32              | >64  | 32               | >64  | 64               | >64  | 4               | 64   | 16              |
| % Inhibited*     | 41.9%| 68.1%           | 17.9%| 46.7%            | 19.7%| 69.9%            | 64.2%| 92.1%           | 23.6%| 79.5%           |
| **OprD Functional (N=47)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 8    | 4               | 32   | 16               | >64  | 16               | 4    | 1               | 32   | 8               |
| MIC<sub>90</sub> | 32   | 16              | >64  | 32               | >64  | 32               | >64  | 2               | 64   | 16              |
| % Inhibited*     | 59.6%| 87.2%           | 10.6%| 38.3%            | 12.8%| 68.1%            | 61.7%| 97.9%           | 12.8%| 76.6%           |
| **OprD Non-Functional (N=163)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 16   | 8               | 32   | 16               | >64  | 16               | 2    | 1               | 32   | 8               |
| MIC<sub>90</sub> | >64  | 32              | >64  | 64               | >64  | 64               | >64  | 8               | 64   | 16              |
| % Inhibited*     | 31.9%| 58.9%           | 17.2%| 46.0%            | 17.8%| 67.5%            | 63.8%| 89.6%           | 23.9%| 79.1%           |
| **MexAB-OprM basal level activity (N=77)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 8    | 2               | 16   | 4                | >64  | 4                | 2    | 0.5             | 16   | 4               |
| MIC<sub>90</sub> | 64   | 4               | >64  | 8                | >64  | 8                | >64  | 1               | 64   | 8               |
| % Inhibited*     | 70.1%| 96.1%           | 44.2%| 96.1%            | 96.1%| 97.4%            | 96.1%| 96.1%           | 39.0%| 93.5%           |
| **MexAB-OprM increased activity (N=145)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 16   | 8               | 64   | 32               | >64  | 16               | 4    | 1               | 32   | 8               |
| MIC<sub>90</sub> | 64   | 32              | >64  | 64               | >64  | 64               | >64  | 8               | 64   | 32              |
| % Inhibited*     | 25.5%| 52.4%           | 0.0% | 17.9%            | 10.3%| 54.5%            | 60.7%| 89.7%           | 15.2%| 72.4%           |
| **OprD Non-Functional MexAB-OprM increased activity (N=105)** |      |                 |      |                  |      |                  |      |                 |      |                 |
| MIC<sub>50</sub> | 32   | 16              | 64   | 32               | >64  | 16               | 4    | 1               | 32   | 8               |
| MIC<sub>90</sub> | >64  | 32              | >64  | 64               | >64  | 64               | >64  | 16              | 64   | 32              |
| % Inhibited*     | 13%  | 40%             | 0%   | 18.1%            | 9.5% | 52.4%            | 58.1%| 86.7%           | 13.3%| 72.4%           |

MEM, meropenem, QPX, QPX7728, ATM, aztreonam; TOL, ceftolozane; FEP, cefepime; PIP, piperacillin.

*: % inhibited at the following concentrations: meropenem: ≤ 8 µg/ml; meropenem/QPX7728: ≤ 8/8 µg/ml; aztreonam: ≤ 8 µg/ml (FDA susceptible breakpoint); aztreonam/QPX7728: ≤ 8/8 µg/ml; ceftolozane: ≤ 4 µg/ml; ceftolozane/QPX7728: ≤ 4/8 µg/ml; cefepime: ≤ 8 µg/ml (FDA susceptible breakpoint); cefepime/QPX7728: ≤ 8/8 µg/ml.
Table S3. In vitro potency of various beta-lactams combined with QPX7728 at 8 µg/ml against the MBL-positive strains from the challenge panel of *Pseudomonas aeruginosa* according to OprD and Efflux due to MexAB-OprM

|               | MEM | MEM+ QPX 8 µg/ml | ATM | ATM + QPX 8 µg/ml | PIP | PIP + QPX 8 µg/ml | TOL | TOL+ QPX 8 µg/ml | FEP | FEP+ QPX 8 µg/ml |
|---------------|-----|------------------|-----|-------------------|-----|-------------------|-----|------------------|-----|------------------|
| **MBL (N=61)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | >64 | 32               | 32  | 16                | >64 | 16                | >64 | 64               | 64  | 32               |
| MIC<sub>90</sub> | >64 | >64              | >64 | 64                | >64 | >64              | >64 | 64               | 64  | 64               |
| % Inhibited*  | 0.0%| 31.1%            | 23.0| 42.6%             | 3.3%| 72.1%            | 3.3%| 27.9%            | 6.6%| 36.1%            |
| **OprD Functional (N=11)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | 32  | 8                | 8   | 8                 | >64 | 8                | >64 | 8                | 64  | 16               |
| MIC<sub>90</sub> | >64 | 64              | 32  | 32                | >64 | 64              | >64 | >64              | 64  | 64               |
| % Inhibited*  | 0.0%| 63.6%            | 54.5| 72.7%             | 0.0%| 81.8%            | 9.1%| 45.5%            | 27.3%| 45.5%            |
| **OprD Non-Functional (N=48)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | >64 | 32              | 32  | 16                | >64 | 16              | >64 | 64               | 64  | 32               |
| MIC<sub>90</sub> | >64 | >64            | >64 | 64                | >64 | >64              | >64 | 64               | 64  | 64               |
| % Inhibited*  | 0.0%| 25.0%           | 16.7| 37.5%             | 4.2%| 70.8%           | 2.1%| 25.0%            | 2.1%| 35.4%            |
| **MexAB-OprM basal level activity (N=21)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | 64  | 4                | 8   | 2                 | >64 | 4                | >64 | 4                | 64  | 8                |
| MIC<sub>90</sub> | >64 | 64              | 32  | 8                 | >64 | 16              | >64 | >64              | 64  | 64               |
| % Inhibited*  | 0.0%| 66.7%           | 66.7| 95.2%             | 9.5%| 90.5%           | 4.8%| 52.4%            | 9.5%| 52.4%            |
| **MexAB-OprM increased activity (N=40)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | >64 | 64              | 32  | 32                | >64 | 16              | >64 | >64              | 64  | 64               |
| MIC<sub>90</sub> | >64 | >64            | >64 | 64                | >64 | >64              | >64 | 64               | 64  | 64               |
| % Inhibited*  | 0.0%| 12.5%           | 0.0%| 15.0%             | 0.0%| 62.5%           | 2.5%| 15.0%            | 5.0%| 27.5%            |
| **OprD Non-Functional MexAB-OprM increased activity (N=33)** |     |                  |     |                   |     |                   |     |                  |     |                  |
| MIC<sub>50</sub> | >64 | >64            | >64 | 32                | >64 | 16              | >64 | >64              | 64  | 64               |
| MIC<sub>90</sub> | >64 | >64            | >64 | 64                | >64 | >64              | >64 | >64              | 64  | 64               |
| % Inhibited*  | 0.0%| 9.1%           | 0.0%| 12.1%              | 0.0%| 60.6%           | 0.0%| 12.1%            | 0.0%| 27.3%            |

