Pharmacodynamics of nine generic products of amikacin compared with the innovator in the neutropenic mouse thigh infection model

Zuluaga et al.
Pharmacodynamics of nine generic products of amikacin compared with the innovator in the neutropenic mouse thigh infection model

Andres F. Zuluaga1,2, Carlos A. Rodríguez1,2, Maria Agudelo1,2,4 and Omar Vesga1,2,3,4*

Abstract

Background: Previously, we validated the mouse thigh infection model to test the therapeutic equivalence of generic antibiotic products. Here, our aim was to compare the in vivo efficacy of amikacin products in clinical use in Colombia using this animal model.

Results: All except one generic product had the same in vitro potency, judging by the lack of differences on MIC and MBC compared with the innovator. However, eight of nine generic products failed in the neutropenic mouse thigh infection model to achieve the innovator’s maximum effect ($E_{\text{max}} \leq 5.65$ for the generics vs. $6.58 \log_{10} \text{CFU/g}$ for the innovator) against Escherichia coli SIG-1, after subcutaneous treatment every 6 h with doses ranging from 1.5 to 3072 mg/kg per day.

Conclusion: As we demonstrated previously with other antibiotics such as vancomycin, gentamicin and oxacillin, the generic products of amikacin failed the in vivo efficacy testing. The therapeutic equivalence should be assessed in vivo before clinical approval of generic products.

Keywords: Amikacin, Animal models, Generics, Therapeutic equivalence, Antimicrobial resistance

Background

Generic substitution of medications is a common practice [1–3]. Worldwide, there is an abbreviated approval pathway for generic drugs of small molecules in which the comparative clinical trials are waived [4, 5], after demonstrating good manufacturing practices and bioequivalence in healthy volunteers [6, 7]. Furthermore, bioequivalence is waived for intravenous generics under the assumption that pharmacological equivalence predicts therapeutic equivalence accurately [8]. This approach has certainly rendered the desired economic results [9], but at the price of neglecting solid evidence documenting the clinical failure of intravenous generics of vancomycin and cefuroxime [10]. Besides, an animal infection model was validated by our group to determine the therapeutic equivalence of antimicrobials [11, 12], in which many generic products of vancomycin [13], oxacillin [14, 15], gentamicin [16], meropenem [17], lincomycin [18], ampicillin [19], and penicillin G [20] failed to kill the same number of microorganisms as the innovators. Of great concern, those generics of vancomycin that failed therapeutic equivalence selected the resistant subpopulation of Staphylococcus aureus [21], whilst therapeutically equivalent generics of ciprofloxacin were indistinguishable from the innovator in terms of selection of resistant Pseudomonas aeruginosa [22].

Amikacin is derived from kanamycin and its structure confers stability towards many enzymes, mainly from Gram negative bacteria, that hydrolyze other aminoglycosides [23]. This quality makes it the preferred aminoglycoside to prescribe along with a β-lactam to treat diverse nosocomial infections. During the execution of this study, the sudden discontinuation of the innovator product (Amikin®, Bristol
Myers-Squibb) forced us to stop the in vivo comparative experiments. In view of the impossibility of obtaining additional data, we decided to publish the available evidence.

**Results**

**Antibiotics**

Table 1 lists the products tested with their pharmaceutical form, lot numbers, manufacturers and distributors. The demonstration of pharmaceutical equivalence for Carlon, Gencol, Pisa, Scalpi and Sigma generic products was published previously by our group [8]. Seven of nine generic products (78%) were produced in Colombia while the other two (Genven and Pisa) were made in Venezuela and Mexico, respectively. The Farmionni-Lubelca consortium manufactured three (Scalpi, Serpharma, Zokumey) of the nine generics tested (33%), but they were analyzed as independent products.

**Susceptibility testing**

Table 2 shows the MIC and MBC of all products against *E. coli* SIG-1 or *P. aeruginosa* ATCC 27853. All but one generic amikacin product exhibited the same in vitro efficacy of the innovator; the exception was Serpharma, which MIC and MBC were 10- and 16-fold higher against both strains (P < 0.05 by Dunn's multiple comparison test). These results were reproducible in assays performed in different days.

**Reliability of the animal model to test therapeutic equivalence**

The repeatability of the PD parameters was assessed in two different days with the same batch of the innovator (batch 99A106). Figure 1 shows that there was no difference in the non-linear regression (NLR) from two independent experiments (P = 0.39 by CFA) with innovator amikacin.

