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Quality Control for β-Lactam Susceptibility Testing with a Well-Defined Collection of *Enterobacteriaceae* and *Pseudomonas aeruginosa* Strains in Spain

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Eighteen *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains, 16 of them with well-defined β-lactam resistance mechanisms, were sent to 52 Spanish microbiology laboratories. Interpretative categories for 8 extended-spectrum β-lactams were collected. Participating laboratories used their own routine susceptibility testing procedures (88% automatic systems, 10% disk diffusion, and 2% agar dilution). Control results were established by two independent reference laboratories by applying the NCCLS microdilution method and interpretative criteria. Interpretative discrepancies were observed in 16% of the results (4.4% for cefepime, 3.0% for aztreonam, 2.8% for piperacillin-tazobactam, 1.7% for cefotaxime [CTX] and ceftazidime, 1.1% for ceftriaxone, 0.9% for meropenem, and 0.3% for imipenem). High consistency with reference values (<5% of major plus very major errors) was observed with (i) American Type Culture Collection quality control strains; (ii) strains with low-efficiency mechanisms inactivating extended-spectrum β-lactams, such as OXA-1-producing *Escherichia coli* or SHV-1-hyperproducing *Klebsiella pneumoniae*; (iii) strains with highly efficient mechanisms, such as SHV-5 porin-deficient *K. pneumoniae*, CTX-M-10 in *Enterobacter cloacae* hyperproducing AmpC, and *P. aeruginosa* with the MexAB OprM efflux phenotype or hyperproducing AmpC. Low consistency (>30% major plus very major errors) was detected in K1-producing *Klebsiella oxytoca*, CTX-M-9-producing *E. coli*, and in OprD$^-$ *P. aeruginosa* strains. Extended-spectrum β-lactamase (ESBL)-producing strains accounted for 86% of very major errors. Recognition of the ESBL phenotype was particularly low in *Enterobacter cloacae* strains (<35%), due to the lack of NCCLS-specific rules in this genus. A K1-producing *K. oxytoca* was misidentified by 10% of laboratories as an ESBL producer. The use of well-defined resistant strains is useful for improving proficiency in susceptibility testing in clinical laboratories.

Antimicrobial susceptibility testing is one of the most important tasks of clinical microbiology laboratories (11). It is performed daily in clinical laboratories by standardized methods. The disk diffusion technique has been extensively used for this objective; however, semiautomated or automated systems based on microdilution techniques are replacing this method, at least for nonfastidious organisms (9).

Quality assurance of antimicrobial susceptibility testing is commonly performed by using internal quality control protocols, which monitor the precision and accuracy of the method, the performance of the reagents, and the performance of the microbiologist or technicians carrying out these procedures (14). These protocols often use organisms susceptible to the majority of antimicrobial agents (24) and, to a lesser extent, resistant strains (36). Additional external quality control assessments (proficiency testing) are necessary in the quality assurance of antimicrobial susceptibility testing methods (2). In this case, organisms with susceptibility defined by reference methods are distributed as unknown strains from a central laboratory to participating laboratories. This approach has been used to compare susceptibility testing results among different laboratories or to demonstrate their ability to detect resistant organisms, both locally and at a national level (1, 31, 32).

In the present study, we performed a multicenter study in Spain on the proficiency of broad-spectrum β-lactam susceptibility testing of a well-defined collection of *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains. Strains were carefully selected on the basis of resistance phenotypes to these β-lactam antibiotics. Moreover, the ability of Spanish laboratories to detect 6 extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, including two ESBL-producing *Enterobacter cloacae* strains, was also evaluated.

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MATERIALS AND METHODS

Bacterial strains and participating laboratories. Thirteen *Enterobacteriaceae* and 5 *P. aeruginosa* strains, coded as strains 1 through 18, were selected from the strain collections of Ramón y Cajal (Madrid, Spain) and Virgen Macarena (Seville, Spain) University Hospitals (Table 1). This batch included 16 strains (strains 2 to 13 and 15 to 18) with different resistance mechanisms, which variably affect broad-spectrum β-lactam antimicrobials, and two reference quality control strains recommended by the NCCLS (strains 1 and 14) (24). Isolates were inoculated onto nutrient agar slants, incubated overnight, and distributed to 52

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TABLE 1. Characteristics of study strains

