Table 1
Association of *iceA* genotypes, clinical outcome and 23S rRNA mutations

iceA genotype	Clinical outcome	23S rRNA mutation		
		A2143G	A2142G	A2142C
iceA1	CG	1 (3.45%)	12 (57.14%)	2 (25%)
	PU	18 (62.07%)	2 (9.52%)	1 (12.5%)
	GC	0	0	0
iceA2	CG	9 (31.03%)	4 (19.05%)	2 (25%)
	PU	0	2 (9.52%)	2 (25%)
	GC	0	0	0
iceA1/*iceA2*	CG	0	1 (4.76%)	0
	PU	0	0	0
	GC	1 (3.45%)	0	1 (12.5%)
Total		29	21	8

CG, chronic gastritis; PU, peptic ulcer; GC, gastric carcinoma.

resistance to clarithromycin. Thus, we hypothesise that identification of *H. pylori* strains based on the clarithromycin susceptibility pattern and the *iceA* gene that circulates in a given geographic area may permit not only the identification of high-risk patients but also define the appropriate regimen for eradication of *H. pylori* and prevention of peptic ulcer disease later in life in patients eventually infected with such strains.

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Effects of β-lactam antibiotics and L-arginine in the treatment of experimental sepsis in rats

Sir,

In this study, we investigated the impact of L-arginine and two β-lactam antibiotics on the treatment of experimental sepsis in order to look for a significant difference in the mechanisms of action of these drugs.

In analysing the formulation of the antibiotics used in this study, it was noted that aztreonam has L-arginine in its formulation (780 mg/g of antibiotic), which is used as a buffer. As L-arginine is a nitric oxide precursor, which is a powerful vasodilator, there is the possibility that this concentration of L-arginine could have some influence on the septic condition. As ceftriaxone, the other β-lactam antibiotic used in our experiments, has no L-arginine in its formulation, the same concentration (39 mg/50 mg of antibiotic) was added. To exclude the possible direct effect of L-arginine, an L-arginine-treated septic group was used as a secondary control.

Six hours after the induction of sepsis [1], rats received an injection of the various treatments: ceftriaxone (50 mg/kg); aztreonam (50 mg/kg); ceftriaxone plus L-arginine (50 mg/kg and 39 mg/kg, respectively); and L-arginine alone (39 mg/kg) (secondary control). The septic control group received a saline injection. Twelve hours after the induction of sepsis (6 h after the treatments), all parameters were analysed and the results are presented in Table 1.

Increased survival was seen in all groups treated with antibiotics, reaching 100% in the group that received ceftriaxone + L-arginine. Survival in the septic and L-arginine-treated control groups was 0.0%, suggesting that this amino acid alone, at the dosage used in our experiment, did not present a therapeutic effect. Despite the different mechanisms of action

Table 1
Effects of β-lactam antibiotics and L-arginine in the treatment of experimental sepsis

Parameter	Septic control (saline) ($n=7$)	Aztreonam ($n=7$)	Ceftriaxone ($n=7$)	Ceftriaxone + L-arginine ($n=7$)	L-Arginine control ($n=7$)
Survival function (%)	0	71.4[a]	85.7[a]	100[a]	0
Bacterial count ($\times 10^6$ CFU/mL)[b]	507.1 ± 375.8	108.7 ± 89.4[*]	9.0 ± 15.3[*]	0.6 ± 0.3[*]	305.7 ± 133.6
Serum $NO_2 + NO_3$ (μM)[c]	54.8 ± 14.5	46.9 ± 12.4	65.3 ± 25.7	89.5 ± 19.3[**]	53.2 ± 9.0
Serum TNF-α (pg/mL)[c]	160.6 ± 24.9	147.5 ± 23.9	142.4 ± 54.1	207.2 ± 43.5	150.9 ± 27.8
Serum IL-1β (pg/mL)[c]	45.6 ± 14.8	126.8 ± 44.3	36.4 ± 10.1	337.2 ± 73.5[***]	142.3 ± 90.1
Serum IL-10 (pg/mL)[c]	92.2 ± 14.0	152.0 ± 32.1[****]	41.7 ± 28.2	199.3 ± 28.1[****]	141.9 ± 39.6[****]

CFU, colony-forming units; TNF-α, tumour necrosis factor-alpha; IL, interleukin; S.D., standard deviation.
[a] Kaplan–Meier survival function curves: test statistics for equality of survival distributions ($P<0.001$).
[b] Values are mean ± S.D. and were analysed by Mann–Whitney statistics: [*]$P<0.01$ compared with the septic and L-arginine-treated control groups.
[c] Values are mean ± S.D. and were analysed by analysis of variance (ANOVA) followed by the Bonferroni test: [**]$P<0.01$ compared with the septic control, aztreonam and L-arginine (secondary control) groups; [***]$P<0.01$ compared with all other groups; [****]$P<0.01$ compared with the septic control and ceftriaxone groups.

of both antibiotics tested (aztreonam acting on penicillin-binding protein (PBP) 3 and ceftriaxone acting on PBP2), they did not present significantly different survival function curves.

As the sepsis model used in this experiment involved the introduction of a capsule containing bacteria into the abdominal cavity, the numbers of viable bacteria following treatments were quantified in the peritoneal liquid. In the groups receiving antibiotics, where survival was increased, a significant reduction in the bacterial count in the peritoneal liquid was observed 6 h after drug administration, whilst in the L-arginine-treated group the bacterial count remained the same as that of the septic control group. These data suggest that the use of β-lactam antibiotics, whatever their mechanism of action, is still an efficient means of controlling Gram-negative bacterial infections.

