Relative Importances of Outer Membrane Permeability and Group 1 β-Lactamase as Determinants of Meropenem and Imipenem Activities against Enterobacter cloacae

GIUSEPPE CORNAGLIA,1,* KIMBERLEY RUSSELL,1 GIUSEPPE SATTAC2 AND ROBERTA FONTANA1

Università degli Studi di Verona, Istituto di Microbiologia, I-37134 Verona,1 and Istituto di Microbiologia, Università Cattolica del Sacro Cuore, I-00168 Rome,2 Italy

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The roles of outer membrane permeability and Bush group 1 β-lactamase activity in determining Enterobacter cloacae susceptibility to either meropenem or imipenem were investigated. A β-lactamase-deficient strain was obtained by mutagenesis from a clinical isolate of E. cloacae, and a porin-deficient strain was selected from this mutant with cefoxitin. Both strains were transformed with the plasmid pAA20R, which contained the gene coding for the carbapenem-hydrolyzing CphA β-lactamase, and the carbapenem permeability coefficients were measured by the Zimmermann and Rosselet technique (W. Zimmermann and A. Rosselet, Antimicrob. Agents Chemother. 12:368–372, 1977). The permeability coefficient of meropenem was roughly half that of imipenem in the normally permeable strain and almost seven times lower than that of imipenem in the porin-deficient strain. In the porin-deficient strain, the virtual absence of porins caused the MICs of meropenem to increase from 8 to 16 times, while it did not affect the MICs of imipenem. Conversely, the β-lactamase affected imipenem but not meropenem activity: meropenem showed similar activity in the parent strain and in the β-lactamase-deficient mutant with both a low- and high-density inoculum, whereas imipenem was 16 times less active against the parent strain when the high-density inoculum was used. It is concluded that outer membrane permeability and stability to group 1 β-lactamase have different impacts on the activities of meropenem and imipenem against E. cloacae.

The high levels of activity of carbapenem antibiotics (such as meropenem and imipenem) against gram-negative bacteria have been ascribed to their high degrees of affinity for PBP 2, their high levels of resistance to β-lactamase hydrolysis, and a high rate of penetration through the outer membrane. This latter property appears to be peculiar to these antibiotics, since carbapenems have been shown to penetrate rapidly through the outer membrane of Pseudomonas aeruginosa via a specific porin pathway (32), and in Escherichia coli, too, in which a specific pathway is apparently absent (7), the permeability coefficients of meropenem and imipenem are 5 and 14 times higher, respectively, than that of cephaloridine, one of the noncarbapenem β-lactams endowed with the highest penetration rates (7). Less information is available on the mechanism by which carbapenems penetrate in other species of the family Enterobacteriaceae, although a considerable amount of evidence suggests that in many species the lack of certain outer membrane proteins is associated with the acquisition of resistance to these antibiotics (5, 10, 12, 16, 20, 24, 25, 33). A study of carbapenem influx under conditions which, as far as possible, preserve the physiological status of the cells seems to be of great importance for comparing carbapenem penetration in different species and for assessing the respective weights of permeability and other plasmiplastic factors, such as β-lactamase activity, in determining susceptibility or resistance to these antibiotics. Although a number of carbapenemases have been described (18, 23, 37), their overall incidence in this bacterial family still seems to be low. Nonetheless, attention has increasingly focused on chromosomal cephalosporinases assigned to class I of the scheme of Richmond and Sykes (26), class C of Ambler’s classification (13), and group 1 of the classification of Bush (3). These β-lactamases exhibit a very low hydrolytic activity for carbapenems but may take on a significant role in vivo because of the interplay between them and impaired outer membrane permeability.

Enterobacter cloacae is largely resistant to β-lactams because of production of constitutive and inducible β-lactamases (30, 35). Although a specific carbapenemase has recently been described in this species (23), the β-lactamase most commonly produced by the species is a Bush group 1 enzyme. This β-lactamase has been reported to produce carbapenem resistance either alone (31) or in association with defects in outer membrane proteins (4, 15, 16, 25). In the latter case, a reduction in carbapenem permeation was reported on the basis of reconstituted liposome assays (15, 25) or was inferred from the indirect quantitation of aztreonam permeation (4) or a reduction in the permeability coefficient for cephaloridine and in the uptake of [14C]meropenem (16), but direct measurement of carbapenem permeation through the outer membranes of intact cells has never been performed.

