Comparative MIC evaluation of a generic ceftriaxone by broth microdilution on clinically relevant isolates from an academic hospital complex in South Africa

Warren Lowman, Naseema Aithma, Johan F Coetzee, Adriano G Dusè, Mervyn Mer

We evaluated the *in vitro* microbiological efficacy of a generic ceftriaxone product against several clinically significant organisms collected from sterile sites. The minimum inhibitory concentration (MIC) of each was determined simultaneously with the reference and the generic ceftriaxone product. Comparative analysis of MICs between the two products for each isolate was performed using both categorical (interpretive) agreement and essential (actual MIC value) agreement. A total of 260 isolates were tested. Overall, there was categorical agreement of 98.9% and essential agreement of 95.8%. The categorical agreement for all isolates (96.7 - 100%) accorded with international standards, as no very major errors were seen and the major error rate was less than 3%. Of the 90 isolates of *E. coli* (40), *Klebsiella* spp. (40) and *Salmonella* spp. (10), 87.6% had an MIC less than or equal to 0.12 mg/l. The generic ceftriaxone product showed equivalent efficacy by MIC determination to the reference formulation. Ceftriaxone remains a viable and useful antimicrobial agent against a variety of clinically relevant organisms in our setting.

S Afr Med J 2012;102:102-103.

The use of generic pharmaceutical agents is entrenched in South Africa, with resource limitation being the primary motivation. The clinical efficacy, *in vivo* performance and subsequently the role of generic antimicrobial formulations in treating serious bacterial infections, is much debated.[1] Ceftriaxone is an extended-spectrum cephalosporin, first released in South Africa under the brand Rocephin. Since then, many generics have been marketed, including Fresenius Kabi's in 2005. To our knowledge, the only microbiological comparison of a generic product with Rocephin was done by Liebowitz and Slabbert in 2005.[2] Ceftriaxone has since fallen out of favour as a 'workhorse antibiotic' as a result of concerns over collateral damage.[3] Because of the escalating problem of drug-resistant pathogens and the limited armamentarium with which to treat them, it is important to re-evaluate and consider the role of ceftriaxone.

We compared the minimum inhibitory concentrations (MICs) of several clinical isolates from various sites, between the Fresenius Kabi (FK) generic ceftriaxone (intravenous) and a pharmaceutical-grade reference ceftriaxone powder.

Department of Clinical Microbiology and Infectious Diseases, School of Pathology, University of the Witwatersrand; and Infection Control Services Laboratory, National Health Laboratory Services, Johannesburg
Warren Lowman, MB BCh, MMed (Microbiol), FCPath (SA) (Microbiol)
Adriano G Dusè, MB BCh, DTM&H, MSc (Med), MMed (Microbiol), FCPath (Microbiol) (SA)

Infection Control Services Laboratory, National Health Laboratory Services
Naseema Aithma, MT

Department of Anaesthesiology and Critical Care, Stellenbosch University, Tygerberg, W Cape
Johan F Coetzee, BSc, MB ChB, MMed (Anes), FCA (SA), PhD

Department of Medicine, Charlotte Maxeke Johannesburg Academic Hospital and University of the Witwatersrand
Mervyn Mer, MB BCh, Dip PEC (SA), FCP (SA), MMed (Int Med), FCCP, FRCP (Lond)

Corresponding author: *W Lowman (warren.lowman@wits.ac.za)*

Materials and methods

Isolates

A total of 260 clinical isolates were tested including: Enterobacteriaceae (112), *Streptococcus pneumoniae* (52), *Streptococcus pyogenes* (5), *Streptococcus agalactiae* (3), viridans streptococci (2), *Staphylococcus aureus* (30) and *Haemophilus influenzae* (56). Phenotypic identification of all isolates was done according to standard microbiological procedures.[4]

Antimicrobial susceptibility testing (AST)

Broth microdilution (BMD) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines[5,6] was utilised. All BMD plates were prepared in duplicate, one containing the FK generic ceftriaxone, and the other the pharmaceutical-grade ceftriaxone reference powder (Abtek, Liverpool, UK). Plates were thawed prior to testing, inoculated with a standardised inoculum, and then incubated for 16 - 20 hours (Enterobacteriaceae and *S. aureus*) or 20 - 24 hours (streptococci and haemophilus). MICs were read independently by 2 observers. Batches of isolates were tested in conjunction with appropriate reference quality control strains.

Analysis of AST

Comparison of MIC was done using categorical and essential agreement. All MICs were interpreted according to CLSI clinical breakpoints, as stated in the guideline *Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement, M100-S19.*[6]

A very major error (VME) constitutes a resistant isolate by reference ceftriaxone designated susceptible by generic ceftriaxone. A major error (ME) constitutes a susceptible isolate by reference ceftriaxone designated resistant by generic ceftriaxone. A minor error (mE) is designated by intermediate susceptibility according to reference ceftriaxone and generic ceftriaxone, either sensitive or resistant. Percentage error rates were calculated accordingly:

VME = (no. VME/no. resistant strains tested) x 100

ME = (no. ME/no. sensitive strains tested) x 100

mE = (no. mE/total no. strains tested) x 100

Essential agreement was based on the number of generic MICs within one doubling dilution of the reference MIC. The accepted international standard for essential agreement between two systems is ≥90%.[7]

Results

Overall levels of categorical and essential agreement for the Enterobacteriaceae were 99.1% (111/112) and 97.3% (109/112) respectively (Table I); 57 of the Enterobacteriaceae isolates were obtained from blood cultures.