**Therapeutic equivalence testing**

Untreated animals had $7.04-7.34\log_{10} \text{CFU/g}$ when treatment started (0 h) and $9.07-9.78\log_{10}\text{CFU/g}$ 24 h later when therapy was finished (net growth $= 2.24 \pm 0.29\log_{10}\text{CFU/g}$). All products tested yielded valid non-linear regressions describing the dose–response relationships obtained by Hill's Equation (Fig. 2). The PD parameters for the innovator were $E_{max} = 6.58 \pm 0.40\log_{10}\text{CFU/g}$, $ED_{50} = 272 \pm 44.6 \text{mg/kg}$ per day, and $N = 1.02 \pm 0.12$, while the magnitudes of primary ($E_{max}$, $ED_{50}$, $N$) and secondary ($BD$, $1LKD$, and $2LKD$) parameters of the other nine products are summarized in Table 3.

Except for Carlon product (Fig. 2, panel a), the remaining eight generics failed to reach the innovator’s $E_{max}$ which ranged from 5.10 to 5.65 $\log_{10}\text{CFU/g}$; in the best case, it was one order of magnitude lower than the innovator. It means that the innovator killed ~3.80 million microorganisms per gram of tissue at the maximal total dose used, whilst the most effective generic killed only 0.45 million. Although two generics (Gencol and Pisa) had greater potency than the innovator comparing their bacteriostatic dose ($\leq 110 \pm 8.30$ vs. $144 \pm 12.7 \text{mg/kg}$ per day), both also had significantly lower $E_{max}$ (P = 0.0003).

### Table 1 General description of the amikacin products studied

| Amikacin product | Form | Demonstrated pharmaceutical equivalence | Batch | Manufacturer | Distributor |
|------------------|------|------------------------------------------|-------|--------------|-------------|
| BMS (innovator)  | 1 g in 4 ml | Not applicable | 99A106 | Grove, Ecuador | The manufacturer |
|                  | 0.5 g in 2 ml | | 00G030 | Bristol-Myers Squibb, Ecuador |
|                  |                | | 05H091A | | |
| Carlon           | 0.5 g in 2 ml | Yes | 111V0203 | Carlon, Colombia | The manufacturer |
| FormasG          | 0.5 g in 2 ml | No | 02525 | Vitropharma, Colombia | Formas genericas farmaceuticas, Colombia |
| Gencol           | 0.25 g in 2 ml | Yes | 0100 | Chalver, Colombia | The manufacturer |
| Genven           | 0.5 g in 2 ml | No | 0200 | | |
| Pisa             | 0.5 g in 2 ml | Yes | 904037 | Leti for Genven, Venezuela | The manufacturer |
| Quimicol         | 0.5 g in 2 ml | No | 060865 | PiSa, Mexico | ECAR, Colombia |
| Scalpi           | 0.5 g in 2 ml | Yes | 011306 | Farmionni-Lubelca, Colombia | Farmionni scalpi, Colombia |
| Serpharma        | 0.25 g in 2 ml | No | 3780199 | Quimicol, Colombia | The manufacturer |
| Serpharma        | 0.1 g in 2 ml | No | AK300072 | Farmionni-Lubelca, Colombia | Serpharma, Colombia |
| Sigma (reference)| 1 g powder | Yes | 120K1643 | Sigma Chemical Co, USA | The manufacturer |
| Zokumey          | 0.25 g in 2 ml | No | AK020035 | Farmionni-Lubelca, Colombia | Zokumey pharma, Colombia |

* The pharmaceutical equivalence (same potency and concentration of the active ingredient) tested by microbiological assay was published elsewhere [8]
Discussion

Here, our results with amikacin indicate that almost all generics (eight of nine products) failed therapeutic equivalence in a head-to-head in vivo comparison with the innovator, independently of their pharmaceutical equivalence. These data are similar to previous results with other antibiotics [13, 15, 17], reinforcing the idea that therapeutic equivalence of generic antimicrobials cannot be predicted from pharmaceutical equivalence or in vitro testing and therefore requires in vivo studies [11, 12].