Strain	Characteristic(s)	Reference
E. coli ATCC 25922	NCCLS quality control strain	24
E. coli HUS 31-94	Hyperproduction of AmpC β-lactamase plus impermeability (OmpF$^-$)	19
K. pneumoniae JCH1	Hyperproduction of SHV-1 β-lactamase	6
E. coli RYC9765550	OXA-1 β-lactamase	27
E. coli RYC5H/9	TEM-27 ESBL plus TEM-1 β-lactamase	22
E. coli RYC97056888	CTX-M-9 ESBL plus TEM-1 β-lactamase	Coque et al.[a]
K. oxytoca RYC9147/8	K1 β-lactamase	36
K. pneumoniae LB3	SHV-5 ESBL plus impermeability (OmpK35$^-$)	20
K. pneumoniae LB4	SHV-5 ESBL plus impermeability (OmpK35$^-$ and OmpK36$^-$)	20
E. cloacae RYC11439/9 I	AmpC β-lactamase	36
E. cloacae RYC11439/9 SDM	Hyperproduction of AmpC β-lactamase	36
E. cloacae RYC97095983 I	AmpC β-lactamase plus CTX-M-10 ESBL	4
E. cloacae RYC97095983 SDM	Hyperproduction of AmpC plus CTX-M-10 ESBL	This study
P. aeruginosa ATCC 27853	NCCLS quality control strain	24
P. aeruginosa HUS C28	Hyperproduction of AmpC β-lactamase	13
P. aeruginosa PAO1 OprD$^-$	AmpC β-lactamase plus impermeability (OprD$^-$)	35
P. aeruginosa HUS3 MUT3	Hyperproduction of AmpC plus impermeability (OprD$^-$)	18
P. aeruginosa HUSC9	AmpC β-lactamases plus hyperproduction of OprM (MexAB OprM)	13

[a] T. M. Coque, M. C. Varela, A. Oliver, M. I. Morosini, F. Baquero, and R. Cantón, 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-298, 2001.

participating Spanish clinical laboratories. Slants were labeled with the number of the strain (1 through 18) and the bacterial species name (i.e., *Escherichia coli* or *Klebsiella oxytoca*), blinding the susceptibility pattern or the identified resistance mechanisms. Instructions to the participating laboratories indicated that organisms would be studied for antimicrobial susceptibility testing by the routine method in each laboratory. A report form was provided to fill in quantitative (zone diameters or MICs) and qualitative (susceptible, intermediate, or resistant) results for eight broad-spectrum β-lactam antibiotics (piperacillin-tazobactam, ceftriaxone, cefotaxime, ceftazidime, cefepime, meropenem, and imipenem). The form also included a questionnaire to provide hospital (number of beds) and laboratory information (routine test method for susceptibility testing, interpretive criteria, and organization of antimicrobial susceptibility testing). Laboratories were encouraged to provide the susceptibility testing results with the interpretation (susceptible, intermediate, or resistant) reported to the clinicians and also to include corrections performed after detecting resistance mechanisms (i.e., detection of an ESBL in *E. coli* and correction of apparently susceptible cephalosporin MICs). Both reference laboratories checked the stability of resistance mechanisms in the tested strains by serial passage and exchanged the studied strains twice.

Characterization of resistance mechanisms. Most of these resistant strains have been published elsewhere (see references in Table 1). Resistance mechanisms characterization included β-lactamase (isoelectric focusing, specific enzymatic activity, substrate profile determinations, PCR, and sequencing) and outer membrane profile studies. The efflux resistance mechanism was inferred in *P. aeruginosa* on the basis of resistance phenotype and on identification of OprM by Western blotting (kindly performed by N. Gotoh, Kyoto, Japan).

Reference antimicrobial susceptibilities and interpretation of results. The antimicrobial susceptibility profiles (reference values) of the bacterial strains were studied by the NCCLS (20) broth microdilution and disk diffusion methods (25, 26) at the Ramón y Cajal and Virgen Macarena University Hospitals. Cefoxitin MICs were also determined for *E. coli* and *Klebsiella pneumoniae* isolates in order to facilitate phenotypic analysis of resistance. Isolates were defined as susceptible, intermediate, or resistant by using the NCCLS criteria (24). Identifications were also confirmed at these institutions by using the API strip (bioMerieux, Marcy l'Etoile, France) and standard biochemical test when necessary (23).

MICs, zone sizes, and interpretative results from participating laboratories were compared with reference values. Interpretative discrepancies were classified as very major, major, or minor errors. Very major errors occurred when the results obtained by the participant were in the susceptible category, whereas they were in the resistant category with the reference method; the number of susceptibility testing determinations with the reference resistant interpretation result was used as the denominator (8). Conversely, major errors occurred when the results obtained by the participant were in the resistant category, whereas they were in the susceptible category with the reference method; the number of susceptibility testing determinations with the reference susceptible interpretation result was used as the denominator (8). Finally, minor errors occurred when the results obtained by the participant were susceptible or resistant and intermediate by the reference method or when the results obtained by the participant were intermediate and susceptible or resistant by the reference method.

