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# Evaluation of Efficiency of Screening Extended-Spectrum β-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in Hospitals Where the Bacteria Are Increasingly Prevalent

K. LEE,[1] J. K. LIM,[1] D. YONG,[1] J. YUM,[1] Y. CHONG,[1]* R. OKAMOTO,[2] AND M. INOUE[2]

*Department of Clinical Pathology, Research Institute of Bacterial Resistance, and BK21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul, Korea,[1] and Department of Microbiology, Kitasato University School of Medicine, Sagamihara City, Japan[2]*

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**The disk screening methods for extended-spectrum β-lactamase-producing strains were evaluated. The confirmatory work is reduced significantly in settings such as those in this study, by changing the cefpodoxime breakpoint to ≤20 mm and by not testing cefoxitin-resistant isolates. Cefotaxime and ceftazidime disk screening is reliable, and the laboratory-prepared cefotaxime- and ceftazidime-clavulanic acid disks are stable at −20°C for 12 weeks.**

The detection of extended-spectrum β-lactamase (ESBL)-producing strains is important for optimal therapy of infected patients. The screening and confirmation of ESBL-producing isolates using the methods of the NCCLS (12) are easily performed. A survey in Korea suggested a high prevalence of ESBL-producing isolates (7), and with the increase of cefpodoxime disk screening-positive isolates, laboratories are confronted with an increasing burden of confirming results. Particularly, the almost daily preparation of clavulanic acid solution-supplemented ceftazidime (ceftazidime-clavulanic acid) and cefotaxime (cefotaxime-clavulanic acid) disks requires time.

Isolates producing certain ESBL types with low hydrolytic activity (4) are difficult to detect (14). In Korea, only TEM-52-, SHV-2a-, and SHV-12-type ESBLs have been reported (6, 13), and the cefpodoxime disk screening-positive isolates have often been ESBL nonproducers (Y. Chong, J. K. Lim, D. Yong, J. W. Yum, K. Lee, R. Okamoto, and M. Inoue, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1608, 2000). In this study, we evaluated the efficiency of NCCLS disk screening methods in Korea and determined the stability of the laboratory-prepared cefotaxime- and ceftazidime-clavulanic acid disks in storage.

TABLE 1. Cefpodoxime disk zone diameter distribution for ESBL-producing and -nonproducing isolates

| Cefpodoxime zone diam (mm) | No. of isolates for species, cefoxitin susceptibility, and ESBL status[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* ($n$ = 256) | | | | *K. pneumoniae* ($n$ = 109) | | | |
| | Cefoxitin S (I) | | Cefoxitin R | | Cefoxitin S (I) | | Cefoxitin R | |
| | ESBL positive ($n$ = 20) | ESBL negative ($n$ = 174) | ESBL positive ($n$ = 6) | ESBL negative ($n$ = 56) | ESBL positive ($n$ = 33) | ESBL negative ($n$ = 58) | ESBL positive ($n$ = 8) | ESBL negative ($n$ = 10) |
| <13 | **14 (1)** | **0 (1)** | **5** | **53** | **31 (1)** | **1 (1)** | **8** | **8** |
| 13–14 | **2** | **2 (1)** | | | **1** | | | |
| 15–16 | **1** | | **1** | **1** | | | | |
| 17–18 | **2** | **5** | | **2** | | | | |
| 19–20 | | **4** | | | | | | |
| 21–22 | | **17 (2)** | | | | **2** | | **2** |
| 23–24 | | 38 (1) | | | | 10 (1) | | |
| 25–26 | | 42 | | | | 14 (1) | | |
| 27–28 | | 31 | | | | 10 | | |
| >28 | | 30 | | | | 18 | | |
| No. (%) screening positive | 20 (7.8) | 32 (12.5) | 6 (2.3) | 56 (21.9) | 33 (30.2) | 4 (3.7) | 8 (7.3) | 10 (9.2) |

[a] Boldface indicates isolates which were cefpodoxime-screening positive. Abbreviations: S (I), susceptible (intermediate); R, resistant.

* Corresponding author. Mailing address: Department of Clinical Pathology, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-ku, Seoul, 120-752, Korea. Phone: 82-2-361-5866. Fax: 82-2-313-0908. E-mail: whonetkor@yumc.yonsei.ac.kr.