In the work described here we measured the permeability coefficients of intact E. cloacae cells for meropenem and imipenem directly by means of the method devised for this purpose by Zimmermann and Rosselet (40) and others (22) by transferring the CphA carbapenemase (19) into a β-lactamase-deficient E. cloacae strain and its porin-deficient mutant. We also investigated the correlation between the antibiotic influx kinetics, the group 1 β-lactamase content, and the susceptibility patterns shown by these strains in order to evaluate the respective roles of β-lactamase activity and outer membrane permeability in determining the activities of the two carbapenems against E. cloacae.
MATERIALS AND METHODS

Bacterial strains. The bacterial strain E. cloacae EB VR-11 is a clinical isolate whose β-lactamase activity is entirely due to a group 1 β-lactamase.

The cephalosporinase-deficient strain MALI-0 was obtained by mutagenesis from the wild-type E. cloacae EB VR-11. Cells were grown until an optical density of 0.8 was reached and harvested by centrifugation. They were washed twice with saline and were resuspended in a half volume of 0.2 M Tris (pH 7.5) to which 20% minimum salts was added. Two milliliters of this suspension was added to 0.2 ml of ethyl methanesulphonate, and the mixture was incubated at 37°C for 60 min. After mutagenesis, the cells were harvested, washed twice with Tris buffer, and plated onto Luria-Bertani (LB) agar plates containing 2% soluble starch. After overnight incubation, plates were flooded with 3 ml of an iodine solution prepared by adding 13 ml of iodine and 15 ml of potassium iodide to 1 ml of 0.05 M phosphate buffer (pH 6.4) and eventually (on the same day as the experiment) adding benzylpenicillin to a final concentration of 50 mg/ml. Colonies not producing decolorization in the agar were picked up and then checked for β-lactamase production by monitoring the hydrolysis of nitrocefin with a Beckman DU-7 spectrophotometer.

The porin-deficient strain MALI-11 was obtained by serial transfer of the cephalosporin-deficient strain MALI-0 onto LB plates to which increasing concentrations of cefoxitin were added over 48-h incubation periods at 37°C. Strains KRGC-0 and KRGC-11 were constructed by transforming E. cloacae MALI-0 and its porin-deficient derivative MALI-11 with the plasmid pAA20R, which contained the gene coding for a carbapenem-hydrolyzing β-lactamase (CphA) cloned from the Chromobacter属的菌（19）。（The alkaline denaturing methods described by Kado and Liu (11) and Birnboim and Doly (2) were used for plasmid extraction. Cells were made competent for transformation as described by Sambrook et al. (28), i.e., by a variation of the calcium chloride procedure of Cohen et al. (10).）The original procedure described by Hanahan (8), which included the use of rubidium chloride instead of potassium chloride in the TFB buffer recipe. Selective tetracycline concentrations were 50 and 100 µg/ml, respectively. Each strain was stored frozen at −80°C until use in brain heart infusion broth to which 10% glycerol was added.

Culture media and growth conditions. LB broth containing 5 mM MgSO4 was used for all tests. LB was 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract (Difco), and 1% NaCl adjusted to pH 7.5 with NaOH.

Antibiotics. Working solutions were prepared on the day of use from laboratory standard powders of all compounds as specified by the manufacturers. M overcrowned was provided by Zeneca (Milan, Italy). Cilastatin-free imipenem was provided by Merck Sharp & Dohme (Rome, Italy). All other antibiotics were from commercial sources.

Susceptibility tests. Antibiotic susceptibility tests were performed by serial twofold dilution in Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) essentially as described by Sambrook et al. (28). The MIC was defined as the lowest concentration that prevented visible growth after incubation for 18 h at 35°C in air.

Permeability assays with intact cells. Since the magnitude of the native (Bush group 1) β-lactamase activity against carbapenems was not sufficient to guarantee measurable induction of permeability mutants, we performed our analysis with the group 1 β-lactamase-deficient strains containing the plasmid for the carbapenem-hydrolyzing CphA β-lactamase (see above).