RESULTS

Participating hospital methods and interpretive criteria. Eighty-eight percent of participating laboratories used automatic systems for routine susceptibility testing: 19 laboratories used the Combo 1S microdilution panels with MicroScan WalkAway (18 laboratories) or AutoScan (1 laboratory) systems (Dade MicroScan, Inc., West Sacramento, Calif.); 13 laboratories used the Wider system, a computer-assisted image-processing device adapted to read and interpret microdilution panels (5), with 6- and 5-W microdilution panels (Francisco Soria Melguizo, Madrid, Spain); 7 laboratories used the VITEK system with the GNS-518 and GNS-519 cards for *Enterobacteriaceae* and *P. aeruginosa*, respectively (bioMèrieux); 5 laboratories used the VITEK2 system with AST-N010 and AST-N011 cards for *Enterobacteriaceae* and *P. aeruginosa*, respectively (bioMèrieux); and 2 laboratories used Sensititre with EMIZA8EF panels (Trek Diagnostic Systems, Westlake, Ohio). Ten percent (5 laboratories) used the disk diffusion technique, and 2% (1 laboratory) used the agar dilution technique. All participating laboratories interpreted susceptibility testing results by following the NCCLS criteria (24), but 15% performed corrections according to the Spanish Antibiogram Committee (Mesa Española para la Normalización de la Sensibilidad y Resistencia a los Antimicrobianos [MENSURA] group) (21). In our study, the MENSURA corrections extended the NCCLS interpretive categorization for cephalosporins and aztreonam in ESBL-producing *E. coli* and *Klebsiella* spp. to other *Enterobacteriaceae* isolates.

Proficiency results. The reference broth microdilution MICs of piperacillin-tazobactam, ceftriaxone, cefotaxime, ceftazidime, cefepime, meropenem, and imipenem for the study strains are shown in Table 2. Reference interpretative categories were those obtained after applying the NCCLS guidelines (24) (Table 2). The distribution of interpretative discrepancies and categorical error rates by organisms and antibiotics are

TABLE 2. Reference susceptibility testing result and interpretive category according to NCCLS criteria[a]

Strain	Characteristic(s)	MIC (μg/ml) of[b]:								
		P/T	FOX	CTX	CRO	CAZ	FEP	ATM	IMP	MER
E. coli ATCC 25922	ATCC QC	2/4 (S)	2 (S)	0.06 (S)	0.06 (S)	0.25 (S)	0.03 (S)	0.12 (S)	0.12 (S)	≤0.01 (S)
E. coli HUS 31-94	AmpC ↑[c] + OmpF$^-$	64/4 (I)	>256 (R)	16 (I)	16 (I)	256 (R)	1 (S)	32 (R)	0.25 (S)	≤0.01 (S)
K. pneumoniae JCH1	SHV-1 ↑	16/4 (S)	2 (S)	0.25 (S)	0.25 (S)	1 (S)	0.12 (S)	0.25 (S)	0.06 (S)	≤0.01 (S)
E. coli RYC9765550	OXA-1	32/4 (I)	2 (S)	0.25 (S)	0.12 (S)	0.5 (S)	1 (S)	0.12 (S)	0.12 (S)	≤0.01 (S)
E. coli RYC5H/9	TEM-27 + TEM-1	1/4 (S)	2 (S)	0.5 (R)	0.5 (R)	>512 (R)	1 (R)	256 (R)	0.12 (S)	≤0.01 (S)
E. coli RYC97056888	CTX-M-9 + TEM-1	1/4 (S)	2 (S)	8 (R)	16 (R)	0.5 (R)	4 (R)	1 (R)	0.06 (S)	≤0.01 (S)
K. oxytoca RYC9147/8	K1	128/4 (R)	2 (S)	0.12 (S)	1 (S)	0.12 (S)	0.06 (S)	4 (S)	0.06 (S)	≤0.01 (S)
K. pneumoniae LB3	SHV-5 + OmpK35$^-$	8/4 (S)	2 (S)	8 (R)	4 (R)	128 (R)	1 (R)	128 (R)	0.06 (S)	≤0.01 (S)
K. pneumoniae LB4	SHV-5 + Omp35$^-$/36$^-$	512/4 (R)	64 (R)	128 (R)	64 (R)	>512 (R)	32 (R)	512 (R)	0.12 (S)	0.06 (S)
E. cloacae RYC11439/9 I	AmpC	1/4 (S)	NA	0.25 (S)	0.25 (S)	0.25 (S)	0.06 (S)	0.06 (S)	0.12 (S)	0.03 (S)
E. cloacae RYC11439/9 SDM	AmpC ↑	256/4 (R)	NA	>256 (R)	>256 (R)	256 (R)	4 (S)	128 (R)	0.12 (S)	0.06 (S)
E. cloacae RYC97095983 I	AmpC + CTX-M-10	1/4 (S)	NA	64 (R)	64 (R)	2 (S)[d]	8 (S)[d]	16 (I)[d]	0.12 (S)	0.03 (S)
E. cloacae RYC97095983 SDM	AmpC ↑ + CTX-M-10	64/4 (I)	NA	256 (R)	256 (R)	64 (R)	32 (R)	64 (R)	0.12 (S)	0.06 (S)
P. aeruginosa ATCC 27853	ATCC QC	2/4 (S)	NA	NA	NA	2 (S)	2 (S)	4 (S)	2 (S)	0.25 (S)
P. aeruginosa HUS C28	AmpC ↑	256/4 (R)	NA	NA	NA	256 (R)	16 (1)	64 (R)	1 (S)	0.25 (S)
P. aeruginosa PAO1 OprD-	AmpC + OprD$^-$	1/4 (S)	NA	NA	NA	1 (S)	1 (S)	2 (S)	16 (R)	2 (S)
P. aeruginosa HUS3 MUT3	AmpC ↑ + OprD$^-$	512/4 (R)	NA	NA	NA	32 (R)	8 (S)	16 (I)	16 (R)	4 (S)
P. aeruginosa HUSC9	AmpC + MexABOprM	8/4 (S)	NA	NA	NA	32 (R)	8 (S)	16 (I)	2 (S)	4 (S)