Isolates of *Escherichia coli* and *Klebsiella pneumoniae* were collected in 1999 in Korea: in phase I, from a tertiary-care hospital, and in phase II, from 28 hospitals with various bed capacities and locations. Antimicrobial susceptibility was tested by the NCCLS disk diffusion method (11) using Mueller-Hinton agar plates and antimicrobial disks (Becton Dickinson Microbiology Systems, Sparks, Md.). ESBL production was screened using cefpodoxime disks (12). To confirm ESBL production, the isolates were tested by double-disk synergy tests (8) using an amoxicillin-clavulanic acid disk plus cefotaxime, ceftazidime, and aztreonam disks and by comparing the MICs (10, 12) of cefotaxime and ceftazidime with or without 4 μg of clavulanic acid (Smith-Kline Beecham, Worthing, United Kingdom) per ml.

Some characteristics of ESBLs were determined using transconjugants obtained by agar mating (2) using recipient *E. coli* RG 176 ($Nal^r$), RG 488 ($Rif^r$) (kindly provided by Dong Taek Cho, College of Medicine, Kyungpook National University, Taegu, Korea), or J53 ($Az^r$) (kindly provided by Chik Hyun Pai, College of Medicine, Ulsan University, Ulsan, Korea). PCR was performed using heat-extracted templates as described previously (5). The isoelectric point (pI) of β-lactamase (9) was determined by electrophoresis of the cell sonicate using a pH 3 to 10 gel and a ThermoFlow unit (Novex Experimental Technology, San Diego, Calif.) and staining with 0.7 mg of nitrocefin (Unipath, Basingstoke, United Kingdom) per ml. The cefotaxime- and ceftazidime-clavulanic acid disks, prepared as recommended by the NCCLS (12), were placed in vials with desiccant and stored at 4° and −20°C, and the inhibition zone diameters were determined using ESBL-producing strains: *K. pneumoniae* ATCC 700603, two clinical isolates of *K. pneumoniae*, and one clinical isolate of *E. coli*.

In the phase I study, 114 of 256 (44.5%) *E. coli* and 55 of 109 (50.5%) *K. pneumoniae* isolates were cefpodoxime disk screening positive (Table 1). Among them, 62 (24.2%) *E. coli* and 18 (16.5%) *K. pneumoniae* isolates were cefoxitin resistant, and among the cefoxitin-susceptible or -intermediate isolates, only 20 (7.8%) *E. coli* and 33 (30.2%) *K. pneumoniae* isolates were ESBL positive. ESBL-positive isolates of *E. coli* and *K. pneumoniae* showed cefpodoxime zone diameters of ≤18 and ≤14 mm, respectively.

Efficiencies of screening of ESBL-producing isolates with disks of aztreonam, cefotaxime, and ceftazidime were compared (Table 2). All of the three antimicrobial disks detected all ESBL-producing isolates, but two and four isolates of *E. coli* were just within the breakpoint of aztreonam (27 mm) and ceftazidime (22 mm), respectively. Among the cefoxitin-nonresistant and ESBL-negative isolates, only 23 (13.2%), 22 (12.6%), and 12 (6.9%) *E. coli* isolates and 17 (29.3%), 20 (34.5%), and 3 (5.2%) *K. pneumoniae* isolates were positive by screening with aztreonam, cefotaxime, and ceftazidime, respectively.

In the phase II study (Table 3), the proportion of ESBL-positive isolates of *E. coli* was relatively higher (35.6%) than that in the phase I hospital, because of lower prevalence of cefoxitin-resistant isolates, but for *K. pneumoniae*, the rates were fairly similar in the two phases. The MICs of cefotaxime or ceftazidime were 2 μg/ml for six and two ESBL-producing isolates of *E. coli* and *K. pneumoniae*, respectively (data not shown), but all of them were positive by screening with cefpo-

TABLE 2. Aztreonam, cefotaxime, and ceftazidime disk zone diameter distribution for ESBL-producing and -nonproducing isolates

