JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 155–158 Vol. 41, No. 1
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.155–158.2003

Occurrence and Phenotypic Characteristics of Extended-Spectrum β-Lactamases among Members of the Family *Enterobacteriaceae* at the Tel-Aviv Medical Center (Israel) and Evaluation of Diagnostic Tests

Shiri Navon-Venezia,[1] Orly Hammer-Munz,[1] David Schwartz,[2] Dan Turner,[3] Boris Kuzmenko,[1] and Yehuda Carmeli[1,3]*

Laboratories for Molecular Epidemiology[1] and Microbiology[2] and Division of Infectious Diseases,[3] Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Received 7 August 2002/Returned for modification 1 October 2002/Accepted 20 October 2002

We assessed the prevalence and phenotypic characteristics of extended-spectrum β-lactamase (ESBL) producers among cefuroxime-resistant (CXM-R) (MIC ≥ 32 μg/ml) members of the family *Enterobacteriaceae* in our institution. The 438 CXM-R clinical isolates obtained from nonurine sources among inpatients were screened. ESBL production was confirmed by disk diffusion assay using cefpodoxime (CPD), cefotaxime (CTX), and ceftazidime (CTZ) with and without clavulanate (CLAV). A difference of ≥5 mm in the size of the zone of inhibition in the presence of CLAV for at least one of the agents was considered representative of the ESBL phenotype: 186 isolates (42.5%) were confirmed as ESBL producers. The isolates tested and the rates of ESBL producers were as follows: *Klebsiella* spp. ($n = 81$), 79%; *Proteus* spp. ($n = 58$), 62%; *Escherichia coli* ($n = 64$), 53%; *Enterobacter* spp. ($n = 69$), 42%; *Serratia* spp. ($n = 70$), 14%; *Citrobacter* spp. ($n = 25$), 24%; *Providencia* spp. ($n = 21$), 24%; *Morganella* spp. ($n = 41$), 5%; and *Kluyvera* ($n = 3$), 0%. The overall sensitivity of isolated ESBL confirmatory tests was 79% for CPD-CLAV, 66% for CTZ-CLAV, and 91% for CTX-CLAV. Sensitivities of CTZ-CLAV confirmatory tests for *Klebsiella* spp., *Proteus* spp., *E. coli*, and *Enterobacter* spp. were 84, 22, 76, and 62%, respectively, and those for CTX-CLAV were 95, 97, 94, and 83%, respectively. They were 90% for CPD-CLAV and CTZ-CLAV, 95% for CPD-CLAV and CTX-CLAV, and 100% for CTZ-CLAV and CTX-CLAV. ESBL production was highly prevalent among *Enterobacteriaceae*. Using resistance to CXM as an ESBL screening criterion is a suitable option in high-incidence areas where *Klebsiella* spp. are not the dominant ESBL producers. This screening criterion may simplify the screening test and improve its sensitivity, although at the price of testing more isolates. The CTX-CLAV combination confirmed ESBL producers better than the CTZ-CLAV combination, with sensitivity varying between species. Combined CTZ-CLAV and CTX-CLAV testing detected all these strains; CPD-CLAV provided no additional benefit.

Production of plasmid-mediated extended-spectrum β-lactamases (ESBLs) has emerged as an important mechanism of resistance to β-lactam antibiotics among members of the family *Enterobacteriaceae* (10). Over 100 such enzymes, which differ in genotype and substrates affinities, have been described to date (see http://www.lahey.org/studies/webt.htm). Several lines of evidence have suggested that the production of ESBL has an important clinical impact. Studies on the inoculum effect (8), animal experiment data (18), and limited clinical data (3, 11, 13; L. B. Rice, J. D. C. Yao, K. Klimm, G. M. Eliopoulos, and R. C. Moellering, Jr., Letter, Antimicrob. Agents Chemother. **35:**1243-1244, 1991) have raised the possibility that using cephalosporins and penicillins to treat infections caused by ESBL-producing organisms may result in adverse treatment outcomes (15). Detection of ESBL producers poses a special challenge for clinical microbiology laboratories: although ESBL producers are able to hydrolyze extended-spectrum penicillins, cephalosporins, and aztreonam, the MICs of some and perhaps even all of these agents may be within the susceptible range.

