and co-trimoxazole ($P = 0.0398$) and for *S. aureus* with respect to oxacillin ($P = 0.0013$). GAS resistance to erythromycin was remarkably lower in Group B, although not significantly ($P = 0.0863$). The resistance rates of *S. pneumoniae* to penicillin were almost equally high in both groups (35.7% for Group A and 40% for Group B).

The predominance of *S. aureus* in our report could be partly due to the establishment of the *S. pneumoniae* vaccine since the year 1995 as a recommended routine preventive vaccination in Greece.

Resistance to erythromycin of *H. influenzae* and GAS was significantly lower in Group B (Table 1). The same could be observed for *S. aureus* with respect to oxacillin. However, penicillin-resistant *S. pneumoniae* strains were in equal rates in both periods, reaching ca. 40% (Table 1) and remaining at about the same levels as reported by other studies on Greek children for the periods 2001–2004 and 2004–2006 [4,5].

The growing resistance of many pathogens and, moreover, the emergence of multidrug-resistant strains, such as those of *S. pneumoniae*, complicate the management of AOM, and the empirical drug choice increases the risk of failure. Many study groups and competent organisations have tried to establish consensus recommendations for the empirical management of AOM. Some recent reports are suggesting amoxicillin at conventional or high doses as an appropriate choice for first-line therapy, with second-line alternatives amoxicillin/clavulanic acid, cefuroxime and ceftriaxone [3,6]. Regarding our data, where *S. aureus* appears to be the causative agent in approximately one-half of the examined child population, these suggestions should be adapted accordingly.

Antimicrobial treatment guidelines should be revised and readapted on an on-going basis according to the epidemiological data provided by each geographic region, taking into consideration various factors such as the resistance patterns of the isolated pathogens as well as the individual antimicrobial treatment policies.

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References

[1] Faden H, Duffy L, Boeve M. Otitis media: back to basics. Pediatr Infect Dis J 1998;17:1105–13.

[2] Teele DW, Klein JO, Rosner B; Greater Boston Otitis Media Study Group. Epidemiology of otitis media during the first seven years of life in children in Greater Boston: a prospective, cohort study. J Infect Dis 1989;160:83–94.

[3] Dowell SF, Butler JC, Giebink GS, Jacobs MR, Jernigan D, Musher DM, et al. Acute otitis media: management and surveillance in an era of pneumococcal resistance—a report from the Drug-resistant *Streptococcus pneumoniae* Therapeutic Working Group. Pediatr Infect Dis J 1999;18:1–9.

[4] Paraskakis I, Kafetzis DA, Chrisakis A, Papavasilliou H, Kirikou H, Pangalis A, et al. Serotypes and antimicrobial susceptibilities of 1033 pneumococci isolated from children in Greece during 2001–2004. Clin Microbiol Infect 2006;12:478–95.

[5] Poulakou G, Katsarolis I, Matthaiopoulou I, Tsiodras S, Kanavaki S, Hatzaki D, et al. Nationwide surveillance of *Streptococcus pneumoniae* in Greece: patterns of resistance and serotype epidemiology. Int J Antimicrob Agents 2007;30:87–92.

[6] Hoberman A, Marchant CD, Kaplan SL, Feldman S. Treatment of acute otitis media consensus recommendations. Clin Pediatr (Phila) 2002;41:373–90.

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High resistance to fourth-generation cephalosporins among clinical isolates of Enterobacteriaceae producing extended-spectrum β-lactamases isolated in Portugal

Sir,

Here we report the molecular and antimicrobial susceptibility profile of extended-spectrum β-lactamase (ESBL)-producing strains found in the Portuguese northern occidental coast region (Minho). For this purpose, bacteria isolated from clinical hospitalised and non-hospitalised patients over a period of 2 years were identified and minimal inhibitory concentrations (MICs) were determined by microdilution methods according to the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) guidelines on Enterobacteriaceae. Additionally, ESBL phenotypic identification was confirmed by the Etest (AB BIODISK, Solna, Sweden). Various methods of molecular identification of the β-lactamase (*bla*) genes, involving polymerase chain reaction (PCR) and sequencing strategies, were used in this study.

