Supporting information for Golemi *et al.* (November 27, 2001) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.241442898.

Methods

Fitting of the pH dependence data. The data obtained from the pH dependence of the kinetic parameters were fitted with the GRAFIT software (Erithacus Software, Middlesex, U.K.). The adequacy of the models was assessed by *F*-test provided by the same software. The pH dependence of k_{cat}/K_m was obtained from fitting to the bell-shaped curves described by Eq. **1**, where k_{lim} is the limiting value of k_{cat}/K_m:

$$k_{obs} = \frac{k_{li}}{1+10^{(pK_a - p\ )} + 10^{(p\ - pK_b)}} \qquad [1]$$

In the case where the pH-dependence curve displayed a tail on the acidic limb, the curve was fitted to Eq. **2**, which describes a model with two active species, a limiting value at lower pH, k_1, a limiting value at high pH, k_2, and two pK_a values:

$$k_{obs} = \frac{k_1 * 10^{(pK_a - pH\ } + k_2}{1+10^{(pK_a - p\ )} + 10^{(p\ - pK_b)}} \qquad [2]$$

The pH dependence of k_{cat} was fitted to a modified bell curve (Eq. **2** or Eq. **3)**:

$$k_{obs} = \frac{k_1 + k_2 * 10^{(p\ - pK_b)}}{1+10^{(pK_a - p\ )} + 10^{(p\ - pK_b)}} \qquad [3]$$

The pH dependence of K_m was fitted to:

$$k_{obs} = \frac{k_1 + k_2 * 10^{(p\ - pK_b)}}{1+10^{(p\ - pK_b)}} \qquad [4]$$

The pH dependence of kinetic parameters of OXA-10 β-lactamase with penicillin and nitrocefin fitted to the above equations are given in Fig. 5.

Concentration of OXA-10 β-lactamase *in vivo*. Clinical strains *Pseudomonas aeruginosa* AP 153 and *P. aeruginosa* McCL were individually grown overnight at 37°C in Antibiotic Medium 3 (Difco). The cultures were diluted 100-fold in fresh growth medium (200 ml) and were allowed to grow to the stationary phase (8–10 hr). The cells were harvested by centrifugation at 7,600 × *g* for 20 min at 4°C. The periplasmic content was liberated by three rounds of freeze-thaw procedure, and the solution was concentrated by passing through a DEAE anion-exchange

column (1 × 10 cm). The enzyme was eluted with 10 mM Tris buffer (pH 7.6), supplemented with 100 mM K_2SO_4. Samples were analyzed by SDS/PAGE, together with known quantities of the purified OXA-10 β-lactamase. The gels were silver stained and read using NIH IMAGE 1.62 software (http://www.nih.gov). The identity of the band corresponding to the OXA-10 enzyme was confirmed by running nondenaturing isoelectric focusing gels. Furthermore, we isolated the enzyme from the gel by electro-elution and showed by nondenaturing gel that it corresponded to the molecular weight of the purified enzyme (a dimer) and that the sample possessed β-lactamase activity as shown by nitrocefin staining of the gel. We have used the known mass and volume for bacteria from the literature in calculations of the periplasmic concentration of the enzyme in the clinical strains (1, 2). The volume of the periplasmic space was assumed to be 5–20% of the total cell volume (3, 4).

References:

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