Identification of ESBL producers by clinical laboratories is based on their phenotypic characteristics, i.e., the presence of an increased MIC of extended-spectrum cephalosporins and reduction in the MIC in the presence of a beta-lactamase inhibitor (9). Since these tests are expensive and time-consuming, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended that these specialized tests be performed based on screening criteria which include an elevated MIC of one of few cephalosporins and/or aztreonam. The NCCLS guide recommends that ESBL producers be reported as resistant to all penicillins and cephalosporins as well as to aztreonam, even when they are shown to be susceptible to these agents by conventional tests (12). These guidelines refer specifically to *Klebsiella pneumonia*, *Klebsiella oxytoca*, and *Escherichia coli*. Indeed, clinical attention has focused on the occurrence of ESBLs among these genera, the ones most likely to be ESBL producers in the United State (19). However, ESBL producers have been found in many other genera, including other members of the family *Enterobacteriaceae*, such as *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, and *Serratia* species (2).

ESBLs are widespread all over the world, but the prevalence

* Corresponding author. Mailing address: Divisions of Infectious Diseases and Epidemiology, Tel-Aviv Sourasky Medical Center, 6 Weizman St., Tel-Aviv 64239, Israel. Phone: 972-3-692 5644. Fax: 972-3-6974996. E-mail: ycarmeli@caregroup.harvard.edu.

and phenotypic characteristics among clinical isolates may vary between geographical areas (19). In this study, we aimed to systematically examine the prevalence of ESBL producers among members of the *Enterobacteriaceae* family in our institution, to characterize their phenotypes, and to evaluate the diagnostic tests that are used to detect ESBL production.

MATERIALS AND METHODS

Setting and study design. The Tel-Aviv Sourasky Medical Center is a 1,200-bed tertiary-care university-affiliated hospital located in central Israel. The clinical microbiology laboratory is ISO 9002 certified and processes over 85,000 clinical cultures annually. All gram-negative bacteria isolated during the study period were identified to the species level, and their susceptibilities to various antibiotics—including cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CTZ), cefepime, and aztreonam—were determined by means of an automated identification and microdilution system using an overnight panel (Microscan; Dade International Inc., West Sacramento, Calif.). The results were recorded and interpreted according to NCCLS guidelines (12).

The bacterial isolates selected for this study included 438 clinical nonurine isolates, all members of the family *Enterobacteriaceae* for which the MIC of CXM is ≥32 μg/ml. They were collected at convenient times during a 9-month period (April 2000 to December 2000) and stored at −70°C in brain heart infusion containing 10% glycerol until further workup.

ESBL detection tests were based on the Kirby-Bauer disk diffusion test methodology (1). For determining the ESBL phenotype, the zones of inhibition of each isolate were tested on Mueller-Hinton agar plates (Hy-Labs, Rehovot, Israel) using disks containing 30 μg of cefpodoxime (CPD), CTX, and CTZ, either alone or in combination with 10 μg of clavulanic acid (catalogue numbers: CT1612B, CT407B, CT1629B, CT1596B, CT1598B, and CT1597B, respectively; Oxoid, Hampshire, England). An organism was classified as having an ESBL producer phenotype if the zone of inhibition differed by ≥5 mm between at least one of the combination disks and its corresponding standard antibiotic disk. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used daily as negative and positive controls for ESBL production, respectively. The sensitivity of each agent to detect an ESBL phenotype was determined relative to all agents in combination.

Based upon these results, the percentage of ESBL producers for a given species was calculated by multiplying the percentage of isolates with an ESBL phenotype among CXM-resistant isolates of the same species by the percentage of CXM-resistant isolates among nonurine hospital isolates of the same species.