The cells were grown until the mid-exponential growth phase and were harvested by centrifugation. They were washed twice with 10 mM sodium phosphate buffer (pH 7) supplemented with 5 mM MgCl2, resuspended in the same buffer, and sonicated with a Labsonic 2000 ultrasonic disrupter (B.Braun Melsungen AG, Melsungen, Germany) by three to four 15-s pulses of sonication with an intervening 30 s in ice to minimize damage to the β-lactamase. Centrifugation or filtration to remove cellular debris was omitted, since both of these procedures involved substantial loss of enzyme activity (roughly 50%); the untreated sonic fluid caused no scattering or other drawbacks when spectrophotometric assays were performed, and thus, we used it as the crude enzyme preparation.

The hydrolysis rates of 10 µM antibiotic by intact cells and sonic extracts of cells were obtained with a Beckman DU-7 UV spectrophotometer, with readings recorded at 242 nm for 5 min. The spectrophotometer was operated at the wavelength at which the difference between the extinction values of the hydrolyzed and nonhydrolyzed molecules was maximum (this wavelength was 298 nm for both imipenem and meropenem). To measure the extent of leakage of enzyme, the rate of β-lactamase hydrolysis was measured with supernatants obtained by centrifugation of the intact cell suspension, and the hydrolysis rates of intact cells were corrected for the contribution of extracellular enzyme, (ranging from 1 to 5% of the rates for intact cells). The permeability coefficients were calculated by the method of Miyamoto et al. (22) with the modifications proposed by Yoshimura and Nikiado (38).

The permeability coefficient of cephalexin could not be calculated for strains KRGC-0 and KRGC-11, since cephalexin is not a valid substrate for the CphA β-lactamase (37). Thus, it was calculated for strain EB VR-11 by using the kinetic parameters of the Bush group 1 β-lactamase (at a wavelength of 266 nm). However, it could not be calculated at all for the cephalosporinase-deficient EB VR-11 derivatives MALI-0 and MALI-11, whose hydrolytic activities proved to be too low.

β-Lactamase catalytic properties. The β-lactamase was confirmed to be a β-lactamase of group 1 enzyme on the basis of its substrate and inhibitor profiles and a pI of 9.6. The Michaelis constant (Km) was determined by the Eadie-Hofstee plot of initial velocity (v) at six different substrate concentrations. Crude cellular extracts diluted 20-fold were used as the enzyme sources, and the hydrolysis of β-lactam antibiotics was followed by spectrophotometric assay as described above. Alternatively, the Km values were obtained directly from a time course of the hydrolysis data as described previously (21, 34), with no significant differences in the values obtained.

Induction studies. Group 1 β-lactamase induction was measured after exposure of logarithmic-phase cells to high MIC of either meropenem or imipenem, respectively, for 2 h. Enzyme activity was measured against 100 µM nitrocefin and was standardized against total protein, as determined with a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.).

Characterization of outer membrane proteins. Outer membranes were prepared by the rapid procedure described for P. aeruginosa by Trias et al. (32) and for E. coli by Corniglia et al. (7), yielding acceptable degrees of purification. Cells were grown in LB broth up to the late exponential phase of growth, harvested by centrifugation, washed once with 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)–NaOH buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and were resuspended in the same buffer. The suspension was sonicated with the aforementioned ultrasound disrupter by four 30-s pulses of sonication with an intervening 30 s in ice. The unbroken cells were removed by centrifugation at 1,500 × g for 15 min, and the membranes were pelleted by centrifugation of the supernatant at 19,000 × g for 30 min. The protein contents of the samples were determined with a protein assay kit (Bio-Rad). Finally, the pellets were resuspended in 1 ml of HEPES-NaOH buffer (20 mM; pH 7.4), and the solutions were stored at −80°C. Outer membrane proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) essentially as described by Laemmli (14), except that solid urea was added to both stacking and resolving gels (4 M) and to the sample buffer (8 M). Proteins were detected by Coomassie blue staining.

RESULTS

Isolation and properties of E. cloacae cephalosporinase and porin mutants. Selection of a cephalosporinase-deficient mutant was carried out with the twofold purpose of studying the role of this enzyme in the susceptibility of E. cloacae to both carbapenems and constructing a strain suitable for permeability studies. After mutagenesis of E. cloacae EB VR-11, mutants which did not show a positive reaction (i.e., decolorization around the colonies) in the iodometric assay were isolated at a frequency of 4 × 10−4. All of these negative clones, when they were tested for their β-lactamase activities by the nitrocefin assay, showed greater or lesser degrees of residual hydrolytic activity. We selected the strain (MALI-0) with the lowest hydrolysis rate (45 times lower than that of the parent strain; Table 1) for further experiments.

