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.

**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.

**Antimicrobial susceptibility testing (AST)**
Broth microdilution (BMD) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines was utilised. All BMD plates were prepared in duplicate, one containing the FK generic ceftriaxone, and the other the pharmaceutical-grade reference 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. 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:

\[
\text{VME} = \left( \frac{\text{no. VME/ no. resistant strains tested}}{100} \right) \\
\text{ME} = \left( \frac{\text{no. ME/ no. sensitive strains tested}}{100} \right)
\]
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 demonstrated 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<sub>50</sub> 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 vivo efficacy.

In our study, the majority of the Klebsiella spp., E. coli and Salmonella spp. isolates that tested resistant to ceftriaxone were extended-spectrum β-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 its extended-spectrum antimicrobial options.

In contrast, only 40.9% (9/22) of Enterobacter spp. had an MIC <0.12 mg/l, which probably reflects chromosomal AmpC β-lactamase production, in addition to ESBL production.

The S. pneumoniae isolates demonstrated acceptable levels of concordance for meningeval and non-meningeval isolates. This is important, given the different breakpoints depending on the site of infection. The bulk of ceftriaxone use is aimed at treating meningeval 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<sub>50</sub> ≤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, fail 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 pharmacopoeia 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 may 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.

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