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Trends in Production of Extended-Spectrum β-Lactamases among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey

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Results of a 2003 survey carried out in Italy to evaluate the prevalence of extended-spectrum β-lactamase (ESBL)-producing enterobacteria are presented. Eleven Italian Microbiology Laboratories investigated 9,076 consecutive nonreplicate isolates (inpatients, 6,850; outpatients, 2,226). ESBL screening was performed by MIC data analysis. Confirmation was obtained using the double-disk synergy test and the combination disk test based on CLSI methodology. ESBL determinants were investigated by colony blot hybridization and confirmed by sequencing. Results were compared to those of the 1999 Italian survey (8,015 isolates). The prevalence of ESBL producers was 7.4% among isolates from inpatients (in 1999, 6.3%) and 3.5% among outpatients (no data were available for 1999). Among hospitalized patients, the most prevalent ESBL-positive species was *Escherichia coli* (*Klebsiella pneumoniae* in 1999). *Proteus mirabilis* was the most prevalent ESBL-positive species among outpatients. In both groups, most ESBL-positive pathogens were obtained from urinary tract infections. TEM-type ESBLs were the most prevalent enzymes (45.4%). Non-TEM, non-SHV determinants emerged: CTX-M-type in *E. coli* and *K. pneumoniae*, and PER-type in *P. mirabilis*, *Providencia* spp., and *E. coli*. With the exception of 3/163 *P. mirabilis* isolates and 1/44 *Providencia stuartii* isolate (all of which were intermediate for imipenem), carbapenems were active against all ESBL-positive enterobacteria. Susceptibility to other drugs was as follows: 84.7% for amikacin, 84.4% for piperacillin-tazobactam, 48.0% for gentamicin, and 32.8% for ciprofloxacin. Carbapenems appear to be the drug of choice. Amikacin and β-lactam/β-lactamase inhibitor combinations represent an alternative in non-life-threatening infections. The appearance of ESBL-positive enterobacteria in the community makes it mandatory that family physicians learn how to treat these pathogens.

Extended-spectrum β-lactamases (ESBLs) are enzymes capable of hydrolyzing a wide range of expanded-spectrum β-lactams, including most recent cephalosporins, but which are inactive against cephamycins and carbapenems (15). To date, more than 150 different natural ESBL variants (which are detected most frequently in enterobacteria) are known, and they represent a worldwide problem in hospitalized patients (29). Infections caused by ESBL-positive organisms often involve compromised patients, making it difficult to eradicate these pathogens in high-risk wards such as intensive care units (1).

The overall prevalence of ESBL-positive enterobacteria varies greatly among different geographical areas, with the highest reported value (44.9%) in Latin America (32). According to published reports, in Europe, ESBLs appeared to be increasing among enterobacteria in the periods 1997 through 1999 to 2001 and 2002 (3, 21). However, the prevalence of ESBLs differs from country to country, with the highest percentages in Greece (27.4%) and Portugal (15.5%) and the lowest in The Netherlands and Germany (2.0 and 2.6%, respectively) (3). *Klebsiella pneumoniae* and *Escherichia coli* are the most common ESBL-positive species, but all enterobacteria can harbor plasmid-mediated ESBL genes (4). Recently, ESBL-positive isolates of *Proteus mirabilis* have been reported from France (7), different European countries (21), and New York City (26). In enterobacteria, classical ESBLs evolved from the TEM and SHV families (4). In recent years, several new ESBLs of non-TEM, non-SHV types emerged, such as enzymes of the CTX-M, PER, VEB, and GES lineages (13). Particularly, CTX-M-type enzymes increased in *E. coli* and *K. pneumoniae* isolates from Spain, the United Kingdom, and Russia (2, 8, 16).

In Italy, a nationwide survey carried out in 1999 among hospitalized patients (28) showed the following: (i) 6.3% of enterobacterial isolates were ESBL positive; (ii) ESBL determinants were more often detected in *K. pneumoniae* and *P. mirabilis*; and (iii) in the large majority of isolates (92.4%), enzymes of the TEM and/or the SHV family accounted for the ESBL phenotype.

