SHORT COMMUNICATIONS

Isolation of multidrug-resistant *Enterobacter cloacae* and comparison among clavulanate-tazobactam and sulbactam-synergy by using a double-disk synergy test

Vincenzo Savini[1], Fabio Febbo[1], Andrea Balbinot[1], Marzia Talia[1], Chiara Catavitello[1], Assunta Manna[1], Fabio Di Berardino[1], Francesca Fioritoni[3], Daniela Carlino[3], Giovanni Di Bonaventura[2], Domenico D'Antonio[1]

[1]Unità Operativa Complessa di Microbiologia e Virologia Clinica, Dipartimento di Medicina Trasfusionale, Ospedale Civile "Spirito Santo", Pescara, Italy

[2]Dipartimento di Microbiologia Clinica, Centro Studi Invecchiamento (Ce.S.I.), Università degli Studi "Gabriele d'Annunzio", Chieti, Italy

[3]Dipartimento di Ematologia Clinica, Ospedale Civile "Spirito Santo", Pescara, Italy,

Isolation of multidrug-resistant *Enterobacter cloacae* and comparison among clavulanate–tazobactam and sulbactam-synergy by using a double-disk synergy test

Key Words: *Enterobacter cloacae*, ESBLs, double-disk synergy test

SUMMARY

False negative results of *double-disk synergy* test with *Enterobacter cloacae* are common, as AmpC-enzymes may mask ESBLs elaboration. We increased the sensitivity of the method by using both *clavulanate*- and *tazobactam*/*sulbactam*; hence, we suggest to use all the three inhibitors to screen ESBLs in AmpC ß-lactamases producing organisms.

Received July 16, 2007 ***Accepted January 9, 2008***

INTRODUCTION

Enterobacter cloacae is an emerging nosocomial pathogen; it is responsible for bacteremia, pneumonia, lower and upper urinary tract infections, and necrotizing enterocolitis (12). It generally overproduces chromosomal AmpC ß-lactamases as a cause of ß-lactam resistance, and, less frequently, plasmid-mediated ESBLs. Carbapenemase-producing *E. cloacae* strains have been uncommonly reported (15).

Palsmid-mediated *Extended-Spectrum ß-lactamases (ESBLs)* have been found in *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Proteus* spp., *Salmonella* spp., *Acinetobacter* spp., but rarely in *non-fermenting* Gram negative organisms (*Pseudomonas aeruginosa* resistance to cephalosporines is mostly mediated by efflux mechanisms) (3, 5, 7, 8) and are able to idrolyze all b-lactams (broad-spectrum penicillins, aztreonam, cephalosporines), except for temocillin, cefoxitin, cefotetan, carbapenems, and betalactams/?-lactamases inhibitors combinations, as ESBLs are inhibited *in vitro* by clavulanate-, sulbactam-, and tazobactam (2).

ESBL expression is often associated to multidrug-resistance, particularly to fluoroquinolones, aminoglycosides, tetracycline, cotrimoxazole, and carbapenems, whilst susceptibility to colistin, tigecycline, nitrofurantoin and fosfomycin does not seem to be related to ESBL expression (14).

STUDY

An E. cloacae strain from a leukemic patient faeces showed susceptibility to cefepime (MIC 8 μg/ml), imipenem (MIC ≤ 1 μg/ml), meropenem (MIC ≤ 0.25 μg/ml), amikacin (MIC ≤ 2 μg/ml), cotrimoxazole (MIC ≤ 20 μg/ml), but resistance to ampicillin (MIC ≥ 32 μg/ml), piperacillin (MIC ≥ 256 μg/ml), amoxicillin/clavulanate (MIC = 32 μg/ml), ampicillin/sulbactam (MIC ≥ 32 μg/ml), piperacillin/tazobactam (MIC ≥ 128 μg/ml), cefuroxime (MIC ≥ 64 μg/ml), cefixime

Corresponding author: **Vincenzo Savini**
Unità Operativa Complessa di Microbiologia e Virologia Clinica, Dipartimento di Medicina Trasfusionale,
Ospedale Civile Spirito Santo - Via Fonte Romana 8 - 65100 Pescara (PE)
Cell.: 320-6265740 - E-mail: **vincsavi@yahoo.it**