RESULTS

Prevalence of ESBL-producing strains. A total of 438 CXM-resistant members of the family *Enterobacteriaceae* were studied (51 blood isolates and 387 from pus, wounds, or sputum). The isolates were tested by using the three combination disks, and 186 isolates (42.5%) were confirmed as being ESBL producers (Table 1). Among the CXM-resistant isolates, the ESBL-producing phenotype was found most frequently among *Klebsiella* species (*K. pneumoniae* [n=57], *K. oxytoca* [n=4], *Klebsiella ornithinolytica* [n = 1], and other *Klebsiella* species [n = 2]), followed by *Proteus* species (*Proteus mirabilis* [n=29], *Proteus penneri* [n=2], and other *Proteus* species [n = 5]), *E. coli*, and *Enterobacter* species (*Enterobacter cloacae* [n=17], *Enterobacter aerogenes* [n = 7], *Enterobacter gergoviae* [n=1], and other *Enterobacter* species [n = 3]). The ESBL phenotype was least prevalent among *Serratia*, *Morganella*, and *Kluyvera* species. When the prevalence of the ESBL producer phenotype among all hospital isolates was calculated, *Klebsiella* species and *Proteus* species showed the highest rate of ESBL producers (35%), followed by *Enterobacter* spp. (22%) and then *Providencia* spp., *Citrobacter* spp., *Serratia* spp., and *E. coli*, with rates ranging between 12 and 18% (Table 1).

TABLE 1. Occurrence of ESBL-producing phenotypes among members of the family *Enterobacteriaceae*

Organism	No. of CXM-resistant isolates studied		% of all hospital isolates that were:	
	Total	Isolates with ESBL phenotype[a]	CXM resistant	Calculated to have ESBL phenotype[b]
Klebsiella sp.	82	64 (78)	45	35
Serratia sp.	72	10 (14)	100	14
Enterobacter sp.	69	28 (41)	54	22
E. coli	65	34 (52)	23	12
Proteus sp.	59	36 (61)	58	35
Morganella sp.	41	2 (5)	90	4.5
Citrobacter sp.	25	7 (28)	56	16
Providencia sp.	22	5 (23)	80	18
Kluyvera sp.	3	0	54	0
Total	438	186 (42.5)		

[a] Values in parentheses are percentages.
[b] Calculated by multiplying the percentage of isolates with an ESBL phenotype among CXM-resistant isolates of the same species to the percent of CXM-resistant isolates among nonurine hospital isolates of the same species.

Performance of screening tests. The MICs for all hospital isolate members of the family *Enterobacteriaceae* were determined for CTX, CTZ, and aztreonam. For all hospital isolates that are CXM susceptible (or intermediate susceptible), the MICs of each of these agents was <2 μg/ml. Of the 438 CXM-resistant isolates, the MIC of CTX for 278 (63.5%) was ≥2 μg/ml, the MIC of CTZ for 246 (56.2%) was ≥2 μg/ml, and the MIC of aztreonam for 236 (53.9%) was ≥2 μg/ml. Among the isolates confirmed to have an ESBL producer phenotype, for 15 (8%) the MIC of CTX was <2 μg/ml, for 41 (22%) the MIC of CTZ was <2 μg/ml, and for 31 (16.7%) the MIC of aztreonam was <2 μg/ml. The distributions according to genera are presented in Table 2. The respective sensitivities, specificities, and negative predictive values for screening CXM-resistant isolates with each agent were 91.9, 57.5, and 90.6% for CTX; 78, 59.9, and 51.7% for CTZ; and 83.3, 67.9, and 84.7% for aztreonam. Among the ESBL-producing strains, for 9 the MICs of aztreonam and CTX were <2 μg/ml, for 27 the MICs of aztreonam and CTZ were <2 μg/ml, for 8 the MICs of both CTX and CTZ were <2 μg/ml, and for 7 the MICs of all three agents were <2 μg/ml.