Monitoring the ESBL prevalence and type in enterobacteria of clinical interest may contribute to delineating the breadth of the problem and to defining appropriate therapeutic options (29). The present nationwide survey aimed at evaluating the prevalences of different ESBL types and the associated resistance determinants in enterobacterial isolates obtained from both inpatients and outpatients. The goal was to analyze

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TABLE 1. Inpatients: prevalence of ESBL-positive strains among enterobacterial isolates[a]

Species	Total no. of isolates	ESBL-positive isolates		
		No. of isolates	% of total[b]	% in species[b]
Escherichia coli	3,648	161	31.9 (10.8)	4.4 (1.2)
Proteus mirabilis	514	132	26.2 (25.7)	25.7 (16.3)
Klebsiella pneumoniae	748	76	15.1 (37.1)	10.2 (20.0)
Enterobacter aerogenes	212	38	7.5 (6.0)	17.9 (20.5)
Providencia stuartii	98	36	7.1 (5.3)	36.7 (28.1)
Klebsiella oxytoca	213	17	3.4 (4.9)	8.0 (15.1)
Enterobacter cloacae	478	15	3.0 (2.4)	3.1 (2.9)
Citrobacter freundii	148	12	2.4 (2.4)	8.1 (4.7)
Serratia marcescens	196	10	2.0 (2.2)	5.1 (4.9)
Citrobacter koseri	91	4	0.8 (1.2)	4.4 (12.2)
Morganella morganii	189	1	0.2 (1.8)	0.5 (4.7)
Citrobacter amalonaticus	12	1	0.2 (0.0)	8.3 (0.0)
Providencia rettgeri	11	1	0.2 (0.0)	9.1 (0.0)
Other species[c]	292	0	0.0 (0.0)	0.0 (0.0)
Total	6,850	504	7.4 (6.3)	

[a] Isolates shown to produce ESBLs by synergy tests and molecular methods.
[b] In parenthesis, the percentage of ESBL-positive isolates detected in the 1999 survey is shown for comparison.
[c] Other species included *Salmonella enterica*, *Citrobacter braakii*, *Enterobacter sakazakii*, *Serratia liquefaciens*, *Proteus vulgaris*, *Proteus penneri*, and *Hafnia alvei*.

TABLE 2. Outpatients: prevalence of ESBL-positive strains among enterobacterial isolates[a]

Species	Total no. of isolates	ESBL-positive isolates		
		No. of isolates	% of total	% in species
Proteus mirabilis	209	31	39.2	14.8
Escherichia coli	1,454	27	34.2	1.9
Providencia stuartii	58	8	10.1	13.8
Klebsiella pneumoniae	192	5	6.3	2.6
Enterobacter aerogenes	48	4	5.1	8.3
Morganella morganii	44	2	2.5	4.5
Klebsiella oxytoca	31	1	1.3	3.2
Citrobacter koseri	16	1	1.3	6.3
Other species[b]	174	0	0.0	0.0
Total	2,226	79	3.5	

[a] Isolates shown to produce ESBLs by synergy tests and molecular methods.
[b] Other species included *Salmonella enterica*, *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter freundii*, and *Proteus vulgaris*.

changes in ESBL types and antimicrobial susceptibilities as compared to the 1999 survey.

MATERIALS AND METHODS

Design of the study. Eleven clinical microbiology laboratories distributed throughout Italy were enrolled in the study. Hospitals of the following cities were represented: Bergamo, Milano, Novara, Varese, Verona, Firenze, Ancona, Napoli, Sassari, Catania, and Palermo. From September to December 2003 (4 months), up to 1,000 consecutive, nonreplicate enterobacterial isolates were collected at each laboratory (750 from inpatients and 250 from outpatients). Each isolate was identified to the species level. Susceptibilities to different β-lactams were evaluated by automated methods using either the Vitek system (bioMérieux, Marcy l'Etoile, France) or the Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD). Ampicillin-susceptible strains of *E. coli*, *P. mirabilis*, *Salmonella enterica* spp., and *Shigella* spp. were regarded as non-ESBL producing and were not further investigated. Ampicillin-resistant isolates of the above strains, as well as any other enterobacterial species, were suspected to produce ESBLs when showing a MIC of ≥2 μg/ml for ceftazidime and/or cefotaxime and/or ceftriaxone and/or aztreonam. The isolates were stored at −70°C at each laboratory and shipped to reference centers (Varese and Catania) for controlling species identification and defining the resistance phenotype. A case report form containing the laboratory code, patient's data, ward of admission, specimen source, and species identification was shipped with each suspected ESBL-positive isolate.