The *S. pneumoniae* isolates were all from sterile sites and demonstrated an overall level of categorical and essential agreement of 98.1% (51/52) and 92.3% (48/52) respectively. A single minor error (error rate = 1.9%) was noted (Table I).

The *H. influenzae* isolates demonstrated an overall level of categorical and essential agreement of 100% (56/56) and 94.6% (53/56) respectively (Table I); 43 isolates were obtained from sterile sites.

The *S. aureus* isolates gave a 96.7% (29/30) level of categorical and essential agreement and demonstrated a single ME (error rate of 4.4%). The range of MICs for *S. aureus* was from 4 to >64 mg/l, with an MIC_{50} of 4 mg/l.

Discussion

The MIC is an *in vitro* microbiological assay used worldwide to determine the susceptibilities of micro-organisms to particular agents. The decision on whether or not to use a particular antimicrobial agent is based on the information derived from MICs. It would therefore seem prudent to evaluate a generic product using this same platform that is used daily in clinical microbiology laboratories. Comparing the MICs of the generic and the originator is indicative of *in vitro* efficacy.

In our study, the majority of the *Klebsiella* spp., *E. coli* and *Salmonella* spp. isolates that tested resistant to ceftriaxone were extended-spectrum ß-lactamase (ESBL) producers. In the absence of ESBL production, ceftriaxone remains a useful antimicrobial option for these isolates, as 86.7% of isolates had an MIC <0.12 mg/l. These low MICs highlight that ceftriaxone requires consideration because of diminishing therapeutic options and efforts to conserve the limited armamentarium of antimicrobials. Isolates were not preselected, and were collected as consecutive isolates from routine cultures. In contrast, only 40.9% (9/22) of *Enterobacter* spp. had an MIC <0.12 mg/l, which probably reflects chromosomal AmpC ß-lactamase production, in addition to ESBL production.

The *S. pneumoniae* isolates demonstrated acceptable levels of concordance for meningeal and non-meningeal isolates. This is important, given the different breakpoints depending on the site of infection. The bulk of ceftriaxone use is aimed at treating meningeal pathogens; therefore we tested insufficient numbers of other streptococci to make firm comparative conclusions. There was, however, 100% categorical agreement for the 10 isolates tested.

The absence or rare occurrence of resistant strains of *H. influenzae* means that there are no defined resistant breakpoints for this organism. Isolates are typically exquisitely sensitive to ceftriaxone, and this was highlighted by a MIC_{50} ≤0.015 mg/l for all isolates.

An overall categorical agreement of 98.9% (257/260) and essential agreement of 95.8% (249/260) have highlighted the comparable *in vitro* efficacy of a generic ceftriaxone. Categorical agreement is determined by defined breakpoints, and this determines whether or not an antimicrobial agent would be prescribed on the basis of antimicrobial susceptibility testing.

There are limitations concerning conclusions from this study. The MIC is a microbiologically defined static end-point that does not address the pharmacokinetic and pharmacodynamic considerations of antimicrobial action. Of greater concern are generic agents that fail to demonstrate bio-equivalence and, in the case of antimicrobial agents, failure to demonstrate *in vitro* microbiological efficacy. The inferior quality of some generic medicines in Nigeria was highlighted, with almost 50% of agents not complying with set pharmacopoeial standards.[8] There are few published reports on the quality of generic antimicrobial agents in South Africa and, with the increasing supply and demand for generic substitution, the necessary controls must be in place to monitor their quality. Furthermore, in the absence of therapeutic efficacy studies, post-marketing surveillance is crucial. There may be publication bias in the reporting of generic antimicrobial agent studies, given the controversy surrounding the issue.

In summary: we demonstrated an excellent level of concordance, using the MIC as a measure of microbiological activity, between a generic ceftriaxone formulation and a reference pharmaceutical-grade powder. Taking into account the controversy and reports of inferior quality of some generic antimicrobial agents, we believe that comparative MIC determination serves as a basis for their initial evaluation.

Acknowledgements. We thank Dr Olga Perovic for assisting in development of the study protocol; the National Institute of Communicable Diseases' GERMS-SA programme for donating *H. influenzae* isolates; and the Fresenius Kabi Advisory Board for their guidance in this project.

Funding. This work was supported by Fresenius Kabi.

Competing interests. The authors received honoraria from Fresenius Kabi. Editorial assistance was provided by Samantha Hopfield, Editorial Director of Anatomy Publishing Partners, and was funded by Fresenius Kabi.

References

1. Schito GC, Keenan MH. Predicting the clinical efficacy of generic formulations of ceftriaxone. J Chemother 2005;17 Suppl 2:33-40.
2. Leibowitz L, Slabbert M. Comparative *in vitro* activity of generic injectable cephalosporins. Southern African Journal of Epidemiology and Infection 2005;20:82-84.
3. Paterson DL. "Collateral damage" from cephalosporin or quinolone antibiotic therapy. Clin Infect Dis 2004;38 Suppl 4:S341-345.
4. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. Manual of Clinical Microbiology, 7th ed. Washington DC: ASM Press, 2007.
5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Seventh Edition. Wayne, Pennsylvania: CLSI WVR, 2006.
6. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement, M100-S19. Wayne, Pennsylvania: CLSI WVR, 2006.
7. Jorgensen JH. Selection criteria for an antimicrobial susceptibility testing system. J Clin Microbiol 1993;31(11):2841-2844.
8. Taylor RB, Shakoor O, Behrens RH, et al. Pharmacopoeial quality of drugs supplied by Nigerian pharmacies. Lancet 2001;357(9272):1933-1936.

Accepted 19 September 2011.