[a] NCCLS interpretive criteria are given in reference 24.
[b] P/T, piperacillin-tazobactam; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IMP, imipenem; MER, meropenem; S, susceptible; I, intermediate; R, resistant; NA, not applicable.
[c] ↑, hyperproduction.
[d] The presence of an ESBL in these isolates led reference laboratories to consider cephalosporins and aztreonam as resistant; however, interpretation of results has been performed considering the indicated interpretive categories according to NCCLS criteria (24).

shown in Tables 3 and 4, respectively. Only interpretative results were used for further analysis of the proficiency results. Quantitative susceptibility values were not considered to be different; automatic systems use different range concentrations of tested antibiotics, which makes further comparison impossible. For instance, the Wider system users employ panels (reference, 5 W) which include 8 concentrations of ceftriaxone (range, 0.12 to 16 μg/ml), whereas some MicroScan users employ panels (reference, Combo breakpoint gram-negative 1S) which only include 2 concentrations of the same antibiotic (8 and 32 μg/ml). As a result of the exclusion of MICs, zone sizes were also not used in the analysis and only interpretative categories were considered. With these criteria, high consistency, less than 5% of major plus very major (MVM) discrepancies, was found for *E. coli* ATCC 25922 and *E. coli* with OXA-1 β-lactamase, *K. pneumoniae* with SHV-1 β-lactamase, *K. pneumoniae* with SHV-5 ESBL and porin OmpK35 and OmpK36 deficiency, *E. cloacae* hyperproducing AmpC plus CTX-M-10 ESBL, *P. aeruginosa* ATCC 27853, *P. aeruginosa* hyperproducing AmpC, and a *P. aeruginosa* strain with the efflux phenotype (MexAB OprM). An MVM rate between 5 and 10% was found in AmpC-hyperproducing *E. coli*, AmpC-inducible *E. cloacae*, and a *P. aeruginosa* strain deficient in porin OprD$^-$. Strains with an MVM rate higher than 10% but lower that 20% were SHV-5 ESBL-producing *K. pneumoniae* and a porin OmpK35-deficient and AmpC-hyperproducing *E. cloacae* strain, whereas strains with an MVM rate higher than 20% and lower than 30% were *E. coli* with TEM-27 ESBL and *E. cloacae* with CTX-M-10. The highest discrepancy was detected (an MVM rate higher than 30% and lower than 40%) in *E. coli* with CTX-M-9 ESBL, K1 β-lactamase-producing *K. oxytoca*, and the AmpC-hyperproducing *P. aeruginosa* porin-deficient (OprD$^-$) strain.

It is worth noting that 86% of very major errors were observed in ESBL strains, particularly with TEM-27 and CTX-M-9 ESBL-producing strains (Table 3). The highest major error rates were observed with the AmpC-hyperproducing plus porin-deficient (OprD$^-$) *P. aeruginosa* (25.5%) and the K1 β-lactamase-producing *K. oxytoca* (22.5%) strains (Table 3). In the former, these results were mainly (>75%) due to cefepime and meropenem, and in the latter, these results were due to ceftriaxone, cefepime, and aztreonam interpretive values. Moreover, this *P. aeruginosa* strain (strain 17) had the highest rate of minor errors (27.1%).