The reliability of the thigh infection model to test the efficacy of antibiotics was assessed in two independent experiments with the innovator, exhibiting the same PD profile (Fig. 1). Besides, the similar in vivo lower efficacy of three generic products from the same manufacturer (all produced by Farmionni-Lubelca) confirmed the consistency of the model’s findings.

Generic drugs are necessary to regulate drug price. But the scant information provided in the abbreviated way used by generic manufacturers have arisen some theoretical concerns [10, 24, 25] that are experimentally supported by our results. In this context, the “contamination” of bioequivalent generic heparin with oversulfated chondroitin sulfate killed approximately 1000 patients around the world [26], but generic antibiotics may entail an even worse problem: antimicrobial resistance [27, 28]. We already demonstrated that so called “bioequivalent” generics of vancomycin devoid of therapeutic equivalence do enrich resistant subpopulations of S. aureus after exposure for only 12 days in the neutropenic murine thigh infection model [21]. In contrast, fully equivalent generic products of ciprofloxacin do not exhibit difference on resistance profile [22]. Here, similar to the vancomycin case, the therapeutically nonequivalent generics of amikacin do not sterilize the thighs even at the highest dose (3072 mg/kg per day), leaving at least 3 million bacterial cells per gram of tissue exposed to the antibiotic, but alive. Then, the risk of resistance is not a minor point [29].

The study by Miller et al. supports our hypothesis about the impact on resistance of the massive use of generic products of amikacin failing therapeutic

Table 2 Comparison of the in vitro biological potency of the amikacin products studied

| Product   | Escherichia coli SIG-1 | Pseudomonas aeruginosa ATCC 27853 |
|-----------|------------------------|----------------------------------|
|           | MIC Min Max            | MBC Min Max                      | MIC Min Max |
| BMS       | 1.59 1.00 2.00         | 1.78 1.00 2.00                   | 4.00 4.00 4.00 |
| Carlon    | 4.00 4.00 8.00         | 8.00 8.00 8.00                   | 5.66 4.00 8.00 |
| FormasG   | 4.00 4.00 4.00         | 4.00 4.00 4.00                   | 5.66 4.00 8.00 |
| Gencol    | 2.00 2.00 2.00         | 2.00 2.00 2.00                   | 4.00 4.00 4.00 |
| Gerven    | 2.00 2.00 2.00         | 2.00 2.00 2.00                   | 5.66 4.00 8.00 |
| Fisa      | 2.00 2.00 2.00         | 2.83 2.00 2.00                   | 4.00 4.00 4.00 |
| Quimicol  | 2.83 1.00 8.00         | 4.00 2.00 8.00                   | 3.36 2.00 4.00 |
| Scapli    | 2.83 2.00 4.00         | 2.83 2.00 2.00                   | 4.00 4.00 4.00 |
| Serpharma** | 19.03 16.00 32.00     | 22.63 16.00 32.00                | 38.05 32.00 64.00 |
| Sigma     | 1.41 1.00 2.00         | 2.83 2.00 2.00                   | 4.00 4.00 4.00 |
| Zokumey   | 2.00 2.00 2.00         | 4.00 2.00 2.00                   | 4.00 4.00 4.00 |

Concentrations are expressed as geometric mean and range (Min. and Max) in mg/L.

MIC minimal inhibitory concentration, MBC minimal bactericidal concentration

** p value <0.05 by Dunn’s multiple comparison test

Fig. 1 Reliability of the neutropenic murine thigh infection model with the innovator of amikacin (BMS) in two independent experiments. The non-significant P value (0.393) from global curve fitting analysis (CFA) indicates that the underlying populations are better described by a single curve, confirming the model’s reliability for testing therapeutic equivalence.
equivalence [30]. They demonstrated that the aminoglycoside resistance mechanisms changed with the time (comparing studies before and after 1990’s decade) and geographical region, according to the increased usage of these drugs. According to Miller et al., the baseline resistance level of Citrobacter spp., Enterobacter spp. or Klebsiella spp. to amikacin was lower than 20 % before 1987 (when generic consumption was limited), moment at which a continuous rise was observed reaching 60 % in countries like Chile, Uruguay, Mexico and Venezuela. They also found that it was precisely during this period of massive use of generic antimicrobials (after 1987), that new enzymes capable to degrade amikacin appeared, such as AAC(6′)-I alone or combined with other enzyme like AAC(6′)-I + AAC(3)-II. Although only speculative with the available data, the possibility that therapeutic nonequivalent generics could enhance enzymatic resistance deserves scientific testing [29, 31].