Confirmatory tests. The performance of each of three confirmatory tests (i.e., CPD, CTX, and CTZ with and without clavulanate) to detect an ESBL phenotype among members of the family *Enterobacteriaceae* was evaluated in comparison to the performance of all three in combination. The overall sensitivities of the tests were 79% for CPD with and without clavulanate, 66% for CTZ with and without clavulanate, and 91% for CTX with and without clavulanate. We also evaluated the performance of using two combination disks as a confirmatory test: combining CPD and CTZ (each with and without clavulanate) resulted in a test sensitivity of 90%, combining CPD with CTX resulted in 95% sensitivity, and combining CTZ with CTX resulted in 100% sensitivity.

The performance of the ESBL confirmatory tests in each of the four major ESBL producer genera in our institution was evaluated (Table 3). Confirmatory tests based on CTX were

TABLE 2. Phenotypes of isolates studied according to three screening determining agents

Organism	All isolates tested				Isolates with ESBL-producing phenotype			
	Total	No. of isolates for which MIC was ≥2 μg/ml			Total	No. (%) of isolates for which MIC was <2 μg/ml		
		CTX	CTZ	AZT[a]		CTX	CTZ	AZT
Klebsiella sp.	82	69	66	69	64	4 (6.3)	4 (6.3)	3 (4.7)
Serratia sp.	72	22	20	15	10	2 (20)	2 (20)	3 (30)
Enterobacter sp.	69	54	51	53	28	0	0	1 (3.6)
E. coli	65	49	46	45	34	3 (8.8)	4 (12)	4 (12)
Proteus sp.	59	45	17	23	36	3 (8.3)	27 (75)	18 (50)
Morganella sp.	41	11	13	7	2	1 (50)	2 (100)	2 (100)
Citrobacter sp.	25	16	14	14	7	1 (14)	1 (14)	1 (14)
Providencia sp.	22	10	17	8	5	1 (20)	1 (20)	0
Kluyvera sp.	3	2	2	2	0			
Total	438	278	246	236	186	15 (8)	41 (22)	31 (17)

[a] AZT, aztreonam.

more sensitive for each genus examined than were the tests based on CTZ or CPD. This was most prominent in confirming ESBL production among *Proteus* spp.

DISCUSSION

We sought to assess the prevalence and phenotypic characteristics of ESBL producers among CXM-resistant members of the family *Enterobacteriaceae* in our institution. Our findings revealed overall high prevalence. We found isolates that expressed an ESBL producer phenotype among every genus that was tested, with the exception of *Kluyvera* spp., of which only few isolates were available for testing. The prevalence among genera varied, with rates ranging between 35% among *Klebsiella* spp. and *Proteus* sp. isolates and from 12 to 22% for all other genera (except for *Morganella* spp., which expressed an ESBL phenotype in 4.5% of the isolates). Thus, in contrast to the attention paid mostly to ESBLs in *E. coli* and *Klebsiella* spp., our results pointed to the ESBL producer phenotype as being common in our institution among the various members of the family *Enterobacteriaceae*: it was equally prevalent among *Proteus* spp. and *Klebsiella* spp., and its occurrence in most other genera was at least within the same high range as in *E. coli*.

The prevalence of strains expressing the ESBL phenotype may vary across geographical regions (5, 16, 17, 19). Most previous studies had focused on *K. pneumoniae*, and high rates of ESBL production have been reported among strains in Latin America (45%), Italy (37%), in New York City, N.Y. (44%) (16, 19). However, the overall rate of CTZ resistance in the United States as a whole, as stated in the Centers for Disease Control and Prevention Project ICARE report, is 8% (6). This discrepancy emphasizes the great geographical diversity of ESBL occurrence even within one country, as well as the difficulty in presenting comprehensive data on this issue.

