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Antimicrobial susceptibility studies

Performance in detection and reporting β-lactam resistance phenotypes in Enterobacteriaceae: a nationwide proficiency study in Italian laboratories

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Abstract

We evaluated the ability of 60 Italian clinical microbiology laboratories in detecting and reporting β-lactam resistance phenotypes in Enterobacteriaceae. Laboratories received 5 well-characterized isolates producing extended-spectrum β-lactamases (ESBLs), 2 hyperproducers of chromosomal enzymes, and 3 quality control strains. The performances in antimicrobial susceptibility testing (AST) were different depending on the species and type of ESBL produced. High rates of very major errors (up to 56%) were observed for ESBL producers when testing cephalosporins and aztreonam, especially in the case of CTX-M-1–producing *Escherichia coli* and TEM-52–producing *Proteus mirabilis*. Isolates hyperproducing chromosomal enzymes were erroneously reported as ESBL producers in approximately 20% of cases. Detection of ESBLs is still a problem for clinical microbiology laboratories. Overall, performances in AST appear to be better with *Klebsiella* spp. producing well-known enzymes (e.g., SHV type) than with strains producing emerging enzymes (e.g., CTX-M type) or organisms not well recognized as ESBL producers (e.g., *P. mirabilis*).

Keywords: β-Lactamases; ESBL; Antimicrobial resistance; AST; Enterobacteria

1. Introduction

Susceptibility testing to antimicrobials is one of the most important tasks performed by clinical microbiology laboratories because it evaluates bacterial resistance in vitro and allows to choose the most appropriate antibiotic treatment (Jorgensen and Ferraro, 1998). The relentless evolution of bacterial resistance mechanisms, however, requires a periodical revision of the interpretative criteria of susceptibility testing results and of testing procedures.

The worldwide dissemination of extended-spectrum β-lactamases (ESBLs) among members of Enterobacteriaceae is a typical example of evolution of bacterial resistance that required major revisions in testing and reporting susceptibility to extended-spectrum β-lactams (Bradford, 2001; Stürenburg and Mack, 2003; Bonnet, 2004). The detection issue is because of the awareness that the use of extended-spectrum cephalosporins in treating infections caused by ESBL producers is strongly associated to clinical failures, even when MIC values of the infecting isolate remain lower than the conventional interpretative breakpoints for resistance (Paterson et al., 2001, 2004; Wong-Beringer et al., 2002; Endimiani et al., 2005). For this reason, modified breakpoints have been established for suspecting ESBL production, specific tests have been developed to confirm ESBL production, and specific reporting guidelines have been issued for confirmed ESBL producers (Jarlier et al., 1988; Clinical and Laboratory Standards Institute [CLSI], 2005; Health Protection

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Agency, 2005). However, some debate is still ongoing concerning strategies for screening and confirmation of ESBL producers, whereas awareness of the ESBL detection and reporting issue is still limited among clinical microbiology laboratories (Cantón et al., 2003). A few large multicenter studies have been performed to evaluate the ability of clinical microbiology laboratories to detect ESBL producers, most of which were designed as proficiency quality control studies (Tenover et al., 1999, 2001; Cantón et al., 2003; Stevenson et al., 2003).

The purpose of this study was to evaluate, at a countrywide level, the proficiency of Italian laboratories to detect and correctly report β-lactam resistance phenotypes, including those mediated by ESBLs, in clinical isolates of Enterobacteriaceae.

2. Materials and methods

2.1. Bacterial strains

Ten well-characterized strains were selected for this proficiency study: 5 ESBL producers (including the ESBL-

Table 1
Strains used for proficiency testing and expected susceptibility results for test drugs

Strain	Species and characteristics	MIC (μg/mL) and susceptibility categories according to CLSI[a]											
		CRO	CTX	CAZ	FEP	ATM	AMX	TZP	IPM	MEM	AMK	GEN	CIP
PT-01	*K. pneumoniae* VA-212/99 producing the SHV-12 ESBL	8 (R)	8 (R)	>64 (R)	2 (R)	>64 (R)	4 (S)	4 (S)	<0.5 (S)	<0.5 (S)	0.5 (S)	0.5 (S)	<0.5 (S)
PT-02	*K. oxytoca* VA-1151/99 producing the SHV-12 ESBL	16 (R)	8 (R)	64 (R)	2 (R)	64 (R)	4 (S)	2 (S)	<0.5 (S)	<0.5 (S)	2 (S)	0.5 (S)	0.5 (S)
PT-03	*P. mirabilis* VA-1134/99 producing the TEM-52 ESBL	8 (R)	16 (R)	4 (R)	8 (R)	<0.5 (R)	4 (S)	0.5 (S)	2 (S)	<0.5 (S)	2 (S)	>64 (R)	>32 (R)
PT-04	*E. coli* VA-1924/01 producing the CTX-M-1 ESBL	64 (R)	>64 (R)	2 (R)	8 (R)	8 (R)	8 (S)	1 (S)	<0.5 (S)	<0.5 (S)	2 (S)	1 (S)	<0.5 (S)
PT-05	*K. pneumoniae* ATCC 700603 producing the SHV-18 ESBL	4 (R)	8 (R)	32 (R)	1 (R)	64 (R)	8 (S)	8 (S)	<0.5 (S)	<0.5 (S)	2 (S)	8 (I)	<0.5 (S)
PT-06	*E. coli* VA-1436/99 hyperproducing AmpC β-lactamase	8 (S)	16 (I)	64 (R)	0.5 (S)	16 (I)	>64 (R)	>64 (R)	<0.5 (S)	<0.5 (S)	2 (S)	1 (S)	<0.5 (S)
PT-07	*K. oxytoca* VA-362/02 hyperproducing the K1 enzyme	16 (I)	8 (S)	0.5 (S)	4 (S)	>64 (R)	>64 (R)	>64 (R)	<0.5 (S)	<0.5 (S)	1 (S)	1 (S)	>32 (R)
PT-08	*E. coli* ATCC 25922 QC for susceptibility testing	0.5 (S)	<0.5 (S)	<0.5 (S)	<0.5 (S)	<0.5 (S)	2 (S)	2 (S)	<0.5 (S)	<0.5 (S)	2 (S)	1 (S)	<0.5 (S)
PT-09	*P. aeruginosa* ATCC 27853 QC for susceptibility testing	NA	NA	2 (S)	1 (S)	8 (S)	NA	2 (S)	4 (S)	<0.5 (S)	1 (S)	0.5 (S)	<0.5 (S)
PT-10	*E. coli* ATCC 35218 QC for susceptibility testing	NA	NA	NA	NA	NA	4 (S)	1 (S)	NA	NA	NA	NA	NA

CRO = ceftriaxone; CTX = cefotaxime; CAZ = ceftazidime; FEP = cefepime; ATM = aztreonam; AMX = amoxicillin–clavulanic acid; TZP = piperacillin–tazobactam; IPM = imipenem; MEM = meropenem; AMK = amikacin; GEN = gentamicin; CIP = ciprofloxacin; NA = not applicable.

[a] Interpretative criteria were from the reference CLSI document (NCCLS, 2003b).