Identification and phenotypic characterization of ESBL-positive isolates. At reference centers, presumptive ESBL-positive isolates were reidentified to the species level by the API system (bioMérieux). Bacteriological media and susceptibility disks were obtained from Oxoid Limited, Basingstoke, United Kingdom. Confirmatory tests for ESBL production were performed according to species groups.

***E. coli*, *Klebsiella* spp., and *P. mirabilis*.** Unless specified otherwise, 10-μg clavulanate disks were used throughout. (i) For quantitative assay, based on the methodology of the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (20), susceptibilities to cefotaxime (30 μg) and ceftazidime (30 μg) alone and plus clavulanate were determined. As recommended, results were positive when the drug tested in combination with clavulanate produced a ≥5-mm increase in the zone diameter over its zone when tested alone. (ii) For qualitative assay, medium and inoculum were as described above. Disks containing aztreonam (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), and ceftazidime (30 μg) were placed around a disk containing amoxicillin (20 μg) plus clavulanate. Center-to-center distance for *E. coli* and *Klebsiella* spp. was 25 mm and for *P. mirabilis*, 30 mm. Results were interpreted according to the method of Jarlier (14).

Enterobacteria other than *E. coli*, *Klebsiella* spp., and *P. mirabilis*. (i) Quantitative assays were performed and interpreted as above. (ii) For qualitative assay, medium, inoculum, and disks were as described above. The only exception was that ceftriaxone was replaced with cefepime (30 μg), since this drug is more stable than other extended-spectrum cephalosporins to hydrolysis by AmpC β-lactamases (15). Thus, cefepime was used for detecting synergistic activity in enterobacteria overproducing AmpC. The center-to-center distance was 25 mm. *Enterobacter* spp. isolates were tested at both 20 and 25 mm. Results were interpreted according to the method of Jarlier (14).

Two additional combination tests were used for both groups: cefpodoxime (10 μg) alone or with clavulanate (1 μg) and cefpirome (30 μg) alone or with clavulanate (10 μg). Cefpodoxime is regarded as a useful tool for analyzing suspected ESBL production (20), whereas cefpirome (as cefepime) is stable in the presence of AmpC enzymes (15).

Resistance phenotype of ESBL-positive isolates. Susceptibility of ESBL-positive isolates was evaluated by the disk diffusion technique according to CLSI guidelines (20). The following drugs were tested: amoxicillin plus clavulanate (20 plus 10 μg), ampicillin plus sulbactam (10 plus 10 μg), piperacillin plus tazobactam (100 plus 10 μg), cefoxitin (30 μg), imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), gentamicin (10 μg), and ciprofloxacin (5 μg). Results were interpreted on the basis of CLSI criteria (20).

Molecular methods. Isolates confirmed as ESBL producing were investigated by colony blot hybridization, using random-primed ^{32}P-labeled DNA probes for bla_{TEM}-, bla_{SHV}-, bla_{PER}-, and $bla_{CTX\text{-}M}$-type genes essentially as described previously (17, 22, 24). The ESBL nature of TEM- and SHV-type determinants was confirmed by sequencing as described previously (24).

RESULTS

Prevalence of ESBL-producing organisms. A total of 9,076 nonreplicate isolates (6,850 from hospitalized patients and 2,226 from outpatients) were studied during a 4-month period (September to December 2003). Overall, 504/6,850 (7.4%) and 79/2,226 (3.5%) isolates showed synergy between clavulanate and at least one β-lactam. The prevalence of ESBL-positive isolates was different among participating centers (inpatients, range, 3.7 to 12.7%; outpatients, range, 0.9 to 7.3%).