(MIC ≥ 4 μg/ml), cefotaxime (MIC ≥ 64 μg/ml), ceftazidime (MIC ≥ 64 μg/ml), cefoxitin (MIC ≥ 64 μg/ml), ciprofloxacin (MIC ≥ 4 μg/ml), levofloxacin (MIC ≥ 8 μg/ml), gentamicin (MIC = 16 μg/ml), tobramycin (MIC ≥ 16 μg/ml), netilmicin (MIC ≥ 32 μg/ml), and tetracycline (MIC ≥ 16 μg/ml).
(identification and MICs were provided by Vitek2, bioMérieux).
Given the absence of published guidelines, Vitek 2 fails in screening ESBL production in organisms other than *E. coli* and *Klebsiella* spp. Hence, we performed a modified *double-disk synergy test (DDST)* (6), by placing disks of cefotaxime (CTX, 30 μg; Oxoid), ceftazidime (CAZ, 30 μg; Oxoid), and cefepime (FEP, 30 μg; Oxoid), adjacent (20 and 30 mm, center to center) to an amoxicillin/clavulanate disk (AMC, 30 μg; Oxoid). DDST results seemed to indicate a *non-ESBL phenotype*. Anyway, given the MIC of 8 μg/ml for cefepime (11), we repeated the test by placing disks at 15 and 10 mm, and by using also tazobactam (TZP, 110 μg, Oxoid) and sulbactam (SAM, 20 μg, Oxoid) as ß-lactamases inhibitors.
Disk diffusion test with CAZ, CTX, FEP, AMC, SAM, and TZP alone confirmed the Vitek2 results: AMC, SAM, CAZ, CTX did not generated inhibition, whilst diameters for TZP and FEP inhibition zones were 16 and 18 mm, respectively (9, 10).
Double-disk synergy test with AMC provided absence of synergy towards CAZ/CTX/FEP at 30, 20, and 15 mm, but slight potentiation of FEP inhibition zone at 10 mm.
By using TZP as an inhibitor, we obtained no synergy at 30 and 20 mm, but an increasing potentiation of FEP inhibition zone at 15 and 10 mm.
With SAM, *DDST* gave absence of synergy at 30 mm; *elongment* of FEP inhibition zone towards SAM at 20 mm; gradual potentiation of FEP inhibition zone at 15 and 10 mm.
Sulbactam- and tazobactam-synergy were more evident than *clavulanate*-, at 10 mm.
Given the cefoxitin resistance and the greater synergy showed by FEP, rather than CAZ and CTX, we considered the strain as a probable AmpC-type hydrolases producer, too (cefoxitin-resistance due to efflux pumps or reduced permeability of the bacterial cell wall has been reported in ESBL-producing *E. coli* and *Klebsiella* spp., but not in *Enterobacter* spp., so far) (13).

CONCLUSIONS

Molecular ESBL detection, *combination disk method*, and *Etest ESBL screen* (4) are expensive for being used in routinely activity. Hence, the simple *double-disk test* (4) still plays a role, but may provide false negative results, due to co-production of AmpC-enzymes induced by clavulanate (which can inhibit the cephalosporines used in the test); contemporary ESBL elaboration and onset of porines mutations; weak ESBL activity/expression (1). Further, distance between the disks plays a crucial role, depending on what type of ESBL is being produced (2).
Since *E. cloacae* notorely overproduces AmpC-enzymes, rather than ESBLs, utilization of FEP may increase the sensitivity of the DDST (10). Further, we suggest to test all the three ß-lactamases-inhibitors to detect *in vitro* synergy with 3rd and 4th generation cephalosporins and to place disks at 15 mm-distance at least, in order to increase the sensitivity of the method.
As a further consideration, polimicrobic infection/colonization of bladder, ulcers, plagues, wounds, upper airways and gut may be responsible for plasmide-mediated ESBL transmission among different species. Hence, a prompt detection of ESBL-producing organisms would play a great role from both a therapeutical and an epidemiologic point of view. Finally, *in vivo* combined therapy based on administration of cephalosporines plus penicillin/ß-lactamases inhibitor combinations has never been evaluated and could be affected by antagonism between the two types of molecules, so that its use is not suggested.

