Interpretation of Diffusion Susceptibility Data Obtained with 50-μg Carbenicillin Discs Against Gram-Negative Organisms

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A total of 284 clinical isolates of various species of Enterobacteriaceae, Pseudomonas aeruginosa, P. maltophilia, and Acinetobacter anitratum were tested for susceptibility to carbenicillin by the standardized Bauer-Kirby disc diffusion technique and a microtiter broth dilution method. The data obtained led to the following proposed criteria for the interpretation of the results of disc susceptibility tests. Enterobacteriaceae that yield zones of inhibition equal to or greater than 20 mm in diameter around 50-μg discs of carbenicillin are designated as sensitive to the drug; isolates that yield zones measuring from 18 to 19 mm in diameter are reported as of equivocal (intermediate) susceptibility to the drug, whereas those enterobacterial isolates that are characterized by zones of inhibition of 17 mm or less in diameter are interpreted as resistant to carbenicillin. Isolates of P. aeruginosa, P. maltophilia, and A. anitratum yielding zones of 14 mm or more in diameter around 50-μg discs of carbenicillin are reported as sensitive, whereas those isolates that are characterized by zones of 13 mm or less in diameter are reported as resistant to this drug.

Recently, results obtained with the Bauer-Kirby method of disc diffusion susceptibility testing clinical isolates of Enterobacteriaceae and Pseudomonas aeruginosa with 25- and 100-μg discs of carbenicillin were reported from a number of laboratories, including our own (4, 6, 8). Meanwhile, 50-μg discs of this drug have been approved by the Food and Drug Administration (2). In this paper, we report the results obtained for a variety of enterobacterial isolates, as well as isolates of P. aeruginosa, P. maltophilia, and Acinetobacter anitratum, and propose criteria for the interpretation of zones of inhibition obtained around 50-μg discs of carbenicillin based on the results derived through a microtiter broth dilution technique.

MATERIALS AND METHODS

Bacteria. A total of 183 clinical isolates of Enterobacteriaceae were tested; these were identified by previously published criteria (7); 87 isolates of P. aeruginosa were identified as described before (6). The five isolates of P. maltophilia were characterized by motility, oxidative utilization of maltose (Hugh-Leifson O-F test), variable oxidative utilization of glucose, lack of oxidase, liquefaction of gelatin and hydrolysis of deoxyribonucleic acid, reduction of nitrate, and lack of pyocyanin and pyoverdin production. The nine isolates of A. anitratum were identified as follows: lack of oxidase and motility, failure to reduce nitrate, lack of pigment production, and oxidative utilization of glucose and xylose, but not of maltose.

Media. Mueller Hinton broth (MHB) and agar (MHA) were purchased from Difco. The isolates were maintained on Brain Heart Infusion agar slants (Difco).

Carbenicillin. Carbenicillin powder (lot A0026MA) was a gift from Beecham Pharmaceuticals, Clifton, N.J., as were 50-μg carbenicillin discs (BBL; lot 006028). The antibiotic was dissolved in sterile distilled water to an activity of 10,000 μg/ml, membrane-filtered (0.2 μm; Nalge Sybron Corp., Rochester, N.Y.), and dispensed in 2-ml portions into sterile screw-capped vials which were frozen and kept stored at −65 C. The vials were never reused after thawing. Antibiotic susceptibility tests. Disc diffusion susceptibility tests were performed precisely by the technique of Bauer et al. (1), with the exception that the large plates (100 by 15 mm) contained 60 ml of MHA, resulting in an agar depth of roughly 4 mm (3). The microtiter method employed was the same as described previously (6); the exponentially growing isolates were exposed to serial twofold dilutions of carbenicillin over the range of 1,000 to 1 μg/ml (final concentrations). As before, the bacterial inocula were adjusted to yield approximately 1.5 × 10^6 colony-forming units/ml at zero time. Strain Escherichia coli ATCC 25922 served as the control for all susceptibility tests.
DIFFUSION SUSCEPTIBILITY OF CARBENICILLIN DISCS

The plates were incubated for 16 to 18 hr at 35 C. The minimum inhibitory concentration (MIC) of carbenicillin was interpreted as the lowest concentration of antibiotic that completely inhibited bacterial growth as judged by visual inspection (6).

RESULTS

The results obtained are shown in Fig. 1 and 2. The control strain of E. coli was inhibited by 8 μg of carbenicillin per ml and gave zones of...

![Fig. 1. Activity of carbenicillin (MIC values and zones of inhibition around 50-μg discs) against 183 enterobacterial isolates. Numbers in parentheses indicate number of respective isolates examined.](image)

![Fig. 2. Activity of carbenicillin (MIC values and zones of inhibition around 50-μg discs) against 101 isolates of P. aeruginosa, P. maltophilia, and A. anitratum. Numbers in parentheses indicate number of respective isolates examined.](image)
inhibition that measured 25 mm in diameter. As is evident from Fig. 1, all enterobacterial isolates characterized by MIC values of 15.7 μg of carbenicillin per ml (or more) yielded zones of inhibition of 22 mm or more in diameter; three isolates (1 strain each of E. coli, Citrobacter freundii, and Serratia marcescens) required 31.3 μg of drug per ml for inhibition and yielded zones of inhibition that measured 20, 22, and 25 mm in diameter, respectively. Five isolates (two isolates of C. freundii, two isolates of Enterobacter, and one isolate of Klebsiella pneumoniae) required 125 μg of carbenicillin per ml for inhibition; however, the diameters of the zones of inhibition varied widely. The same was true for those enterobacterial isolates that required 250 μg of carbenicillin per ml for inhibition; all but one of these 13 isolates yielded zones of inhibition of 16 mm or less in diameter. Those enterobacterial isolates that were characterized by MIC values of 500 μg/ml or more gave zones of inhibition that measured 16 mm or less.

Six of the 87 isolates of P. aeruginosa required 250 μg of carbenicillin per ml (or more) for inhibition, and the diameters of the zones of inhibition were 8 mm or less (Fig. 2). Six of the isolates were inhibited by 125 μg of carbenicillin per ml; the corresponding zone diameters measured 15 and 16 mm, respectively. All isolates of P. aeruginosa that required 62.5 μg or less of carbenicillin per ml for inhibition gave inhibition zones that measured 14 mm or more in diameter. The five isolates of P. maltophilia were inhibited by 250 to 500 μg of carbenicillin per ml and proved resistant to the drug as determined with the disc diffusion method. The nine isolates of A. anitratus examined proved similar to P. aeruginosa with respect to susceptibility to carbenicillin and resultant zones of inhibition.

**DISCUSSION**

On the basis of the above data, we propose criteria for the interpretation of zone diameters obtained with 50-μg discs of carbenicillin (Table 1). It should be stressed that occasional enterobacterial isolates, that require 125 μg of carbenicillin per ml for inhibition, may yield zones of inhibition measuring less than 18 mm in diameter, as was the case for three of our enterobacterial isolates (Fig. 1); similarly, isolates characterized by MIC values of 250 μg of carbenicillin per ml might on occasion yield zones of inhibition that measure 18 or 19 mm in diameter. It is noteworthy that isolates of K. pneumoniae, Enterobacter (E. cloacae and E. aerogenes), and C. freundii proved problematic in this respect.

Clearly, one must employ different criteria for isolates of P. aeruginosa and A. anitratus, possibly for all Pseudomonadaceae and additional species of nonfermenting gram-negative bacteria. At the moment we have no data other than those for P. aeruginosa, P. maltophilia, and A. anitratus; this is why the proposed criteria of Table 1 were qualified accordingly.

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