The coding regions of the transpeptidase domain of the pbp1a, pbp2b and pbp2x genes of the two GBS isolates were amplified and sequenced according to previously outlined methods. Amino acid sequences were deduced and analysed using the ClustalW alignment tool included in the Lasergene software (DNASTar, Madison, WI, USA). Nucleotide and deduced amino acid sequences were compared with those of the reference penicillin-susceptible strains 2603V/R (GenBank accession number: NC_004116) and NEM316 (GenBank accession number: NC_004368). DNA analysis revealed that the pbp genes of the clinical GBS isolates possessed many amino acid substitutions compared with the corresponding genes of the reference strains 2603V/R and NEM316. Five previously described amino acid substitutions were observed in both the penicillin-susceptible GBS and penicillin G-non-susceptible GBS isolates (S453N and N682D in PBP1a, V625I in PBP2b, and I377V and G627V in PBP2x). However, there were three novel substitutions (T526A in PBP1a, P278L in PBP2b and N575D in PBP2x) found exclusively in the penicillin G-non-susceptible GBS 2007 isolate.

In previous studies, the V405A and Q527E substitutions adjacent to the conserved SSN and KSG motifs in PBP2x, considered to form the active site of the enzyme, were found in 4 invasive GBS with elevated, but still susceptible, MICs of one or multiple β-lactam antibiotics, in 21 penicillin G-non-susceptible GBS isolated from the respiratory tract and in a penicillin G-non-susceptible GBS recurrently isolated from a sacral ulcer. Several amino acid substitutions in PBP1a, PBP2a and PBP2b were also found in penicillin G-non-susceptible GBS isolated from the respiratory tract. However, the PBP2a amino acid substitutions were documented in only two GBS with penicillin MICs of 1 mg/L. In the present study, the penicillin G-non-susceptible GBS 2007 isolate did not harbour the PBP2x V405A and Q527E substitutions previously associated with reduced susceptibility to penicillin. Instead, the penicillin G-non-susceptible GBS 2007 isolate possessed three novel substitutions (T526A in PBP1a, P278L in PBP2b and N575D in PBP2x). At this time it is not known whether these substitutions can actually increase resistance to penicillin since they were not found within or in the proximity of the putative conserved motifs, and their significance needs to be assessed in future studies. Moreover, it is possible that the penicillin G-non-susceptible GBS 2007 isolate harbouring mutations in other pbp or non-pbp genes that are responsible for the observed phenotype. To our knowledge, that is the first report of development of invasive GBS not susceptible to penicillin G and ceftriaxone after prolonged low-dose oral penicillin V.

Acknowledgements
We thank the personnel of the bacteriology section of the Medical Microbiology Laboratory of CHUM-Hôpital Saint-Luc for their technical assistance. The editorial work on this manuscript by Ovid Da Silva, Research Support Office, Research Centre, CHUM, is appreciated.

Funding
The data were generated as part of the routine work of the microbiology laboratory of CHUM and LSPQ/INSPO. M. R. is the recipient of a Research Scholar award from the Fonds de recherche en santé du Québec (FRSQ).

Transparency declarations
None to declare.

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J Antimicrob Chemother 2010
doi:10.1093/jac/dkp488
Advance publication 12 January 2010

Raltegravir: is a 400 mg once-daily dose enough?
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Keywords: atazanavir, HAART, HIV infection, pharmacokinetics

Sir,
In the STARTMRK trial, 400 mg of raltegravir twice daily þ tenofovir/emtricitabine showed non-inferior efficacy versus efavirenz. The lack of teratogenicity and favourable drug–drug interaction profile of raltegravir supports first-line use, but the current cost prevents widespread use of raltegravir for first-line treatment.

In dose-ranging studies, raltegravir has shown strong efficacy at doses of 100–400 mg twice daily, with no clear correlation
between the raltegravir dose used and either reductions in HIV RNA or rises in CD4 counts. Summary results from the three main dose-ranging trials are shown in Table 1.

The efficacy of raltegravir was first evaluated in a 10 day monotherapy study, in 35 treatment-naive, HIV-infected individuals. The doses evaluated were 100, 200, 400 and 600 mg twice daily. After 10 days of dosing, the log10 reductions in HIV RNA and the percentage of patients with HIV RNA <400 copies/mL were similar at the four doses evaluated (Table 1). Subsequently, a 48 week trial in treatment-naive patients showed no differences in efficacy between raltegravir dosing and efficacy. 

A 41-year-old man (CDC A3), HIV infected from 2000, without co-infections (hepatitis B virus and hepatitis C virus infections) and co-morbidities, started first-line antiretroviral therapy in October 2008 for a decline of his CD4 count to <250/mm³ and HIV RNA of 28434 copies/mL. A genotypic resistance test showed no resistance mutations for nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors but resistance mutations for non-nucleoside reverse transcriptase inhibitors (K103N). The patient refused all conventional and approved anti-retroviral regimens due to a fear of lipodystrophy, so we began an NRTI-sparing regimen of raltegravir (400 mg twice daily) with atazanavir/ritonavir 300/100 mg daily. At week 1, the patient came back complaining of jaundice. Clinical chemistry showed a value of total bilirubin of 3.9 mg/dL (grade 3). We decided to reduce the atazanavir dosage to 200 mg daily boosted with 100 mg daily of ritonavir. One month later his CD4 cell count rose to 319 cells/mm³ and HIV RNA was undetectable (<50 copies/mL) with a total bilirubin value of 2.06 mg/dL. After 6 months the CD4 count was 331 cells/mm³ with HIV RNA <50 copies/mL. The patient revealed that he had never taken raltegravir at the correct dosage but he took only one raltegravir tablet daily. The patient continued on raltegravir at the 400 mg once-daily dose thereafter.

After 9 months we performed a pharmacokinetic analysis to assess C trough of raltegravir and atazanavir through a validated HPLC method with UV detection. The results showed an atazanavir C trough of 232 ng/mL, superior to 150 ng/mL (the minimum effective concentration of atazanavir), and a raltegravir C trough of 26 ng/mL which exceeded the concentration required to inhibit 95% of viral replication (IC95) (14.6 ng/mL).

Two months later, HIV RNA was still undetectable and CD4 cells were 383 cells/mm³. After another 2 months (October 2009), HIV RNA was <50 copies/mL, with a CD4 cell count of 459 cells/mm³.

During a year of follow-up, plasmatic total cholesterol and low-density lipoprotein (LDL)-cholesterol rose above the upper limit of the normal range. Total cholesterol rose from 178 mg/dL at baseline to 236 mg/dL (upper limit <200 mg/dL); LDL-cholesterol rose from 91 mg/dL at baseline to 140 mg/dL (upper limit <100 mg/dL). The results from the dose-ranging trials and this case report provide preliminary evidence that a 400 mg once-daily dosage of raltegravir, combined with atazanavir/ritonavir, could be a first-line treatment option. This strategy needs to be confirmed in large, well-powered efficacy trials, compared with a recognized standard of care. At this lower dosage, the NRTI-sparing combination of raltegravir with atazanavir/ritonavir would lower pill counts and cost, and could therefore be suitable for wide-scale use.

### Funding

No specific funding was received.

### Transparency declarations

None to declare.
References