Among inpatients (Table 1), *E. coli* (n = 161; 31.9%) was the most common ESBL-positive species, followed by *P. mirabilis* (n = 132; 26.2%), *K. pneumoniae* (n = 76; 15.1%), *Enterobacter aerogenes* (n = 38; 7.5%), and *Providencia stuartii* (n = 36; 7.1%). When data were expressed as ESBL prevalence within each species, *P. stuartii* and *P. mirabilis* appeared to

TABLE 3. Distribution of gene types among ESBL-producing enterobacteria[a]

Species	No. of ESBL-positive isolates	No. (% within species) of isolates with gene type				
		TEM type only	SHV type only	TEM and SHV	Non-TEM, non-SHV	
					CTX-M[b]	PER[c]
Escherichia coli	188	50 (26.6)	22 (11.7)	12 (6.4)	103 (54.8)	1 (0.5)
Proteus mirabilis	163	158 (96.9)	0 (0.0)	0 (0.0)	0 (0.0)	5 (3.1)
Klebsiella pneumoniae	81	0 (0.0)	47 (58.0)	24 (29.6)	10 (12.4)	0 (0.0)
Providencia stuartii	44	36 (81.8)	0 (0.0)	0 (0.0)	0 (0.0)	8 (18.2)
Enterobacter aerogenes	42	8 (19.0)	24 (57.2)	10 (23.8)	0 (0.0)	0 (0.0)
Klebsiella oxytoca	18	2 (11.1)	14 (77.8)	2 (11.1)	0 (0.0)	0 (0.0)
Enterobacter cloacae	15	2 (13.3)	6 (40.0)	7 (46.7)	0 (0.0)	0 (0.0)
Citrobacter freundii	12	0 (0.0)	7 (58.3)	5 (41.7)	0 (0.0)	0 (0.0)
Serratia marcescens	10	2 (20.0)	4 (40.0)	4 (40.0)	0 (0.0)	0 (0.0)
Citrobacter koseri	5	5 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Morganella morganii	3	2 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
Citrobacter amalonaticus	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)
Providencia rettgeri	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
Total	583	265 (45.4)	124 (21.3)	64 (11.0)	115 (19.7)	15 (2.6)

[a] Isolates shown to encode different ESBL types by hybridization with specific probes.
[b] CTX-M-type enzymes were frequently associated with TEM-type enzymes.
[c] PER-type enzymes were frequently associated with TEM-type enzymes.

harbor these enzymes at the highest frequency (36.7% and 25.7%, respectively). ESBL prevalence in *E. coli* was relatively low (4.4%). Among outpatients (Table 2), *P. mirabilis* ($n = 31$; 39.2%) and *E. coli* ($n = 27$; 34.2%) were the most frequent ESBL producers, followed by *P. stuartii* ($n = 8$; 10.1%) and *K. pneumoniae* ($n = 5$; 6.3%).

Patient population and source of specimens. Overall, 8,926 patients were studied (6,720 inpatients and 2,206 outpatients). The mean age was 58 years (median, 66; standard deviation, ±26). ESBL-positive enterobacteria were isolated from 558 patients. The mean age of patients infected by ESBL producers was 62 years (median, 71; standard deviation, ±28).

Isolates carrying ESBL determinants were obtained most frequently from urine samples in both hospital and community patients (65.8% and 86.1%, respectively). The remaining samples were obtained from respiratory tract infections (10.4%, inpatients; 8.9%, outpatients), surgical wounds (8.8%, inpatients; none, outpatients), bloodstream infections (6.5%, inpatients; none, outpatients). Among the hospitalized patients, ESBL-positive isolates were most frequently detected in medical (52.4%) and surgical (24.0%) wards but also from ICUs (16.5%), pediatrics (5.3%), and oncohematology wards (1.8%).

Distribution of β-lactamase gene types in enterobacterial species. The presence of β-lactamase genes of the TEM, SHV, CTX-M, or PER type was investigated in ESBL-producing isolates by colony blot hybridization. The ESBL nature of TEM and SHV determinants was confirmed by sequencing. Table 3 shows the distribution of different types of β-lactamase genes in enterobacterial species. TEM-type enzymes were more prevalent than SHV-type enzymes (45.4% versus 21.3%, respectively). Eleven percent of ESBL-positive enterobacteria hybridized with both TEM and SHV probes. In these isolates, at least one of the two gene types accounted for the ESBL phenotype. Hybridization with the CTX-M- or PER-type probes occurred in a remarkable number of isolates (19.7% and 2.6%, respectively). Of interest is that CTX-M-type genes were frequently associated (89%) with TEM-type determinants in *E. coli*. This association was not detected in *K. pneumoniae*. PER-type genes were frequently associated with TEM-type determinants in *P. mirabilis* (100%) and *Providencia* spp. (78%).