Despite the obvious importance of the use of antibiotics in managing sepsis, there is a correlation between them and the pathophysiology of this inflammatory syndrome. Lipopolysaccharide (LPS) released by antibiotics correlates well with the affinities for binding to and inhibition of PBP1, PBP2 and PBP3 [2]. The components released from the cell wall lead to the production of pro-inflammatory and anti-inflammatory cytokines. Kragsbjerg et al. [3] showed an increase in the levels of tumour necrosis factor-alpha (TNFα) when monocytes were incubated with the supernatant of LPS from *Escherichia coli* and antibiotics that act on different PBPs. The levels of TNFα were significantly higher when an antibiotic was used that acted on PBP3 compared with one that acts on PBP2. In our study, there was no difference between any treated group and both control groups. This observation suggests that serum TNFα may not be a good indicator of the pro-inflammatory response when measured 12 h after bacterial infection.

An in vitro study showed that TNFα and interleukin (IL)-1β, in combination with LPS, can stimulate the inducible nitric oxide synthase (iNOS) enzyme and L-arginine uptake, increasing the accumulation of nitrite in cell culture [4]. Analysing our results, we observed an increase in the levels of IL-1β in the septic animals treated with ceftriaxone + L-arginine. This same group presented significantly increased plasma nitrite/nitrate levels. Based on these data, it is possible to suggest that there was iNOS stimulation and/or an increase in L-arginine uptake by the CAT2B cationic amino acid transporter, the activity of which is known to be stimulated by the IL-1β level [3].

In our study, there was a significant rise in the serum concentration of IL-10 in the septic groups treated with ceftriaxone + L-arginine and aztreonam. Our results show that IL-10, which has a protective effect in lethal and sublethal endotoxaemia [5,6], was significantly increased in those groups treated with antibiotics that contained L-arginine (either in its formulation or added), suggesting that this amino acid in some way stimulates an anti-inflammatory response. However, when analysing the levels of IL-1β, nitrite/nitrate and IL-10 in conjunction, it is possible to observe that the IL-1β and nitrite/nitrate levels were only increased in the ceftriaxone + L-arginine group, suggesting that L-arginine stimulates the inflammatory effect of ceftriaxone, leading us to propose that the simultaneous increase in the IL-10 level in this group represents an organic attempt to control the enhanced inflammatory process.

Data from our study suggest that β-lactam antibiotics, whatever their target of action, significantly improved the survival of rats. Although some authors recommend the use of L-arginine in the treatment of sepsis [7], we observed that this amino acid given alone at the low dose used in this study did not have a positive influence on the septic condition and failed to increase the survival rate of the animals. However, when L-arginine was administered together with a β-lactam antibiotic that does not have it in its formulation (i.e. ceftriaxone), it appears to generate an enhanced anti-inflammatory response, indicated by the increase in IL-10, which, eventually, can be beneficial in the treatment of sepsis.

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First report of *Listeria monocytogenes* endocarditis treated with linezolid

Sir,

Listeria endocarditis is a very uncommon entity (69 cases published to date) and carries a related mortality of ca. 35% [1,2]. Advanced age, pregnancy, human immunodeficiency virus (HIV) infection, prior heart valve disease and immunosuppression have all been described as underlying conditions. Only 23 of the reported cases occurred on prosthetic valves. Optimal therapy of *Listeria* endocarditis is not well established and the role of surgery is controversial. Based on in vitro synergy studies and animal models, a combination of ampicillin and gentamicin is usually recommended as first-line therapy. For those intolerant to penicillin, the agents trimethoprim/sulphamethoxazole or vancomycin are alternatives to β-lactam drugs.

In the present study, we report the first case of *Listeria monocytogenes* endocarditis treated with linezolid in a patient who presented intolerance to standard therapy and who was diagnosed by means of molecular techniques performed in the valve tissue.

A 76-year-old man was hospitalised due to relapsing fever. His antecedents included chronic renal insufficiency (creatinine clearance 30 mL/min), rheumatic polymyalgia, transient ischaemic cerebrovascular accident and duodenal ulcer. He had a prosthetic aortic valve since 1996 and a defibrillator since May 2005. He was chronically treated with acenocumarol, lovastatin, enalapril, omeprazole and benzodiazepines.

In October 2005 he had been hospitalised due to a constitutional syndrome and anaemia. A gastroscopy and colonoscopy were performed and revealed a Barret's oesophagus, chronic gastritis, colonic diverticulosis and a colonic polyp that was resected. No endocarditis prophylaxis was administered. The patient was placed on iron therapy due to anaemia.

Since discharge, the patient came on several occasions to the emergency room owing to malaise, fever and abdominal pain. He was re-admitted again to the hospital 2 weeks after discharge and transoesophageal echocardiography demonstrated a prosthetic aortic valve and native mitral valve endocarditis. Blood cultures remained negative. Vancomycin, gentamicin (two doses) and ceftriaxone were started. An abdominal computed tomography (CT) scan demonstrated several lesions in the spleen compatible with infarcts. *Bartonella, Brucella, Coxiella, Mycoplasma, Chlamydia, Legionella* and *Thoperyma* serology remained negative.

The evolution was clearly unfavourable, with development of congestive heart failure and progressive valve dysfunction. An aortic homograft and replacement of aortic and mitral valves were required and surgery revealed the presence of an aortic periannular abscess. Valves and abscess tissues were sent to the microbiology laboratory for polymerase chain reaction amplification of the bacterial 16S rRNA gene and for bacterial, mycobacterial and fungal culture. DNA was extracted from the valves and the amplicon was sequenced and compared with those stored in GenBank database (http://www.ncbi.nlm.nih.gov/). Identification to species level demonstrated the presence of *L. monocytogenes*. Valve culture was negative.

The patient's renal function experienced post-operative deterioration. Vancomycin therapy was stopped and line-