| Zone diam (mm) | No. of isolates for drug, species, cefoxitin susceptibility, and ESBL production status | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aztreonam | | | | | | Cefotaxime | | | | | | Ceftazidime | | | |
| | *E. coli* ($n$ = 256) | | *K. pneumoniae* ($n$ = 109) | | | | *E. coli* ($n$ = 256) | | *K. pneumoniae* ($n$ = 109) | | | | *E. coli* ($n$ = 256) | | *K. pneumoniae* ($n$ = 109) | |
| | Cefoxitin S/I | | Cefoxitin S/I | | | | Cefoxitin S/I | | Cefoxitin S/I | | | | Cefoxitin S/I | | Cefoxitin S/I | |
| | ESBL production positive | ESBL production negative | ESBL production positive | ESBL production negative | Cefoxitin R | | ESBL production positive | ESBL production negative | ESBL production positive | ESBL production negative | Cefoxitin R | | ESBL production positive | ESBL production negative | ESBL production positive | ESBL production negative | Cefoxitin R |
| <11 | **2** | | **12** | **1** | **6** | | **1** | | **2** | | **7** | | **2** | **1** | **11** | | **6** |
| 11–15 | **2** | | **15** | **1** | **2** | | **6** | | **12** | | **5** | | **2** | **19** | **15** | | **8** |
| 16–17 | | **1** | **4** | | **3** | | **5** | **3** | **8** | | **1** | | **5** | **24** | **6** | | **2** |
| 18–19 | **3** | **3** | **1** | | **1** | | | **1** | **7** | | **1** | | **4** | **11** | | | |
| 20–21 | **3** | **15** | | | **2** | | **3** | **3** | **4** | **1** | **2** | | **3** | **2** | **1** | **1** | |
| 22 | **1** | **8** | | | **2** | | **1** | **2** | | **1** | | | **4** | **7** | | **2** | |
| 23–24 | **2** | **10** | **1** | | | | **3** | **3** | | **4** | | | | **10** | **1** | **11** | **1** |
| 25–26 | **5** | **8** | | | | | **1** | **6** | | **6** | **1** | | | **32** | | **13** | **1** |
| 27 | **2** | **7** | | | **1** | | | **4** | **2** | **8** | **1** | | | **11** | **1** | **13** | |
| 28–29 | | **4** | | | | | | **28** | | **12** | | | | **35** | **1** | **9** | |
| >29 | | | | | | | | **124** | | **26** | | | | **74** | | **9** | |
| Total | 20 | 174 | 62 | 33 | 58 | 18 | 20 | 174 | 62 | 33 | 58 | 18 | 20 | 174 | 62 | 33 | 58 | 18 |

[a] Boldface indicates strains that screened positive. Abbreviations: S/I, susceptible or intermediate; R, resistant.

TABLE 3. Efficiency of cefpodoxime disk screening test for ESBL-producing isolates in a tertiary-care hospital and in other hospitals[a]

| Cefpodoxime zone diam (mm) | No. of isolates for species, cefoxitin susceptibility, and study phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* ($n$ = 232) | | | | *K. pneumoniae* ($n$ = 132) | | | |
| | Cefoxitin S/I | | Cefoxitin R | | Cefoxitin S/I | | Cefoxitin R | |
| | Phase I | Phase II | Phase I | Phase II | Phase I | Phase II | Phase I | Phase II |
| <10 | 11 | 33 | 57 | 9 | 25 | 34 | 15 | 12 |
| 11–12 | 4 | 7 | 1 | 3 | 7 | 6 | 1 | 3 |
| 13–14 | 2 | 2 | | | 1 | 1 | | |
| 15–16 | 1 | | 2 | | | | | |
| 17–18 | 2 | | 2 | | | | 2 | |
| Total (%) | 20 (17.5) | 42 (35.6) | 62 (54.4) | 12 (10.2) | 33 (62.3) | 41 (51.9) | 18 (34.0) | 15 (19.0) |

[a] Isolates that were cefoxitin susceptible or intermediate were all positive for ESBL production. Results for cefoxitin-susceptible or -intermediate isolates that were negative for ESBL production are not shown. The numbers of isolates tested for each phase are as follows: phase I, 114 of *E. coli* and 55 of *K. pneumoniae*, and phase II, 118 of *E. coli* and 79 of *K. pneumoniae*. S/I, susceptible or intermediate; R, resistant.

doxime, cefotaxime, and ceftazidime disks. The $bla_{TEM}$ and/or $bla_{SHV}$ sequence was detected in all ESBL-producing transconjugants (Table 4), and their β-lactamases had a pI of 6.0, 7.6, or 8.2, suggesting that they were TEM-52, SHV-2a, or SHV-12 type, respectively. The mean MIC of ceftazidime was higher for isolates with a pI 8.2 β-lactamase.

The inhibition zone diameter produced by the cefotaxime- and ceftazidime-clavulanic acid disks did not change significantly after 1 week at 4°C or after 12 weeks at −20°C (Table 5).

In the phase I study, 12.5% of *E. coli* isolates were cefpodoxime disk screening positive but ESBL negative. By reducing the screening breakpoint by 2 mm to ≤20 mm, the false-positive rate decreased by 59.4% in *E. coli*, but this did not influence the result significantly in *K. pneumoniae* (Table 1). In a U.S. study, 42.2% of cefpodoxime screening-positive *E. coli* isolates were ESBL nonproducers (F. C. Tenover, P. Raney, P. P. Williams, K. L. Brittain, C. D. Steward, S. K. Fridkin, R. P. Gaynes, and J. E. McGowan, Jr., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1606, 2000), which indicates the feasibility of eliminating unnecessary work by adjusting breakpoints in other countries, too.