MEM, meropenem, QPX, QPX7728, ATM, aztreonam; TOL, ceftolozane; FEP, cefepime; PIP, piperacillin.

*: % inhibited at the following concentrations: meropenem: ≤ 8 µg/ml; meropenem/QPX7728: ≤ 8/8 µg/ml; aztreonam: ≤ 8 µg/ml (FDA susceptible breakpoint); aztreonam/QPX7728: ≤ 8/8 µg/ml; ceftolozane: ≤ 4 µg/ml; ceftolozane/QPX7728: ≤ 4/8 µg/ml; cefepime: ≤ 8 µg/ml (FDA susceptible breakpoint); cefepime/QPX7728: ≤ 8/8 µg/ml.
MATERIAL AND METHODS

**MexB expression studies.** Single colonies from overnight grown plates were used to inoculate CaMHB and grown with shaking at 37°C till OD₆₀₀=0.6-0.7. Then, 1.5 ml of cultures were collected by centrifugation and total RNA was isolated using Ambion RiboPure-Bacteria RNA Isolation kit (ThermoFisher, Cat# AM1925) according to manufacturer’s instructions. Residual DNA in the RNA samples was removed by treatment with DNase I. Reverse transcription (RT) was performed using TaqMan® Reverse Transcriptase Reagents kit (ThermoFisher, cat# N8080234) following the manufacturer’s protocol. A mixture of reverse primers for *mexB* and the house-keeping gene *polA*, used as an internal control for quantitative PCR (qPCR) signal normalization, each at 0.5 µM, was used as the RT primers (see Table above). 2µl of RNA samples was added to a total RT reaction volume of 10 µl. qPCR was performed on ABI Prism 7000 Sequence instrument (Applied Biosystems) using SYBR® Select Master Mix (ThermoFisher, cat# 4472919). Each qPCR reaction tube (20 µl total volume) contained 10 µl of SYBR® Select Master Mix (2x), 1 µl of qPCR primer pair mixture resulting in a final concentration of forward and reverse primers at 0.5 µM, and 9 µl of 10-fold dilution (with water) of the RT reaction mixtures. The qPCR was run with the following thermal cycling conditions: 55°C for 2 min, 95°C for 5 min, and followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The qPCR reaction was carried out in duplicate.

The *mexB* gene qPCR results (CT values) were normalized with the housekeeping gene *polA* by subtracting the CT value of *mexB* from the CT value of *polA* of the same RT reaction mixture. To compare the expression of *mexB* between a test strain and the wildtype strain PAM1020 (equivalent to the standard strain PAO1), the normalized CT value of the test strain was subtracted from that of PAM1020, and the difference (ΔCT) was used as a logarithmic power (base=2) to calculate the relative normalized level of mRNA. The average and standard deviation of relative normalized mRNA level in duplicated qPCR reactions from the same RNA sample were calculated.
Primers used in this study

| Primer name | Sequence (5' to 3') | Used in            |
|-------------|---------------------|--------------------|
| PA-mexR-seq-F | CATGGGCCCATATTCCAGAACTGG | mexR sequencing    |
| PA-mexR-seq-R | CATTGCCGTAAGGCGGATA     |                    |
| PA-nalC-seq-F | GAGAACGGCTCTGACGCAAC    | nalC sequencing    |
| PA-nalC-seq-R | TCACTGAGGTGCAAGGCAA    |                    |
| PA-nalD-seq-F | GCAGCATTAGAACAAGGTTGS  | nalD sequencing    |
| PA-nalD-seq-R | CAGGAGGCAATACCATGCAA    |                    |
| PA-oprD-seq-F | CTATCGGAAAAGCAGACTGC   | oprD sequencing    |
| PA-oprD-seq-R | GCAGAGTAAATGAGGAAAGAC  |                    |
| PA-mexB-qF    | CGATAGGCTCCAGGTACAGAC   | mexB RT-qPCR      |
| PA-mexB-qR    | CGTCTTGAAAGCTGAAAGAAG   |                    |
| PA-polA-qF    | ATCCGAAGAAGCTCAAGGTC    | polA RT-qPCR      |
| PA-polA-qR    | ATCTGGTCGAAGGTACAGTTG   |                    |

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