TABLE 3. Sensitivities of ESBL confirmatory tests for various species

Combination disk	Sensitivity (%) for:			
	Klebsiella spp.	*Proteus* spp.	*E. coli*	*Enterobacter* spp.
CPD-clavulanate	95	94	88	34
CTX-clavulanate	95	97	94	83
CTZ-clavulanate	84	22	76	62

We elected to use the SENTRY data (19) for comparing the prevalence of ESBL production in our area to that reported in other geographical areas. The rates of the ESBL producer phenotype were higher in our center than those in the SENTRY report: our respective prevalence of ESBL phenotype in *Klebsiella* species, *Proteus* species, and *E. coli* were 35, 35, and 12%, while those in Latin America were 45, 22, and 8.5%; those in the Western Pacific region were 25, 1.8, and 7.9%; those in Europe were 23, 11, and 5.3%; and those in the United States were 7.6, 5, and 3.3%.

The NCCLS recommendations at the time of the present study were to screen for ESBL production in *E. coli* and *Klebsiella* spp. using the criterion of an MIC of one of the following five agents of ≥2 μg/ml: CPD, CTZ, aztreonam, CTX, or ceftriaxone (12). We evaluated the performance of conducting screening with CTZ, aztreonam, and CTX and found considerable differences between the agents. Specifically, for 15 (8%) of the isolates with an ESBL producer phenotype the MIC of CTX was <2 μg/ml, for 41 (22%) the MIC of CTZ was <2 μg/ml, and for 31 (16.7%) the MIC of aztreonam was <2 μg/ml. Thus, CTX had the highest negative predictive value (91%) in our hospital. Using the criterion of an elevated MIC as being indicative of ESBL to any of the three agents would have detected all 64 ESBL-producing *Klebsiella* species, but seven isolates that were members of other genera which had an ESBL producer phenotype would have been missed. When resistance to CXM was added to the screening criteria, sensitivity for the detection of ESBLs improved to 100%.

Advantages of screening that uses criteria of CXM resistance. Screening that uses criteria of CXM resistance has several advantages. (i) Monitoring of CXM resistance is more feasible than continuously monitoring the MICs of several agents for multiple genera. (ii) It improves the negative predictive value for genera other than *Klebsiella* species. (iii) No isolates for which the MIC of any of the extended spectrum cephalosporins was ≥2 μg/ml occurred among CXM-susceptible isolates in our study. Using this screening criterion, how-

ever, will increase the number of isolates that will be selected for confirmatory testing. Nevertheless, we believe that this screening criterion is a suitable option in high-incidence areas where *Klebsiella* spp. are not the dominant ESBL producers.

We evaluated three agents for the use in ESBL confirmatory testing. Overall, CTX performed best in detecting ESBL producers. Its superiority was most apparent for confirming ESBL production in *Proteus* species, probably as a result of the existence of ESBLs with high affinity to this agent. In contrast to our results, Winokur et al. reported that CTZ appeared to be a better reagent for detecting an ESBL than ceftriaxone (19). This discrepancy might be due to differences in dominant enzymes in various geographical areas.

Our results demonstrate the importance of adapting the diagnostic tests for ESBL detection to a particular geographical area, a particular hospital, and particular genera. Moreover, we believe that these tests need to be evaluated periodically, given that their performance may change with the introduction of new enzymes.

Note that by being based on phenotypic testing, the tests we conducted may have shown false-negative results due to the coexistence of chromosomal beta-lactamases (4, 7) or to the presence of plasmid-mediated enzymes that are not inhibited by beta-lactamase inhibitors (5, 14). In addition, it is very likely that the particular enzymes harbored by the tested organisms might influence test performance.

This systematic assessment of diagnostic tests aimed at detecting an ESBL phenotype among members of the *Enterobacteriaceae* revealed a high incidence of this phenotype among various genera in our institution. It also highlighted the need for local evaluation of the tests chosen in a particular setting as well as the need for guidelines for ESBL testing of species other than *E. coli* and *Klebsiella* spp.

ACKNOWLEDGMENTS

Esther Eshkol is thanked for editorial assistance.