CMY-1-producing strains continue to be prevalent in Korea (1), and AmpC β-lactamases have been reported worldwide, including the United States (3). It may not be significant to detect ESBL producers among cefoxitin-resistant isolates, as AmpC β-lactamase producers are resistant to all β-lactams except carbapenem, temocillin, and mecillinam (8). If so, 54.4% of cefpodoxime screening-positive *E. coli* isolates in the phase I study did not require a confirmatory test (Table 3). The reduction was only 10.2% in phase II hospitals, but such isolates may increase in the near future. In a U.S. study, 16.3% of cefpodoxime screening-positive *E. coli* isolates were ESBL nonproducers and were possibly AmpC β-lactamase producers (Tenover et al., 40th ICAAC).

Additional testing of cefpodoxime susceptibility for only *E. coli* and *Klebsiella* isolates may hinder laboratory workflow, as the species identification and susceptibility testing are usually performed simultaneously. If cefotaxime, ceftazidime, or aztreonam susceptibility is reliable for screening ESBL producers, its use can help streamline laboratory workflow, as the drugs are usually used for susceptibility testing of all aerobic gram-negative bacilli. In this study, some isolates were inhibited by 2 μg of cefotaxime or ceftazidime per ml, which is much lower than the >16 μg of ceftazidime per ml in a U.S. study (Tenover et al., 40th ICAAC), but all were screening test positive with cefotaxime and ceftazidime disks. However, two and four ESBL-producing *E. coli* isolates were just within the breakpoints of aztreonam and ceftazidime, respectively, suggesting that the current breakpoints may not be high enough in Korea.

In the past, preparation of screening disks has been a limiting factor in routine testing for ESBL-producing enteric organisms. The process was time-consuming for a busy laboratory, and the long-term stability of the disks was unknown. Recently, such disks have been reported to be stable for at least 14 days at −20°C (15), and in this study we have extended that observation for up to 12 weeks at −20°C. Although clavulanic acid-containing ceftazidime and cefotaxime disks have

TABLE 4. Comparison of MICs of expanded-spectrum cephalosporin and aztreonam for transconjugants by possession of TEM and SHV genes and pI patterns of β-lactamase

| Gene(s) detected | No. of isolates | | | β-Lactamase with (pI)[a]: | | | | Mean MIC (μg/ml) of drug[b]: | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* | *K. pneumoniae* | Total (%) | 5.4 | 6.0 | 7.6 | 8.2 | CAZ | CTX | CRO | ATM |
| $bla_{TEM}$ | 6 | 4 | 10 (20.0) | + | + | | | 53 | 18 | 10 | 5 |
| $bla_{SHV}$ | 2 | 2 | 4 (8.0) | | | + | | 84 | 44 | 38 | 11 |
| $bla_{SHV}$ | 7 | | 7 (14.0) | | | | + | 144 | 8 | 7 | 36 |
| $bla_{SHV}$ | | 7 | 7 (14.0) | | | | + | 237 | 42 | 33 | 96 |
| $bla_{TEM}$ + $bla_{SHV}$ | 7 | 15 | 22 (44.0) | + | | | + | 151 | 11 | 10 | 47 |

[a] Possible β-lactamase types were TEM-52 (pI 6.0), SHV-2a (pI 7.6), and SHV-12 (pI 8.2).
[b] Abbreviations: CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; AZT, aztreonam.

TABLE 5. Effect of storage on the stability of laboratory-prepared ceftazidime- and cefotaxime-clavulanic acid disks