As shown in Table 3, TEM-type ESBLs were particularly prevalent in *P. mirabilis*, *P. stuartii*, *Morganella morganii*, and *Citrobacter koseri*, whereas SHV-type enzymes (either alone or in association with TEM-type determinants) were widely distributed in *Klebsiella* and *Enterobacter* species. CTX-M-type enzymes were found most frequently in *E. coli* and *K. pneumoniae*. Notably, in *E. coli* these enzymes accounted for more than 50% of ESBL determinants. Finally, PER-type determinants were detected (though at a low rate) in *P. mirabilis*, *Providencia* spp., and *E. coli*.

In vitro susceptibility to clinically relevant drugs. Drugs potentially active against ESBL-positive *Enterobacteriaceae* include β-lactam/β-lactamase inhibitor combinations, cephamycins, carbapenems, aminoglycosides, and fluoroquinolones. As shown in Table 4, meropenem (100%) and imipenem (99.3%) were the most active drugs against ESBL-positive isolates. The only exception was represented by three *P. mirabilis* isolates and one *P. stuartii* isolate classified as intermediate to imipenem. Cefoxitin, which is not hydrolyzed by ESBLs, was active against 83.9% of isolates. High cefoxitin resistance was found in *Enterobacter* spp. (>85%), *Serratia marcescens* (100%), and *Citrobacter freundii* (100%), likely due to the expression of the chromosomal AmpC β-lactamase. Among β-lactam/β-lactamase inhibitor combinations, ampicillin-sulbactam was the least effective (49.1%), whereas amoxicillin-clavulanate and piperacillin-tazobactam were more active (64.2% and 84.4%, respectively). *P. mirabilis* and *P. stuartii* isolates were highly susceptible to piperacillin-tazobactam (98.8 and 100%, respectively). Susceptibility to gentamicin was fairly low (48.0%), whereas 84.7% of ESBL-producing enterobacteria were susceptible to amikacin. Most of the amikacin-resistant isolates belonged to *K. pneumoniae*, *E. aerogenes*, or

TABLE 4. ESBL-positive strains: percent susceptibilities to potentially active drugs for different species[a]

Species	No. of isolates	% Susceptible to:								
		AMC	TZP	SAM	FOX	IPM	MEM	AMK	GEN	CIP
Escherichia coli	188	73.4	84.6	31.9	95.2	100	100	92.6	60.6	24.5
Proteus mirabilis	163	91.4	98.8	87.1	96.9	98.2	100	95.7	31.9	24.5
Klebsiella pneumoniae	81	65.4	70.4	38.3	90.1	100	100	51.9	43.2	56.8
Providencia stuartii	44	18.2	100	59.1	100	97.7	100	90.9	20.5	15.9
Enterobacter aerogenes	42	11.9	64.3	28.6	14.3	100	100	59.5	78.6	28.6
Klebsiella oxytoca	18	61.1	55.6	22.2	94.4	100	100	100	72.2	72.2
Enterobacter cloacae	15	13.3	60.0	13.3	13.3	100	100	86.7	20.0	60.0
Citrobacter freundii	12	0.0	58.3	25.0	0.0	100	100	83.3	50.0	58.3
Serratia marcescens	10	0.0	80.0	0.0	0.0	100	100	60.0	70.0	50.0
Other species[b]	10	80.0	100	60.0	100	100	100	100	80.0	60.0
Total	583	64.2	84.4	49.1	83.9	99.3	100	84.7	48.0	32.8

[a] Drugs are as follows: AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; SAM, ampicillin-sulbactam; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin.
[b] Other species included the following: *Citrobacter koseri* ($n = 5$), *Morganella morganii* ($n = 3$), *Citrobacter amalonaticus* ($n = 1$), and *Providencia rettgeri* ($n = 1$).

S. marcescens species. Resistance to fluoroquinolones was widely spread among ESBL-positive enterobacteria: overall, two-thirds of isolates were resistant to ciprofloxacin. It is noted that *E. coli*, *P. mirabilis*, and *P. stuartii* isolates were resistant to this drug in more than 70% of cases.