The ESBL-producing strains ($n = 193$) were isolated from urine ($n = 127$), sputum ($n = 42$), bronchoalveolar lavage ($n = 14$), blood ($n = 7$) and ascitic fluid ($n = 3$). The most frequent ESBL-producing organism isolated in the present study was *Escherichia coli* (67.9%; $n = 131$), followed by *Klebsiella pneumoniae* (30.6%; $n = 59$), *Klebsiella oxytoca* (0.5%; $n = 1$), *Enterobacter aerogenes* (0.5%; $n = 1$) and *Citrobacter freundii* (0.5%; $n = 1$). The ESBL detected in the present study were the TEM type (40.4%), CTX-M type (36.8%) and SHV type (22.8%).

TEM-52 and TEM-24 were the most frequent TEM types (20.2% and 12.9%, respectively). Members of TEM-10 (4.1%) and TEM-116 (2.1%) were also detected.

Within the CTX-M family, CTX-M-9 group was represented by CTX-M-9 (13.5%) and CTX-M-14 (8.4%). In the CTX-M-1 group, CTX-M-15 was the most frequent type (12.4 %), followed by CTX-M-1 (2.1%), CTX-M-3 (0.5%) and CTX-M-32 (0.5%). Regarding CTX-M types, it appears that CTX-M-14 is widespread among the northwestern Iberian Peninsula [1]. *Klebsiella pneumoniae* harbouring a CTX-M-15 enzyme was described for the first time in Portugal in 2005 [2] in the Lisbon area, but CTX-M-15 enzyme has also recently been found by us in the north of Portugal in another Enterobacteriaceae member, isolated from bloodstream infections [3] among seven patients in two different hospitals. Other ESBL-producing species (not *E. coli* or *K. pneumoniae*) were also found. This is the first time that *C. freundii* has been described as a producer of CTX-M-32 in this country.

The SHV enzymes occurred only in 23.3% of all ESBL-producing organisms. Within this type, the most frequent type was SHV-12 (12.4%), followed by SHV-5 (8.8%) and finally SHV-2 (2.1%).

Some isolates co-produced more than one ESBL type: TEM-52/CTX-M-14 (0.5%); TEM-116/CTX-M-14 (0.5%); and TEM-116/CTX-M-15 (0.5%).

MIC testing showed that isolates producing ESBLs were mostly susceptible to carbapenems (100%) and amikacin (99.5%). In con-

trast, ESBL-producing strains presented low susceptibility rates to cefepime and quinolones. Indeed, 98.9% of the ESBL-producing strains were cefepime-resistant and 85.4% were resistant to quinolones (ciprofloxacin and norfloxacin). In the generality, these high levels of resistance to quinolones were more conspicuous in members of the CTX-M family (98.1%) than TEM and SHV types (80.8% and 72.1%, respectively).

In this study, cefepime presented a surprisingly low activity against ESBL-producing microorganisms. Recent literature refers to the inoculum effect exhibited by cefepime [4]. Nevertheless, we believe that this should not be pointed out as a single explanation once MIC determination is performed using inoculum concentrations of 0.5 McFarland standard. In our sample, only two *K. pneumoniae* harbouring SHV-2 ESBL were susceptible to cefepime. All the other clinical isolates (98.9%) expressing the ESBL phenotype were resistant to cefepime. It seems interesting that a recent study showed that cefepime was successfully administered to three patients (two females and one male) aged between 47 years and 87 years carrying a Gram-negative ESBL-positive strain [5]. Nevertheless, other studies worldwide have begun to describe the emergence of high resistance to cefepime among Gram-negative ESBL-producers [6].

The present work showed a high diversity of ESBL enzymes occurring in the north of Portugal. In this country, the most prevalent type is still the TEM type, but CTX-M is growing rapidly [7]. The emergence of ESBL-producers resistant to cefepime in Portugal is a matter of concern. We believe that the uncontrolled use of cephalosporins may have an important role in the acquisition of resistance mechanisms, particularly the production of ESBL enzymes. Establishment of policies to monitor drug delivery in hospital and ambulatory pharmacies as well as implementation of public health defence strategies towards health promotion and drug resistance prevention appear to be urgent.

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References

[1] Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165–74.

[2] Conceição T, Brízio A, Duarte A, Lito LM, Cristino JM, Salgado MJ. First description of CTX-M-15-producing *Klebsiella pneumoniae* in Portugal. Antimicrob Agents Chemother 2005;49:477–8.