| Storage (wk) | Zone diam (mm) for isolate, drug, and temp[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *K. pneumoniae* ATCC 700603 | | | | | | *K. pneumoniae* 99-3-R251 | | | *K. pneumoniae* 99-3-R256 | | | *E. coli* 99-3-U609 | | |
| | CAZ | CAZ-CLA | | CTX | CTX-CLA | | CAZ | CAZ-CLA | | CAZ | CAZ-CLA | | CAZ | CAZ-CLA | |
| | | 4°C | −20°C | | 4°C | −20°C | | 4°C | −20°C | | 4°C | −20°C | | 4°C | −20°C |
| 0 | 13 | 22 | 22 | 18 | 23 | 23 | 16 | 25 | 25 | 10 | 25 | 25 | 6 | 25 | 25 |
| 1 | 14 | 21 | 23 | 20 | 23 | 23 | 17 | 25 | 25 | 11 | 25 | 25 | 6 | 24 | 24 |
| 2 | 14 | 21 | 23 | 20 | 23 | 23 | 17 | 25 | 25 | 12 | 22 | 25 | 6 | 23 | 25 |
| 3 | 14 | 22 | 22 | 18 | 23 | 23 | 17 | 23 | 23 | 12 | 22 | 25 | 6 | 21 | 23 |
| 4 | 13 | 22 | 23 | 18 | 23 | 23 | 18 | 26 | 27 | 10 | 23 | 25 | 6 | 21 | 25 |
| 6 | 14 | 21 | 23 | 19 | 23 | 23 | 18 | 20 | 23 | 10 | 20 | 25 | 6 | 19 | 24 |
| 8 | 13 | 20 | 23 | 19 | 23 | 25 | 18 | 25 | 27 | 12 | 19 | 25 | 6 | 20 | 23 |
| 12 | 14 | 20 | 22 | 20 | 24 | 24 | 18 | 24 | 26 | 12 | 18 | 25 | 6 | 18 | 24 |
| 16 | 12 | 18 | 21 | 19 | 25 | 25 | 15 | 21 | 23 | 10 | 16 | 22 | 6 | 18 | 23 |
| 20 | 13 | 17 | 20 | 20 | 24 | 24 | 15 | 22 | 23 | 11 | 16 | 22 | 6 | 15 | 22 |

[a] Ceftazidime and cefotaxime disks were those used for routine susceptibility testing. Abbreviations: CAZ, ceftazidime; CAZ-CLA, ceftazidime-clavulanic acid; CTX, cefotaxime; CTX-CLA, cefotaxime-clavulanic acid.

become commercially available, they may be too expensive for some laboratories to purchase and, hence, those laboratories may still have to prepare their own. The method described herein enables laboratories to prepare large batches of disks at one time and store them for several months. By using preprepared and stored disks, laboratories will have a cost- and time-saving alternative for the routine confirmation of ESBL-producing isolates.

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## REFERENCES


1. **Bauernfeind, A., Y. Chong, and K. Lee.** 1998. Plasmid-encoded AmpC β-lactamases: how far have we gone 10 years after the discovery? Yonsei Med. J. **39:**520–525.
2. **Bergquist, P. L.** 1988. Incompatibility, p. 37–78. *In* K. G. Hardy (ed.), Plasmids. A practical approach. IRL Press, Oxford, United Kingdom.
3. **Bradford, P. A., C. Urban, N. Mariano, S. J. Projan, J. J. Rahal, and K. Bush.** 1997. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β-lactamase, and the loss of an outer membrane protein. Antimicrob. Agents Chemother. **41:**563–569.
4. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
5. **Chong, Y., K. Lee, R. Okamoto, and M. Inoue.** 1997. Characteristics of extended-spectrum β-lactam hydrolyzing activity of *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from clinical specimens. Korean J. Infect. Dis. **29:**477–485.
6. **Kim, J., Y. Kwon, H. Pai, J. W. Kim, and D. T. Cho.** 1998. Survey of *Klebsiella pneumoniae* strains producing extended-spectrum β-lactamases: prevalence of SHV-12 and SHV-2a in Korea. J. Clin. Microbiol. **36:**1446–1449.
7. **Lee, K., C. L. Chang, N. Y. Lee, H. S. Kim, K. S. Hong, H. C. Cho, and Korean Nationwide Surveillance of Antimicrobial Resistance Group.** 2000. Korean nationwide surveillance of antimicrobial resistance of bacteria in 1998. Yonsei Med. J. **41:**497–506.
8. **Livermore, D. M.** 1995. β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. **8:**557–584.
9. **Matthew, M., A. M. Harris, M. J. Marshall, and G. W. Ross.** 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. **88:**169–178.
10. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2–A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing; ninth informational supplement. M100–S9. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. **Pai, H., S. Lyu, J. H. Kim, J. M. Kim, Y. Kwon, J.-W. Kim, and K. W. Choe.** 1999. Survey of extended-spectrum β-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae:* prevalence of TEM-52 in Korea. J. Clin. Microbiol. **37:**1758–1763.
14. **Thomson, K. S., C. C. Sanders, and E. S. Moland.** 1999. Use of microdilution panels with and without β-lactamase inhibitors as a phenotypic test for β-lactamase production among *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens*. Antimicrob. Agents Chemother. **43:**1393–1400.
15. **Watt, C., M. Louie, and A. E. Simor.** 2000. Evaluation of stability of cefotaxime (30-μg) and ceftazidime (30-μg) disks impregnated with clavulanic acid (10 μg/ml) for detection of extended-spectrum β-lactamases. J. Clin. Microbiol. **38:**2796–2797.