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LETTERS TO THE EDITOR

Risk of misidentification of Enterobacter aerogenes inducible cephalosporinases

Dear Editor,

Enterobacter aerogenes is a Gram negative bacterium, frequently harboured as a part of the commensal flora in the human respiratory and enteric tracts, [9] but able to cause urinary infections, pneumonia and bacteremia. [1,12,15] A case of cryptogenic abdominal aortitis has also been reported. [12] Particularly, this organism has recently emerged as a nosocomial pathogen, generally affecting immunocompromised hosts. [9] E. aerogenes often exhibits high degree resistance to betalactams, due to extended-spectrum beta-lactamase expression, production of AmpC-hydrolases, reduced outer membrane permeability, and development of efflux mechanisms. [1,9,15]

Patients with hematologic malignancies which are admitted to the Department of Hematology of the Spirito Santo Hospital in Pescara, Italy, are routinely screened for colonization with common and uncommon organisms. This has the purpose of early detecting clinically significant bacterial and fungal colonization, monitoring the diffusion of antimicrobial resistance phenotypes and mechanisms within the Department, and aiding clinicians in choosing the most proper empirical antibiotic treatment when infections (especially bacteremias and pneumonias) are diagnosed, while waiting for culture results and susceptibility data. In fact, vigilance and timely recognition of colonization with resistant organisms may be a weapon against multidrug-resistant bacterial population. So, stool, sputum and urine samples, as well as skin, pharyngeal and genital swabs from these patients are collected and studied at least twice or three times a week, even iff ever is absent and no signs of infection at any bodily site are observed. Particularly, drug-resistant isolates belonging to the Pseudomonas genus and the Enterobacteriaceae family are studied and monitored, as these represent the most common agents ofl ife-threatening bloodstream infections among the immuno-compromised patients of the Pescara Hospital Hematology Department.

Five E. aerogenes strains (identification obtained by Vitek2, bioMe´rieux, Italy) were isolated from faeces of hospitalized patients with hematologic malignancies over a six-month period, from 1 January 2008 to 30 June 2008. All of the isolates exhibited susceptibility to piperacillin (MIC $\leq$ 4 mg/ml), piperacillin e tazobactam (MIC $\leq$ 4 mg/ml), cefotaxime (MIC $\leq$ 1 mg/ml), ceftazidime (MIC $\leq$ 1 mg/ml), cefepime (MIC $\leq$ 1 mg/ml), aztreonam (MIC $\leq$ 1 mg/ml), imipenem (MIC $\leq$ 1 mg/ml), meropenem (MIC $\leq$ 0.25 mg/ml), but resistance to ampicillin (MIC $\geq$ 64 mg/ml), ampicillin/sulbactam (MIC $\geq$ 32 mg/ml), amoxicillin/clavulanate (MIC $\geq$ 32 mg/ml), and cefoxitin (MIC $\geq$ 64 mg/ml). MICs were obtained by Vitek2 (bioMe´rieux, Italy). A disc diffusion test (disks by Liofilchem, Italy) confirmed Vitek2 results. [10] Also, a double disk synergy test (DDST) was carried on by placing disks of cefotaxime, ceftazidime, cefepime, and aztreonam adjacent to an amoxicillin/clavulanate and an ampicillin/sulbactam disk. It has been known that sulbactam should be preferred when probable AmpC-producers are screened for ESBLs, as amoxicillin/clavulanate acts as a stronger AmpC inducer than ampicillin/sulbactam, so masking ESBL detection by agar disk methods. [2,8] Neither clavulanate- nor sulbactam-synergy was observed with any of the betalactams tested, so that all of the isolates were labeled as non-ESBL phenotypes. Results were confirmed by an E-test ESBL screen (AB BIODISK, Sweden) which was performed by using ceftazidime/clavulanate and cefotaxime/clavulanate commercial strips. [4,14] A modified disk approximation test (D-test) was also carried out, [5] in order to screen the isolates for the expression ofi nducible hydrolases. We used imipenem e piperacillin, imipenem e cefotaxime, imipenem e ceftazidime, imipenem e aztreonam, imipenem e cefepime, and imipenem e piperacillin/tazobactam, as inducer e substrate combinations. Towards imipenem (induced side), inhibition zone of substrate disks other than cefepime was reduced by > 2 mm, thus revealing the expression ofi nducible beta-lactamases. All of the isolates were then considered as resistant to all beta-lactams, except for cefepime and carbapenems, despite Vitek2 results. Lack ofi nducible cefepime-resistance was expected, as this compound is known to be poorly affected by AmpCs.

AmpC-betalactamases have recently gained remarkable clinical and microbiological importance, as these confer resistance to narrow-, expanded-, and broad-spectrum cephalosporins (including 3rd generation compounds), and aztreonam; further, they are not affected by clavulanate, sulbactam, and tazobactam and commonly leave a few therapeutic alternatives among betalactams (carbapenems). [15] Prevalence of this kind of hydrolases has underwent a significant rise in the recent years, so that drug-resistance due to their expression has become of serious clinical concern. Particularly, Gram negative bacteria producing both extended-spectrum beta-lactamases and AmpCs have been increasingly found world-wide. Also, genes encoding for resistance to antimicrobials