This work was supported by research grants from Neopharm LTD, Petach Tikva, Israel, and from the United States-Israel Binational Science Foundation, Jerusalem, Israel.

REFERENCES

1. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45:**493–496.
2. **Bradford, P. A.** 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14**:933–951.
3. **Brun-Buisson, C., P. Legrand, A Philippon, F. Montravers, M. Ansquer, and J. Duval.** 1987. Transferrable enzymatic resistance to third-generation cephalosporines during nosocomial outbreak of multiresistant *K. pneumoniae*. Lancet **ii:**302–306.
4. **Bush, K., R. K. Flamm, S. Ohringer, S. B. Singer, R. Summerill, and D. P. Bonner.** 1991. Effect of clavulanic acid on activity of β-lactam antibiotics in *Serratia marcescens* isolates producing both a TEM β-lactamase and a chromosomal cephalosporinase. Antimicrob. Agents Chemother. **35:**2203–2208.
5. **Champs, C. D., S. C. Chanal, R. Bonnet, J. Sirot, and the French Study Group.** 2000. A 1998 survey of extended-spectrum β-lactamases in *Enterobacteriaceae* in France. Antimicrob. Agents Chemother. **44:**3177–3179.
6. **Fridkin, S. K.** 2001. Increasing prevalence of antibacterial resistance in intensive care units. Crit. Care Med. **29**(Suppl. 4)**:**64–68.
7. **Huber, T. W., and J. S. Thomas.** 1994. Detection of resistance due to inducible β-lactamase in *Enterobacter aerogenes* and *Enterobacter cloacae*. J. Clin. Microbiol. **32:**2481–2486.
8. **Jacoby, G. A.** 1997. Extended spectrum β-lactamase and other enzymes providing resistance to oxyimino β-lactams. Infect. Dis. Clin. N. Am. **11:**875–887.
9. **Jacoby, G. A., and P. Han.** 1996. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *E. coli*. J. Clin. Microbiol. **34:**908–911.
10. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
11. **Lautenbach, E., J. B. Patel, W. B. Bilker, P. H. Edelstein, and N. O. Fishman.** 2001. Extended-spectrum b-lactamase-producing *E. coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis. **32:**1162–1171.
12. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing. Twelfth informational supplement. Approved standard M100-S12. NCCLS, Wayne, Pa.
13. **Paterson, D. L., W. C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. Mccormack, and V. L. Yu.** 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol. **39:**2206–2212.
14. **Phillipon, A., G. Arlet, and G. A. Jacoby.** 2002. Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents Chemother. **46:**1–11.
15. **Rasheed, J. K., C. Jay, B. Methcode, F. Berkowitz, L. Weigel, J. Crellin, C. Steward, B. Hill, A. A. Medeiros, and F. C. Tenover.** 1997. Evolution of extended-spectrum β-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. **41:**647–653.
16. **Saurina, G., J., M. Quale. V. M. Manikal, E. Oydna, and D. Landman.** 2000. Antimicrobial resistance in *Enterobacteriaceae* in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. J. Antimicrob. Chemother. **45:**895–898.
17. **Spanu, T., F. Luzzaro, M. Perilli, G. Amicosante, A. Toniolo, G. Fadda, and the Italian ESBL Study Group.** 2002. Occurrence of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae* in Italy: implications for resistance to β-lactams and other antimicrobial drugs. Antimicrob. Agents Chemother. **46:**196–202.
18. **Thauvin-Eliopoulos, C., M. F. Tripodi, R. C. Moellering, Jr., and G. M. Eliopolous.** 1997. Efficacy of piperacillin-tazobactam and cefepime in rats with experimental intra-abdominal abscesses due to an extended-spectrum β-lactamase-producing strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **41:**1053–1057.
19. **Winokur, P. L., R. Canton, J. M. Casellas, and N Legakis.** 2001. Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin. Infect. Dis. **32**(Suppl. 2)**:**S94–S103.