Mutant lacking porins were selected from MALI-0 on agar medium to which increasing concentrations of cefoxitin were added. Twenty colonies grown on plates containing cefoxitin (256 µg/ml) were picked up for further analysis. The outer membranes of the aforementioned strains partially purified with HEPES-PMSF and the outer membranes of their parent wild-type strain were solubilized, and their protein profiles were analyzed by SDS-PAGE. All strains showed variously reduced contents of porins of either 37 kDa (OmpF) or 38 kDa (OmpD), but only one of them (MALI-11) was found to apparently have entirely lost both 37- and 38-kDa proteins (Fig. 1), while on monitoring its nitrocefin hydrolysis it was found to have retained its β-lactamase mutation (Table 1).

No differences in the patterns of penicillin-binding proteins could be detected between the mutant and the parental strains (data not shown).

Induction studies. After a 2-h induction period, the group 1 β-lactamase activity increased by four times when imipenem was the inducer, while virtually no induction could be observed when meropenem was used as the inducer. No induction was observed when the periplasmic contents of the repressed cells were analyzed. No qualitative differences were demonstrated when the protein profiles of the induced and uninduced samples were compared with the two-dimensional gel electrophoresis system (26).
observed with either meropenem or imipenem in the β-lactamase-deficient strains MALI-0 and MALI-11.

Construction of strains harboring CphA carbapenemase. Because measurements of the permeability coefficients by the Zimmermann and Rosselet (40) technique require strains which harbor a specific β-lactamase, both MALI-0 and MALI-11 were transformed with the plasmid pAA20R, which carries the CphA carbapenemase gene. MALI-0 cells were easily made competent for transformation by a variation of the calcium chloride procedure of Cohen et al. (6), but the aforementioned method did not work with MALI-11, which required the rubidium chloride procedure described by Hanahan (8) (see Materials and Methods). The transformants obtained from both strains proved to be stable and did not show any autolysis during washing and resuspension. One MALI-0 (KRGC-0) transformant with a seemingly normal OmpF content and one MALI-11 transformant (KRGC-11) with an apparent absence of both porins were selected for determination of their outer membrane permeabilities (Fig. 1). No significant difference in CphA carbapenemase content was observed between the two transformants after spectrophotometric monitoring of imipenem hydrolysis by crude cell extracts (see Materials and Methods) (Table 2).

Effect of outer membrane alterations on carbapenem penetration. In strain KRGC-0, with a normal porin content, the permeability coefficient (P) of meropenem (1,630 nm/s) was roughly half that of imipenem (P = 3,530 nm/s). This ratio was virtually the same as that described previously for E. coli (7); in E. coli, however, permeability proved four times higher than that in E. cloacae. In the porin-deficient strain KRGC-11, meropenem (P = 70 nm/s) penetrated through the outer membrane 25 times more slowly than it did in the parent strain and almost seven times more slowly than imipenem under the same conditions (P = 470 nm/s). Imipenem showed a 7.5-fold reduction in penetration into the porin-deficient strain compared with that into the normal strain. The permeability coefficient of cephaloridine measured in the parent strain EB VR-11 was as low as 110 nm/s.

To explore the possibility of a carbapenem-specific channel, we measured the apparent permeability coefficients at different substrate concentrations in strain MALI-0 (which does not present any apparent porin defect) and strain MALI-11 (in which only trace levels of porin are present and therefore the influence of a possible specific channel should be more appreciable). Figure 2 shows that the measured permeability coefficients were indeed higher as the substrate molarity decreased, in accordance with findings reported previously and discussed by several investigators (9, 17), but no relation could be established between the extent of the differences and the porin contents of the strains. These findings seem to rule out the possibility of carbapenem-specific pathways, at least in the range of concentrations that we considered (namely, 10 to 1,000 μM).

Relationships between β-lactamase contents, permeability defects, and susceptibilities to carbapenems. Table 3 shows that the substantial reduction in cephalosporinase content did not affect meropenem activity at either a low or a high inoculum. Conversely, imipenem was as active in the parent strain as it was in the cephalosporin-deficient mutant when the lower inoculum was used, but it was 16 times less active in the parent strain at the higher inoculum.