other than betalactams and AmpC-genes frequently coexist on the same plasmid; therefore, AmpCs may cause multidrug-resistance, together with loss of penicillin- and cephalosporin-susceptibility. [3,7,11,13] AmpC-enzymes have been mainly reported in Escherichia coli , Klebsiella pneumoniae , Salmonella spp., Citrobacter freundii , Enterobacter cloacae , Serratia spp., and Proteus mirabilis , [6] and can be upregulated by subinhibitory concentrations of betalactams. Ureidopenicillins and oxyimino-cephalosporins act as weak AmpC-inducers, thus leading to selection of stably derepressed mutants which are responsible for overproduction of AmpCs and expression of high-level resistance to betalactams. [5] Clinical laboratories currently screen Gram negative organisms for ESBLs, whereas inducible AmpC-betalactamases are not routinely searched. Also, automatic systems for antibiotic susceptibility testing and standard disk methods may fail in detecting inducible mechanisms of resistance to antimicrobials, so that treatment failure is frequently observed, in vivo . [3,7,13,14] Our brief communication focuses on the risk of misidentification of E. aerogenes inducible betalactamases in routine clinical practice, so that careful evaluation of susceptibility of this organism to betalactams should be required; though isolation of this bacterium is not as frequent as the one of E. cloacae , importance of members of this species as human nosocomial pathogens is emerging, partly due to administration of betalactams as prophylaxis or first-line therapy for hospital infections. But above all, authors would suggest clinicians to routinely require both ESBL and inducible AmpC screening to clinical laboratories. This is needed in order to obtain correct susceptibility data, to provide real informations about diffusion and epidemiology of drug resistances, and to prevent in vivo antimicrobial failure, and reduce morbidity and mortality due to life-threatening infections by Gram negative bacteria.

Acknowledgments

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Co-morbidities increase the risk of serious infections in patients with rheumatoid arthritis treated with TNF a inhibitors

In patients with rheumatoid arthritis (RA), TNF a inhibitors may confer an increased risk for non-tuberculous infection. [1] Some studies found no increase in infection rate, [2,3] whereas others found increased risk of serious infections. [4,5] We prospectively studied serious infections in RA patients treated with TNF a inhibitors in a single Rheumatology Center. All 269 patients with RA followed up in the Rheumatology Clinic of the University General Hospital of Larisa from January 2002 to September 2007 were included in the study. Seventy-three patients (27.1%) were on TNFa inhibitors (mean age ± standard deviation [SD], 56.7 ± 13.5 years; 605.1 patient-years) and 196 patients on DMARDs (61.1 ± 12.1 years; 1448.3 patient-years). For a possible link between infection and drug, patients should have been exposed to or withdrawn from the drug less than seven times the half-life of the drug. All hospitalized patients had basic hematological and biochemistry tests, blood and urine cultures, and serological tests for bacteria and viruses. They also had tests for ANA, anti-dsDNA, serum protein electrophoresis, and cryoglobulins. They underwent bone marrow aspiration for parasites and culture, and bone marrow biopsy. In suspected lung infection, diagnosis was established on the basis of compatible symptoms and signs, typical changes on X-rays, and CT-scan, sputum examination, and, in a few cases, broncho-alveolar lavage (BAL) and transbronchial biopsy. Continuous parameters between the two groups were compared using Mann e Whitney U test and categorical parameters were compared using Fisher's exact test or chi-square test.

Seventeen patients were hospitalized for serious infections (18 events). Six patients were on TNF a inhibitors (8.2%; rate ofi nfection, 0.99/100 person-years) and 11 patients (12 events) were on DMARDs (5.6%; rate ofi nfection, 0.83/100 person-years). There was no difference between the two groups [unadjusted OR Z 1.97, (0.72 e 5.39; adjusted for co-morbidities and disease duration OR Z 2.24 (0.73 e 6.89)) p Z 0.26]. Survival curve (Kaplan e Meier) analysis also revealed no difference between the two groups. Co-morbidity [interstitial lung disease (ILD), diabetes mellitus (DM)], disease duration, age, and gender were tested as potential risk factors for infection by univariate and multivariate logistic regression. ILD [adjusted for co-morbidities and disease duration OR Z 7.3 (95% CI, 2.2 e 24.4), p Z 0.001] and DM [adjusted for co-morbidities and disease duration OR Z 16.7 (95% CI, 2.8 e 98.0), p Z 0.001] were significantly associated with infection. Disease duration [OR Z 0.99 (95% CI, 0.93 e 1.04), p Z 0.19], gender [OR Z 0.7 (95% CI, 0.2 e 2.1), p Z 0.5], or age [OR Z 1.0, (95% CI, 0.9 e 1.0), p Z 0.4], were not associated with increased risk ofi nfection.

Patients with serious infections are shown in Table 1. Among TNFa inhibitor-treated patients with infection, 2 patients (#15, #17) had DM and 3 patients (# 13, #14, #15) had ILD. Patient #14 presented with symptoms and signs of lung infection, the CT-scan showed patchy infiltration in both lungs with interstitial fibrosis of the bases; all cultures and serology for pathogens were negative. The patient received the empiric antibiotic treatment but died of respiratory failure in the Intensive Care Unit. Among DMARD-treated patients with infection, 2 patients had chronic obstructive pulmonary disease (COPD) (# 2, # 3), 2 patients had DM (# 1, # 3), and 4 patients had ILD (# 1, # 5, # 9, # 11). Except for patients # 1, #6, and #11, DMARD-treated patients had an uneventful recovery.

ILD was identified in 23 patients. Five ILD patients were on TNFa inhibitor: three patients had lung infections and all three died. Eighteen ILD patients were on DMARDs: four patients had lung infections and two of these died. The probability of being alive in the two treatment groups was compared using the Kaplan e Meier survival curve and the longrank test. Patients with ILD on TNF a inhibitors were more likely to die (p Z 0.02).

Patients with RA generally may have an increased risk of infection compared to non-RA patients and sites ofi nfections with the highest risk ratios were skin and soft tissue, bone, joints, and the respiratory tract. [6] In a meta-analysis of randomized controlled trials of TNF a inhibitors, an increased risk of serious infections was found in TNF a

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ERRATUM

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