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 β-lactamases as a cause of β-lactam resistance, and, less frequently, plasmid-mediated ESBLs. Carbapenemase-producing E. cloacae strains have been uncommonly reported (15).

Extended-Spectrum β-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 β-lactams (broad-spectrum penicillins, aztreonam, cephalosporines), except for betalactams/beta-lactamase 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 116
(MIC ≥ 4 µg/ml), cefotaxime (MIC ≥ 64 µg/ml), ceftazidime (MIC ≥ 64 µg/ml), cefoxitin (MIC ≥ 4 µg/ml), ciprofloxacin (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-,

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 hydrolyser producer, too (cefoxitin-resistance due to efflux pumps or reduced permeability of the bacterial cell wall has been reported in ESBL-producing 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-lactamas-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, polimicrobial 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 therapeutic 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


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