Association of drug resistance with β-lactamase gene types. When isolates were grouped by gene type, interesting resistance patterns emerged. As shown in Table 5, TEM-positive enterobacteria were widely susceptible to ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam. In contrast, SHV-positive bacteria (carrying either SHV only or TEM plus SHV determinants) showed high rates of resistance to these combinations (up to 78.1%). Even in the latter group, piperacillin-tazobactam was the most effective drug. Enterobacteria carrying CTX-M- or PER-type determinants had similar phenotypes characterized by resistance to ampicillin-sulbactam and amoxicillin-clavulanate but remarkable susceptibility to piperacillin-tazobactam.

Consistent with the notion that ESBL determinants are often associated with other resistance traits (23), TEM- and PER-type determinants were associated in over 70% of cases with both fluoroquinolone and aminoglycoside resistance (data not shown). With regard to isolates carrying other ESBLs, those carrying SHV-type determinants alone or associated with TEM-type genes were frequently resistant to amikacin (Table 5). CTX-M-positive strains were frequently resistant to fluoroquinolones (67.8%) but susceptible to both amikacin and gentamicin in at least two-thirds of cases.

DISCUSSION

The increasing frequency of ESBL-producing enterobacteria among hospitalized patients is an important problem for both microbiologists and clinicians, because of the difficulty in correctly detecting, reporting, and treating infections caused by these organisms (23). In recent years, true community-acquired infections caused by ESBL-producing enterobacteria have also been described (25). This survey defines the current epidemiology of ESBLs among hospitalized and community patients in Italy. In addition, compared to the 1999 survey, the study provides evolutionary data regarding prevalence and distribution of ESBL-producing species, types of produced enzymes, and susceptibility to potentially active drugs.

In 2003, the overall prevalences of ESBL-producing enterobacteria among inpatients and outpatients were 7.4% and 3.5%, respectively. Thus, compared to the 1999 survey, data show a moderate increase of ESBL-producing organisms among hospitalized patients (28). The spread of ESBL-positive enterobacteria in the community presumably started after the 1999 survey, since at that time these pathogens were not detected.

Compared to data for 1999, the prevalence of ESBL-producing *E. coli* was increased among hospitalized patients. This

TABLE 5. ESBL-positive strains: percent susceptibilities to potentially active drugs according to gene type[a]

Gene type	No. of isolates	% Susceptible to:								
		AMC	TZP	SAM	FOX	IPM	MEM	AMK	GEN	CIP
TEM type only	265	82.6	95.1	73.2	95.5	98.5	100	93.2	32.8	20.8
SHV type only	124	62.1	60.5	41.1	68.5	100	100	61.3	61.3	46.0
Both, TEM and SHV	64	45.3	65.6	21.9	45.3	100	100	71.9	62.5	60.9
CTX-M type	115	42.6	93.9	21.7	93.0	100	100	95.7	65.2	32.2
PER type	15	0.0	100	13.3	100	100	100	100	13.3	20.0
Total	583	64.2	84.4	49.1	83.9	99.3	100	84.7	48.0	32.8

[a] Drugs are as follows: AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; SAM, ampicillin-sulbactam; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin.

is currently the most frequent ESBL-positive species, followed by *P. mirabilis*. On the other hand, ESBL-positive isolates of *K. pneumoniae* (the leading species in 1999) showed a significant decrease. Taken together, these findings agree with the current wide diffusion of ESBLs in medical wards and may reflect the positive impact of infection control interventions in high-risk wards. Concerning the community, *E. coli* and *P. mirabilis* appeared to be the most common ESBL-producing strains. This was not unexpected, since most isolates were obtained from urinary tract infections. Notably, *P. stuartii* isolates accounted for approximately 10% of ESBL producers in the community. ESBL-positive *P. stuartii* has been previously detected at high frequency in a large Italian university hospital (30). This species is also frequent among hospitalized patients enrolled in this survey (7.1%). Thus, ESBL-positive *P. stuartii*, an emerging problem in Italy, needs increased attention by clinical microbiologists.