The analysis of interpretative discrepancies by antibiotics revealed that cefepime had the highest rate, 30.5%, when considering the number of susceptibility testing determinations for

TABLE 3. Distribution of interpretive discrepancies and categorical error rates by antimicrobial agent tested

Antimicrobial agent	No. of organism-antimicrobial combinations tested	% of interpretative discrepancies[a]	% of errors of type		
			Minor[a]	Major[b]	Very major[c]
Piperacillin-tazobactam	809	21.2	13.9	9.1	7.7
Ceftriaxone	352	19.6	10.8	15.6	5.2
Cefotaxime	647	17.0	10.2	6.4	8.0
Ceftazidime	892	11.8	4.8	8.9	5.1
Cefepime	903	30.5	13.9	16.9	19.3
Aztreonam	848	22.1	13.9	13.6	6.6
Imipenem	919	2.4	1.6	0.6	1.9
Meropenem	829	6.6	3.6	3.0	0
Total	6,199	15.9[d]	8.8[d]	7.6	7.8

[a] Considering the number of susceptibility testing determinations for each antibiotic as the denominator.
[b] Considering the number of susceptible strains for each antibiotic as the denominator.
[c] Considering the number of resistant strains for each antibiotic as the denominator.
[d] Considering the total number of susceptibility testing determinations as the denominator.

TABLE 4. Distribution of categorical error rates by microorganism tested

Strain	Characteristic(s)[a]	% of errors of type		
		Minor	Major	Very major
E. coli ATCC 25922	ATCC QC	0.3	1.1	0
E. coli HUS 31-94	AmpC ↑ + OmpF$^-$	23.7	6.2	0
K. pneumoniae JCH1	SHV-1 ↑	1.6	0.8	0
E. coli RYC9765550	OXA-1	8.9	5.0	0
E. coli RYC5H/9	TEM-27 + TEM-1	4.4	4.9	17.3
E. coli RYC97056888	CTX-M-9 + TEM-1	4.6	3.6	29.9
K. oxytoca RYC9147/8	K1	6.3	22.5	17.7
K. pneumoniae LB3	SHV-5 + OmpK35$^-$	6.8	5.6	8.9
K. pneumoniae LB4	SHV-5 + OmpK35$^-$/36$^-$	3.0	0	3.3
E. cloacae RYC11439/9 I	AmpC	2.2	7.5	0
E. cloacae RYC11439/9 SDM	AmpC ↑	3.3	13.0	2.3
E. cloacae RYC97095983 I	AmpC + CTX-M-10	10.7	20.9	1.3
E. cloacae RYC97095983 SDM	AmpC ↑ + CTX-M-10	8.1	0	4.2
P. aeruginosa ATCC 27853	ATCC QC	3.3	3.6	0
P. aeruginosa HUS C28	AmpC ↑	17.3	1.1	0
P. aeruginosa PAO1 OprD$^-$	AmpC + OprD$^-$	3.7	5.0	3.8
P. aeruginosa HUS3 MUT3	AmpC ↑ + OprD$^-$	27.1	25.5	4.6
P. aeruginosa HUSC9	AmpC + MexABOprM	2.3	4.9	0

[a] ↑, hyperproduction.

this antibiotic and a rate of 4.4%, when considering the total number of susceptibility testing determinations, followed by piperacillin-tazobactam (21.2 and 2.8%), aztreonam (22.1 and 3.0%), ceftriaxone (19.6 and 1.1%), cefotaxime (17.0 and 1.7%), ceftazidime (11.8 and 1.7%), meropenem (6.6 and 0.9%), and imipenem (2.4 and 0.3%).

Cefepime was also the tested antimicrobial with the highest very major (19.3%) and major (16.9%) error rates. In this antibiotic, all very major, 22% of major, and 25% of minor errors were concentrated on ESBL-producing *Enterobacteriaceae.* Moreover, cefepime minor errors were mainly observed with *P. aeruginosa* strains (69%).

In general, carbapenem discrepancies were low and were mainly concentrated in *P. aeruginosa* strains with a porin deficiency (OprD$^-$) or expressing MexAB OprM: strains 16 (20% of carbapenem discrepancies), 17 (43% of carbapenem discrepancies), and 18 (26% of carbapenem discrepancies).

ESBL recognition and misidentification of non-ESBL producers as ESBL producers. Not all laboratories studied all cephalosporins on ESBL-producing strains, which may have affected their ability to recognize ESBL. Two laboratories did not study cefotaxime, ceftazidime, and cefepime in ESBL-producing *E. coli* or *K. pneumoniae* strains (strains 5, 6, 8, and 9), and three laboratories did not study these antibiotics in ESBL-producing *E. cloacae* strains (strains 12 and 13). In all these strains, ceftriaxone and aztreonam were less frequently reported (Table 5).