In the porin-deficient strain MALI-11, which was obtained from the cephalosporinase-deficient strain MALI-0, the virtual absence of porins caused the MICs of meropenem for MALI-11 to increase 8- to 16-fold at both inocula, while it did not affect the MICs of imipenem at either inoculum size.

The introduction of the CphA β-lactamasms caused a marked increase in the MICs of both carbapenems, which exceeded the MICs for the wild-type strain EB VR-11 by 4 to 8 times when the porin content was preserved and the inoculum was low but which were 64 to 256 times greater than those values at the higher inoculum or for the porin-deficient mutants.

DISCUSSION

Although nonspecific porins have often been suggested as playing a role in E. cloacae permeability to carbapenems and

| Strain   | Phenotype<sup>a</sup> | Hydrolysis rate<sup>b</sup> | K<sub>m</sub> (μM) |
|----------|-----------------------|-----------------------------|-----------------|
|          |                       | Not induced | Induced<sup>c</sup> (MPM) | Induced<sup>c</sup> (IPM) |
| EB VR-11 | OmpF<sup>a</sup> OmpD<sup>a</sup> AmpC<sup>a</sup> CphA<sup>a</sup> | 5.36 | 5.69 | 21.32 | 56.3 |
| MALI-0   | OmpF<sup>a</sup> OmpD<sup>a</sup> AmpC<sup>a</sup> CphA<sup>a</sup> | 0.11 | 0.13 | 0.14 | 48.1 |
| MALI-11  | OmpF<sup>a</sup> OmpD<sup>a</sup> AmpC<sup>a</sup> CphA<sup>a</sup> | 0.09 | 0.08 | 0.09 | 50.3 |

<sup>a</sup> Phenotypes indicate only the mere presence or (relative) absence of the gene products, regardless of the mechanisms underlying their expression.

<sup>b</sup> Hydrolysis rate at 100 μM expressed in nanomoles per minute per milligram of protein. Values are averages of at least three different determinations. MPM, meropenem; IPM, imipenem.

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FIG. 1. Outer membrane proteins of E. cloacae derivatives separated on a 12% acrylamide gel containing 4 M urea. Outer membranes were prepared from various strains grown in LB broth as described in Materials and Methods. Lanes: A, MALI-0 (OmpF<sup>a</sup> OmpD<sup>a</sup> CphA<sup>a</sup>); B, KRGC-0 (OmpF<sup>a</sup> OmpD<sup>a</sup> CphA<sup>a</sup>); C, MALI-11 (OmpF<sup>a</sup> OmpD<sup>a</sup> CphA<sup>a</sup>); D, KRGC-11 (OmpF<sup>a</sup> OmpD<sup>a</sup> CphA<sup>a</sup>); E, molecular weight standards, consisting of bovine plasma albumin (66,000), ovalbumin (45,000), and carbonic anhydrase (29,000).
TABLE 2. Kinetics of carbapenem hydrolysis by the derivatives of E. cloacae EB VR-11 harboring the CphA β-lactamase

| Strain   | Phenotype | MPM     | K_{cat} (μM) | IPM     | K_{cat} (μM) |
|----------|-----------|---------|-------------|---------|-------------|
| KRGC-0   | OmpF" OmpD" AmpC" CphA" | 290.3   | 940         | 192.6   | 235         |
| KRGC-11  | OmpF" OmpD" AmpC" CphA" | 279.7   | 895         | 165.5   | 215         |

* MPM, meropenem; IPM, imipenem.

Hydrolysis rate at 100 μM expressed in nanomoles per minute per milligram of protein. Values are averages of at least three different determinations.

carbapenem resistance in this species has been associated with defects in outer membrane proteins (4, 15, 16, 25), direct measurement of carbapenem permeation through the outer membranes of intact cells has not been used to date to confirm this hypothesis.