Compared to data from the 1999 survey, the prevalence of isolates carrying TEM-type alone or both TEM- and SHV-type determinants was unchanged (45.4% versus 46.8% and 11.0% versus 11.0%, respectively). In contrast, a decrease of isolates carrying SHV-type determinants was observed (21.3% in 2003 versus 34.6% in 1999). This was likely due to the reduced isolation rate of *K. pneumoniae*. The most notable variation in 2003 was the marked increase of isolates harboring non-TEM, non-SHV determinants (22.3% versus 7.6% in 1999). This was mostly due to the emergence and spread of CTX-M-type enzymes in *E. coli* isolates. This finding is in agreement with recent European reports (5, 16) and confirms the current relevance of CTX-M enzymes in pathogenic enterobacteria. Clinical microbiology laboratories should take into account this changing epidemiology and adjust accordingly the screening and confirmatory procedures for ESBL production. The experience of this survey indicates that testing both ceftazidime and cefotaxime is an effective choice for ESBL screening. In fact, CTX-M-type enzymes may be undetected by screening with ceftazidime alone (2), whereas a few TEM-type ESBL enzymes can be lost by using cefotaxime alone (15). Use of both ceftazidime and cefotaxime (alone and in association with clavulanate) is suggested by CLSI for confirmatory tests (6), at least in the case of *E. coli*, *Klebsiella* spp., and *P. mirabilis*.

This survey also demonstrates that PER-type determinants, which previously were restricted to nonfermenting species such as *Pseudomonas aeruginosa* and *Acinetobacter baumanii* (31), are now harbored by *P. mirabilis*, *Providencia* spp., and *E. coli*. PER-positive isolates were found in 5/11 hospitals, thus revealing the spread of this determinant in Italy.

Therapy for infections caused by ESBL-producing enterobacteria is usually difficult, since these organisms not only are resistant to penicillins, cephalosporins, and the monobactam aztreonam but often are characterized by associated resistance to other classes of antimicrobials (27). ESBL-positive enterobacteria isolated in the 2003 survey remained fully susceptible to carbapenems, with only 3/163 *P. mirabilis* and 1/44 *P. stuartii* isolates showing an imipenem MIC of 8 μg/ml. On the contrary, our survey showed that susceptibility to ciprofloxacin had dramatically decreased, from 58% to 32% (1999 and 2003 data, respectively). A marked association between ESBL production and resistance to ciprofloxacin was observed, especially in *E. coli*, *P. mirabilis*, *P. stuartii*, and *E. aerogenes*. Notably, species that frequently harbored TEM-type determinants (i.e., *P. mirabilis* and *P. stuartii*) were characterized by resistance to both ciprofloxacin and gentamicin in more than 70% of cases. Overall, these data support the choice of carbapenems as empirical therapy in the case of life-threatening infections or nosocomial outbreaks (9, 10, 11). Use of fluoroquinolones may be justified only in selected cases of ESBL-related infections.

Excluding carbapenems, amikacin and piperacillin-tazobactam were the most effective drugs in vitro (overall susceptibility, 84.7% and 84.4%, respectively). In both cases, resistance was found in isolates of species carrying SHV-type determinants. Species harboring TEM-, CTX-M-, and PER-type determinants (e.g., *P. mirabilis*, *P. stuartii*, and *E. coli*) were consistently susceptible to amikacin and piperacillin-tazobactam. The latter three species represent two-thirds of ESBL producers and are frequently associated with urinary tract infections. Based on these data, piperacillin-tazobactam alone or together with amikacin would be a useful option for urinary tract infections caused by *P. mirabilis*, *E. coli*, or *P. stuartii*. As suggested previously (33), in the case of non-life-threatening infections and in nonoutbreak situations, it is not necessary to administer carbapenems. This approach is intended to preserve the therapeutic value of these precious drugs. The heavy use of carbapenems, in fact, may favor the selection of *Stenotrophomonas maltophilia* (a species naturally resistant to these drugs) and other nonfermenting gram-negative organisms resistant to carbapenems (17). It is notable that *K. pneumoniae* isolates producing carbapenem-hydrolyzing enzymes are emerging in the United States (34) and that different enterobacteria encoding metallo-β-lactamases have been recently detected in the Mediterranean area (12, 18, 19).

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