The highest rates of ESBL recognition were obtained with the SHV-5-producing and OmpK35-lacking *K. pneumoniae* (strain 8) (71.1%) and the TEM-27-producing *E. coli* (strain 5) (69.2%), for which the ceftazidime was highly affected. This fact can be related with the study of the ceftazidime-clavulanate combination in 67.3% of participating laboratories (data not shown). The CTX-M-9-producing *E. coli* (strain 6) was recognized as an ESBL producer by 61.5% of participating laboratories, decreasing to 40.4% in the case of the SHV-5-producing *K. pneumoniae* lacking the OmpK35 and OmpK36 porins (strain 9). ESBL was less frequently recognized in *E. cloacae* strains (34.6 and 9.6%), probably due to the lack of NCCLS-specific recommendations for screening and confirmation of ESBL in this genus (24) and difficulties in discriminating between the ESBL phenotype and that of the AmpC-hyperproducing phenotype (37).

With the exception of VITEK2, which uses an algorithm to recognize ESBL-producing strains, the other automatic systems used by participating laboratories include the ceftazidime-clavulanate combination for this purpose. In addition, 30 laboratories (57%) also used the double-disk synergy test (25 laboratories) and/or specific E-test (8 laboratories) as ESBL confirmatory tests (7, 10). Misidentification of the K1 β-lactamase-producing *K. oxytoca* strain (strain 7) as an ESBL producer occurred in five laboratories (10%), all of them using the double-disk synergy confirmatory test for ESBL. Moreover, three laboratories (6%) misidentified the AmpC-hyperproducing *E. coli s*train (strain 2) as an ESBL producer, two of them using the ceftazidime-clavulanate confirmatory E-test for ESBL.

DISCUSSION

Different multicenter studies have been performed to study the ability of laboratories to detect specific resistance mecha-

TABLE 5. ESBL-producing isolates and interpretive susceptibility results of cephalosporins and aztreonam reported by participating laboratories[a]

Strain	Characteristic(s)[b]	Cefotaxime				Ceftriaxone				Ceftazidime				Cefepime				Aztreonam			
		Ref MIC	No. of laboratories reporting a result of:			Ref MIC	No. of laboratories reporting a result of:			Ref MIC	No. of laboratories reporting a result of:			Ref MIC	No. of laboratories reporting a result of:			Ref MIC	No. of laboratories reporting a result of:		
			S	I	R		S	I	R		S	I	R		S	I	R		S	I	R
E. coli RYC5H/99	TEM-27 + TEM-1	0.5	16	3	31	0.5	8	4	16	>512	0	0	50	1	14	4	32	256	1	4	42
E. coli RYC97056888	CTX-M-9 + TEM-1	8	3	2	45	16	0	4	22	0.5	23	1	26	4	13	6	31	1	20	2	26
K. pneumoniae LB3	SHV-5 + OprK35$^-$	8	5	4	41	4	2	6	19	128	0	2	48	1	11	5	34	128	1	0	46
K. pneumoniae LB4	SHV-5 + OmpK35$^-$/36$^-$	128	1	2	47	64	0	2	25	>512	1	0	49	32	4	3	43	512	1	0	45
E. cloacae RYC97095983 I	AmpC + CTX-M-10	64	1	7	41	64	0	2	26	2	24	7	18	16	17	9	23	8	11	13	22
E. cloacae RYC97095983 SDM	AmpC ↑ + CTX-M-10	256	1	0	48	256	0	0	27	64	1	0	48	32	6	5	38	64	1	0	45

[a] Ref, reference; S, susceptible; I, intermediate; R, resistant. MICs are given in micrograms per milliliter.
[b] ↑, hyperproduction.

nisms that, when present in infectious pathogens, represent a clinical concern (1, 30, 33). Most of these studies have been designed as proficiency quality control studies, distributing resistant strains from a central laboratory, which normally acts as a reference laboratory, to different participating laboratories. We present the results of a Spanish multicenter study on the proficiency of broad-spectrum β-lactam susceptibility testing of a well-defined collection of *Enterobacteriaceae* and *P. aeruginosa* strains. Strains were carefully selected on the basis of resistance phenotypes to these β-lactam antibiotics. The resistance mechanisms, which included ESBLs, AmpC β-lactamase hyperproduction, permeability defect, and/or efflux-based mechanisms, represent a major challenge to these broad-spectrum antimicrobials (3, 15, 33). Moreover, we considered eight broad-spectrum antimicrobials belonging to different β-lactam groups (cephalosporins, monobactams, carbapenem, and β-lactam and β-lactamase-inhibitor combinations), which were variably affected by these resistance mechanisms (Table 1).