The direct measurement of the penetration rates of meropenem and imipenem into E. cloacae whole cells, which was performed in the present study by the method devised for this purpose by Zimmermann and Rosselet (40) and others (22), has revealed a difference in the role that outer membrane permeability plays in determining susceptibilities to these carbapenems. Our study shows that meropenem penetrates through the outer membrane of E. cloacae at a rate which is roughly twice as slow as that of imipenem and that the penetration rate of meropenem is more affected by the lack of OmpD and OmpF porins (with a 20-fold decrease in the permeability coefficient) than the penetration rate of imipenem is (7-fold decrease in the permeability coefficient). In the porin-deficient strain the ratio of meropenem to imipenem penetration rates is as low as 1:7. Such a difference in the penetration rates of carbapenems was not found by other authors (15, 25), who used an alternative method for measuring outer membrane permeability, namely, the reconstituted proteoliposome swelling assay. In those studies the relative diffusion rates of both carbapenems were found to be reduced by roughly 90% when the outer membrane of a porin-deficient mutant was used to reconstitute proteoliposomes. It should be observed, however, that only one of the two studies (25) attempted an indirect estimation of permeability coefficients, which concerned imipenem alone and was founded on two assumptions, namely, (i) that the E. cloacae outer membrane had a nonspecific permeability equal to or only slightly lower than that of the E. coli outer membrane (quoted from unpublished data) and (ii) that imipenem penetrates through the E. coli OmpF porin only 30% faster than cephaloridine (39). The first assumption is in sharp contrast to previous reports of very different permeabilities to noncarbapenem β-lactams in E. coli and E. cloacae (1, 21) and the findings of the present work (see below), while the second assumption has been confuted in the only work to date determining carbapenem permeability in whole cells of strains of the family Enterobacteriaceae, under which conditions the E. coli permeability coefficient for imipenem was 14 times higher than that for cephaloridine (7). If the factors used to extrapolate results of model membrane studies to whole cells are correct, our findings may indicate that the whole-cell kinetics of imipenem penetration are much faster than those in reconstructed systems, implying (i) the existence of specific channels of the type described in P. aerugi- nosa (32) or (ii) that in intact cells the nonspecific porins are much more permeable to imipenem than when they are purified and used in reconstituted proteoliposomes, which is in contrast to current opinion and experience to date with the noncarbapenem β-lactams (7). In both cases, correct comparison of the influx kinetics of meropenem and imipenem would necessarily imply the use of whole-cell systems of the type that we used in the present study.

Comparison of the carbapenem permeability coefficients measured in E. cloacae (the present study) and in E. coli (7) by the same experimental approach and with the same β-lacta-mase shows that the outer membrane of E. coli is four times more permeable to both meropenem and imipenem, respectively. Moreover, the lack of porins seems to affect meropenem permeation more in E. cloacae (20-fold reduction) than in E. coli (10-fold reduction), while the opposite can be seen in the case of imipenem (a 7.5-fold reduction in E. cloacae versus a 20-fold reduction in E. coli). As a consequence, the ratio between the two carbapenem permeations in normally permeable strains of the two species considered is virtually the same (namely, 2:1 in favor of imipenem), but in the porin mutants this ratio undergoes significant modifications, since the virtual absence of porins in E. coli confers the same low permeability values to both carbapenems upon the outer membrane, while an E. cloacae strain endowed with an apparently similar defect proves to be seven times more permeable to imipenem than to meropenem (see above).

The great advantage in terms of the ease with which the periplasmic space of E. cloacae can be reached is clearly reflected in the susceptibility results, since the MIC of imipenem was not affected at all by the loss of porins even at the higher inoculum, while the MIC of meropenem, under the same con-
ditions, was 16 times higher in the porin-deficient mutant than in the parent strain even at the lower inoculum. It is worth noting, however, that the strain is still in the susceptible interpretative category for meropenem, notwithstanding the increase in the MIC for the strain.

The different behaviors of meropenem and imipenem which we have just described in the porin-deficient strain of *E. cloacae* are completely reversible if one considers the pair of strains differing from each another in their β-lactamase contents. While no variation is detectable at the lower inoculum, at the higher inoculum the meropenem MIC showed no variation, but the imipenem MIC proved to be 16 times higher for the wild-type strain than for the cephalosporinase-deficient mutant. These results agree with the finding that imipenem is a stronger inducer than meropenem (Table 1), although the extent of this difference is not as large as that reported previously (36).

In conclusion, it seems likely that the level of meropenem susceptibility in *E. cloacae* is more dependent on outer membrane permeability status, whereas imipenem susceptibility is more dependent on group 1 β-lactamase activity. The relative importance of these factors in determining high-level resistance, as is found with increasing frequency in clinical isolates, depends on the extent of the porin defect and/or β-lactamase overproduction.

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