[3] Fernandes R, Vieira M, Ferraz R, Prudêncio C. Bloodstream infections caused by multidrug-resistant Enterobacteriaceae: report from two Portuguese hospitals. J Hosp Infect 2008;70:93–5.

[4] Queenan AM, Foleno B, Gownley C, Wira E, Bush K. Effects of inoculum and beta-lactamase activity in AmpC- and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. J Clin Microbiol 2004;42:269–75.

[5] Bhavnani SM, Ambrose PG, Craig WA, Dudley MN, Jones RN. SENTRY Antimicrobial Surveillance Program Outcomes evaluation of patients with ESBL- and non-ESBL-producing *Escherichia coli* and *Klebsiella* species as defined by CLSI reference methods: report from the SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis 2006;54:231–6.

[6] Grover SS, Sharma M, Chattopadhya D, Kapoor H, Pasha ST, Singh G. Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in *Klebsiella pneumoniae*: emergence of high resistance against cefepime, the fourth generation cephalosporin. J Infect 2006;53:279–88.

[7] Hernández JR, Martínez-Martínez L, Cantón R, Coque TM, Pascual A. Spanish Group for Nosocomial Infections (GEIH), Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Spain. Antimicrob Agents Chemother 2005;49:2122–5.

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Statins inhibit *Toxoplasma gondii* multiplication in macrophages in vitro

Sir,

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is a widespread disease affecting primarily immunocompromised and pregnant individuals. As an obligate intracellular parasite, *T. gondii* replicates only inside a host cell in a specialised compartment called the parasitophorous vacuole [1].

Although cholesterol is not synthesised by *T. gondii*, there is evidence for isoprenoid synthesis, a lipid pathway in the apicoplast [2]. This pathway is essential for various cellular functions such as control of cell growth, mitochondrial electron transport and tRNA synthesis, thus revealing an attractive area for therapeutic intervention [3]. In both prokaryotic and eukaryotic cells, the isoprenoid pathway is highly regulated at the level of two sequential enzymes involved in the synthesis of mevalonate, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase [4,5].

Simvastatin (SV), rosuvastatin (RV) and atorvastatin (AV) are commercial drugs of the statin family available as hypocholesterolaemic agents in humans. These drugs are HMG-CoA reductase inhibitors, blocking L-mevalonic acid synthesis and preventing the synthesis of important isoprenoid intermediates of the cholesterol biosynthetic pathway [6].

Despite the impressive increase in our knowledge of *T. gondii* biology, treatment of toxoplasmosis is still limited to only a few available regimens. Unfortunately, standard first-line therapies are often not tolerated or induce significant side effects in patients. Thus, the need for finding alternative anti-*Toxoplasma* strategies remains. The goal of our study was to establish whether the three statins SV, RV and AV are able to inhibit *T. gondii* multiplication in vitro as a tool for interference in the intracellular cycle of the parasite.

Resident macrophages were collected from the peritoneal cavity of Swiss mice as previously described [1]. Monolayers of macrophages were cultivated for 24 h at 37 °C in a 5% CO_2 atmosphere and were then used for the experiments. Tachyzoites from the virulent RH strain of *T. gondii* were maintained by intraperitoneal passage in female Swiss mice. The parasites were obtained as previously described [1]. *Toxoplasma gondii* tachyzoites suspended in Dulbecco's Modified Eagle's Medium (DMEM) were incubated for 1 h in the presence of macrophages using a 5:1 parasite–host cell ratio. Following interaction, the cultures were washed three times with medium to remove extracellular parasites and treated for 24 h with different statin concentrations. SV was tested at 5–40 μg/mL, whilst RV and AV were tested at 5–60 μg/mL. The cultures were then fixed and stained using the Panotic Staining Kit (Laboclin, Paraná, Brazil), composed of a mixture of stains used in haematological analysis. The mean number of macrophages containing more than one parasite per parasitophorous vacuole was determined by examining 200 infected cells. Statistical significance was assessed by analysis of variance (ANOVA) followed by Tukey's test; $P < 0.05$ was regarded as statistically significant. After quantifying *T. gondii* multiplication, the percentage inhibition for each statin