It has been assumed that detection and recognition of the aforementioned resistance mechanisms may be difficult for clinical laboratories when routinely testing organisms, particularly with automated systems (29, 33). Some of these problems may be related to the limit of antibiotics and antibiotic concentrations included in the susceptibility testing panels, which affect the ability of laboratories to detect the resistance phenotypes (30, 32). Moreover, susceptibility testing of ESBL- and AmpC-producing organisms may produce inconsistent or anomalous quantitative results (12, 16, 33), which can also affect interpretative (qualitative) results. This issue becomes even more complex when more than one resistance mechanism is present in a single isolate due to a superimposed resistance phenotype. The lack of appropriate reporting conventions enhances difficulties for the accurate interpretation of susceptibility testing results, affecting the appropriate selection of antimicrobial therapy (3, 33).

In our study, well-characterized resistant strains were useful in demonstrating the proficiency of routine susceptibility testing. In addition, our aim was to detect which β-lactam antimicrobial susceptibility testing had the highest difficulties and for which strains (or the resistance mechanism) difficulties were higher. Neither of the NCCLS recommended quality control strains, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, represented problems in susceptibility testing. Only 0.3 and 1.1% of minor and major errors, respectively, were observed in the former and 3.3 and 3.6%, respectively, in the latter, denoting the high quality of performance of routine susceptibility testing in Spain. This situation was similar with strains expressing resistance mechanisms which slightly affect the studied β-lactams but for which MIC or inhibition zone values fall in the susceptible category (i.e., OXA-1-producing *E. coli* or SHV-1-hyperproducing *K. pneumoniae* strains). This was also the case for those strains expressing resistance mechanisms, which unlike the former, highly affect cephalosporins (i.e., SHV-5-producing *K. pneumoniae* deficient in both OmpK35 and OmpK36 porins, *E. cloacae* hyperproducing AmpC plus CTX-M-10 ESBL, *P. aeruginosa* hyperproducing AmpC, and a *P. aeruginosa* strain with the MexAB OprM efflux phenotype). On the contrary, the highest combined MVM discrepancies (>20%) were detected in K1-producing *K. oxytoca*, *E. cloacae* with CTX-M-10 ESBL, *E. coli* with TEM-27 or CTX-M-9, and an $OprD^-$ AmpC-hyperproducing *P. aeruginosa* strain. It is worth noting that this group included two *E. coli* strains with a clear ESBL phenotype, the TEM-27 and CTX-M-9 producers. The TEM-27 enzyme highly affects ceftazidime but not cefotaxime or ceftriaxone, whereas the CTX-M-9 enzyme does not apparently affect ceftazidime (Table 2). In both strains, as well as in other ESBL producers, cefepime was slightly affected, but a high inoculum size effect for this antibiotic was noted (27, 34), which was partially responsible for these discrepancies. In addition, the inability of some participating laboratories to realize that an ESBL was present in some of these strains was also responsible for the high rate of cefepime-interpretative discrepancies (30.5%). Moreover, all cefepime very major errors (19.3%) were observed in ESBL-producing strains. Although the ability of cefepime to be active against ESBL-producing organisms is controversial (28), in these laboratories, cefepime was reported as susceptible and no categorical modification was performed. This modification is recommended both in the NCCLS and MENSURA criteria (21, 24).

Aztreonam (22.1%) and piperacillin-tazobactam (21.2%) discrepancies were also high, followed by those of ceftriaxone (19.6%), cefotaxime (17.0%), and ceftazidime (11.8%). All of these antibiotics were variably affected by the resistance mechanisms. This was not the case with imipenem and meropenem, which were the antibiotics least affected by the resistance mechanisms and those for which lower interpretative discrepancies, 2.4 and 6.6%, respectively, were observed.

The detection of ESBL was also specifically studied in our multicenter study. In general, the ability of the Spanish laboratories to detect the presence of ESBL-producing isolates and consequently to modify susceptibility testing reports was higher than that observed in other studies (30–32). This fact can be related with the high number of laboratories using automatic susceptibility testing devices incorporating the so-called expert software that helps the microbiologist interpret phenotypes (9, 16) and, in addition, that nearly 60% of laboratories routinely made phenotypic verification of ESBL producers by using confirmatory tests.

In a multicenter study performed among users of the WHONET software during a 3-year period (1996 through 1999), only 1.5% of laboratories reported that a TEM-3 ESBL-producing *K. pneumoniae* was specifically an ESBL isolate (31). In this study, although 88% of laboratories reported that the *K. pneumoniae* isolate was resistant to at least one extended-spectrum cephalosporin, none of them modified the interpretation of the other cephalosporins to resistant, as currently suggested by the NCCLS. Moreover, in a study performed in 1998 in Connecticut, 18.4% of 38 participating laboratories noted on their report forms that at least one of the five organisms used in the study (only three contained an ESBL) contained a presumptive ESBL. In addition, 21.0% of these laboratories failed to detect extended-spectrum cephalosporin or aztreonam resistance in any of the ESBL- or AmpC-producing isolates (32).