REFERENCES

1. Ardanuy C, Linares J, Dominguez MA, et al. Outer membrane profiles of clonally related *Klebsiella pneumoniae* isolates from clinical samples and activities of cephalosporins and carbapenems. Antimicrob Agents Chemother 1998; 42: 1636-40.
2. Bradford PA. Extended spectrum ß-lactamases in the 21st century: characterisation, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001; 14: 933-51.
3. Chia J-H, Chu C, Su L-H, Chiu C-H, Kuo A-J, Sun C-F, Wu T-L. Development of a multiplex PCR and SHV melting-curve mutation detection system for detection of some SHV and CTX-M ß-lactamases of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan. J Clin Microb 2005; 43(9): 4486-91.
4. D'Azevedo PA, Gonçalves ALS, Musskopf MI, Ramos CG, Dias CAG. Laboratory tests in the detection of extended-spectrum ß-lactamase production: National Committee for Clinical Laboratory Standards (NCCLS) screening test, the E-test, the double disk confirmatory test, and cefoxitin susceptibility testing. The Brazilian Journal of Infectious Diseases 2004; 8(5): 372-7.
5. Hoffmann H, Stuerenburg E, Heesemann J, Roggenkamp A. Prevalence of extended-spectrum ß-lactmases in isolates of the Enterobacter cloacae complex from German hospitals. Clin Microbiol Infect 2006; 12(4): 322-30.
6. Jarlier V, Nicolas MH, Fournier G, Philippon A.

Extended broad-spectrum ß-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10: 867-8.
7. Levison ME, Mailapur YV, Pradhan SK, et al. Regional occurrence of plasmid-mediated SHV-7, an extended-spectrum ß-lactmase, in *Enterobacter cloacae* in Philadelphia Teaching Hospitals. Clin Infect Dis 2002; 35(12): 1551-4.
8. Metan G, Zarakolu P, Hascelik G, Akova M. Antimicrobial susceptibility of phenotypically extended spectrum ß-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates. Mikrobiyol Bul 2006; 40(1-2): 23-8.
9. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility testing; 8th informational supplement. 2003 Approved standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 10th informational supplement, Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards Document M100-S10, 2000.
11. Naumiuk L, Samet A, Dziemaszkiewicz E. Cefepime in vitro activity against derepressed extended-spectrum ß-lactamase (ESBL)-producing and non-ESBL-producing Enterobacter cloacae by a disk diffusion method. J Antimicrob Chemother 2001; 48: 321-2.
12. Powell J, Bureau MA, Paré C, Gaildry ML, Cabana D, Patriquin H. Necrotizing enterocolitis. Epidemic following an outbreak of *Enterobacter cloacae* type 3305573 in a neonatal intensive care unit. Am J Dis Child 1980; 134(12): 1152-4.
13. She D, Liu Y. The expression of AmpC and extended-spectrum ß-lactmases among clinical isolates of *Enterobacter cloacae* and its impact on antibiotics susceptibility. Zhonghua Yi Xue Za Zhi 2002; 82(19): 1355-8.
14. Spanu T, Luzzaro F, Perilli M, et al. Occurrence of extended-spectrum ß-lactamases in members of the family *Enterobacteriacea* in Italy: implications for resistance to ß-lactams and other antimicrobial drugs. Antimicrob Agents Chemother 2002; 46: 196-202.
15. Yu YS, et al. First isolation of blaIMI-2 in an *Enterobacter cloacae* clinical isolate from China. Antimicrob Agents Chemother 2006; 50(4): 1610-1.

ACKNOWLEDGMENT

We are grateful to Mrs Annarita Perfetti (Unità Operativa Complessa di Microbiologia e Virologia Clinica, Dipartimento di Medicina Trasfusionale, Ospedale Civile Spirito Santo, Pescara, Italy) for her precious collaboration.