In our study, 71% of participating laboratories were able to infer the presence of an ESBL in the *K. pneumoniae* SHV-5-producing strain. This strain had the typical ESBL resistance profile and produced the typical synergy between ceftazidime and clavulanate included in most of the automated systems

(Wider, VITEK, and MicroScan). The ability of participating laboratories was also high in the TEM-27 ESBL-producing *E. coli* strain (69.2%), which also showed this typical synergy effect. It is also of interest that 61.5% of laboratories were able to infer the presence of an ESBL in the CTX-M-9-producing isolate, which did not produce this typical ceftazidime-clavulanate synergy. Conversely, as shown by both reference laboratories and participating laboratories performing the ESBL confirmatory disk approximation test, this strain showed a clear cefotaxime-clavulanate synergy. Unfortunately, this combination was not present in the automated systems used for susceptibility testing, which would have enhanced the ability to detect the CTX-M-9-producing *E. coli* strain.

The ability to detect an ESBL was dramatically decreased in the CTX-M-10-producing *E. cloacae* strain (34.6%) and particularly in the isogenic AmpC-hyperproducing isolate (9.6%). In both strains, the ESBL phenotype may be confused with the overexpressed AmpC phenotype. In addition, ceftazidime-clavulanate synergy was not clear due to the CTX-M ESBL production, which may interfere with ESBL detection (4, 37). In both strains, a low rate of susceptibility testing report modification was also performed, which was undoubtedly related to the fact that the NCCLS only recommended rules for ESBL-producing *E. coli* and *Klebsiella* spp. but not for ESBL-producing *Enterobacter* or other AmpC-producing isolates (24). Those laboratories using the NCCLS recommendations modified with the MENSURA criteria (21) performed susceptibility testing report modifications in ESBL AmpC-producing strains. In our opinion, the NCCLS should also include screening and confirmatory tests for ESBL not only in *E. coli*, *K. pneumoniae*, and *K. oyxtoca* but also in other *Enterobacteriaceae* organisms.

Misidentification of a non-ESBL producer as an ESBL producer has been previously reported (30). Most of these misidentifications are due to the presence of clavulanate-inhibited β-lactamases that slightly affect expanded-spectrum cephalosporins (i.e., the K1 enzyme in *K. oxytoca*), which may offer synergy results in some confirmatory tests for ESBL (i.e., K1 enzyme-producing *K. oxytoca* in the disk approximation test among aztreonam, ceftriaxone, and/or cefotaxime with amoxicillin-clavulanate) (unpublished results). In our study, five (10%) participating laboratories using the double-disk synergy confirmatory test for ESBL misidentified a K1 β-lactamase-producing *K. oxytoca* as an ESBL-producing strain and made interpretative modifications in all cephalosporins tested and aztreonam and not only in those affected to a higher extent (ceftriaxone or aztreonam). These modifications are a matter of discussion (17), as no clear criteria have been published. In our study, reference values for this *K. oxytoca* strain were those corresponding to the strict NCCLS interpretation of the inhibition zone or MICs. Furthermore, three participating laboratories (6%) misidentified an AmpC-hyperproducing *E. coli* strain as an ESBL producer. This also occurred in a previous study including an AmpC-hyperproducing strain in a batch of 5 strains containing three ESBL-producing strains when no laboratory differentiated the AmpC-producing strain from the other ESBL-producing strains (31). It should be noted that in our study, all misidentification in the *K. oxytoca* and *E. coli* strains occurred in automatic susceptibility testing system users but not in disk diffusion users (data not shown in tables).

In summary, our study demonstrated that well-defined resistant strains were useful for the study of susceptibility testing proficiency at the national level. For broad-spectrum β-lactam antibiotics, antimicrobial susceptibility testing had the highest difficulties for those strains with β-lactam-resistant mechanisms yielding complex phenotypes, the most affected antimicrobials being cefepime, aztreonam, and piperacillin-tazobactam. Moreover, the detection of ESBL-producing strains was more difficult in those strains for which ESBL are atypical (*E. cloacae*) or when superimposed with other resistance mechanisms (ESBL *K. pneumoniae* with porin deficiency). Quality control exercises performed at the national level are essential to estimate the sensitivity of the diagnostic microbiological facilities of the country for the detection of new bacterial threats and the time to react to them. Extensive information has been provided now to the participating laboratories. We hope that this initiative will improve the ability of our diagnostic microbiological laboratories to deal with new threats in antimicrobial resistance.

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