There are at least two hypotheses to explain the findings. First, Bau et al. described the X-ray crystal structure of amikacin [32], establishing that the spatial relationship depends on two bifurcated hydrogen (H) bonds that are

![Figure 2](image-url)
necessary for the internal conformation of the amikacin molecule. The first H-bond is common with the molecule of kanamycin to control the A/B ring orientation but the second H-bond is required to define the conformational angles around the B/C ring junction. Any subtle change in the position of the second H-bond or its lack could reduce significantly the in vivo efficacy of amikacin. To test this hypothesis, one could compare simultaneously the chemical structure of innovator and generic by X-ray crystallography or NMR studies, however, the innovator is no longer available. Second, that impurities or different excipients might explain the failure of amikacin generics [33], but it is less likely because the process for semi-synthesis of amikacin from acylation of kanamycin A is a well-known process [34].

Conclusions
In vitro susceptibility tests do not predict the in vivo efficacy of generic products of amikacin. Considering the potential impact on antimicrobial resistance of non-therapeutically equivalent generics, more studies comparing the molecular and chemical identity, as well as head-to-head studies in validated animal models of infection should be required before approval of generic amikacin products, although therapeutic equivalence will be difficult to establish without a gold standard (innovator product).

Methods
Antibiotics
All amikacin products were bought from reputable drugstores and handled following the instructions of each manufacturer. The innovator drug was included in all experiments as the gold standard [35]. Additionally, the reference powder (Sigma Aldrich, USA), a product not designed for clinical use, was used.

Bacteria and media
E. coli SIG-1 (an ampicillin-resistant clinical isolate) was selected for in vitro and in vivo experiments. For susceptibility testing, Pseudomonas aeruginosa ATCC 27853 was used as control [36]. Culture media included trypticase soy broth and agar for in vivo studies and cation-adjusted Mueller–Hinton broth and agar for susceptibility testing (Becton–Dickinson, USA).

Susceptibility testing
Minimal inhibitory (MIC) and bactericidal (MBC) concentrations of nine generic products, the reference powder and the innovator of amikacin were determined twice by broth microdilution following the Clinical Laboratory Standard Institute method [36]. To compare the in vitro potency, the differences between geometric means were assessed by Kruskal–Wallis (KW) test followed by Dunn’s multiple comparison test (GraphPad Prism 6.05) [37].

Animal model
The University of Antioquia Animal Experimentation Ethics Committee approved the protocol. Six-week-old, 23–27 g, female murine-pathogen free mice of the strain Udea:ICR(CD-1) were used [38]. Mice were rendered neutropenic by injecting two intraperitoneal doses of cyclophosphamide (Cytoxan®, Bristol-Myers Squibb, Puerto Rico) given 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection [39]. An intramuscular injection (0.1 mL) per thigh of a log-phase culture with ~7 log_{10} CFU of E. coli SIG-1 per mL was used. Two hours later (0 h), infected animals began a 24 h-treatment with each amikacin product (N ≥ 10 mice/product), allocating two animals per dose and using at least five total doses that ranged from no effect (1.5 mg/kg per day) to maximal effect (3072 mg/kg per day). Each dose was administered by the subcutaneous route (0.2 mL) every 6 h to optimize fC_{max}/MIC and fAUC/MIC, the pharmacodynamic (PD) indices related to the efficacy of amikacin in mice and humans with normal renal function [40, 41]. Untreated infected control mice were sacrificed just after inoculation (−2 h), at the onset (0 h), and at the end of experiment (24 h), while treated mice were euthanized at 24 h.

To determine antibacterial efficacy, both thighs of each mouse were dissected under aseptic technique and homogenized independently in sterile saline (1:10). After serial dilutions and manual plating, the cultures were incubated for 18 h at 37°C under air atmosphere before colony counting and data registration in a database (Microsoft Excel®, USA). In this model, one thigh weighs 1 g and the limit of detection is 100 CFU/thigh.

Statistical analysis
For each total dose (independent variable), the net antibacterial effect (E, dependent variable) was calculated by subtracting the CFU/g obtained in thighs of infected mice from the 24 h untreated controls. Nonlinear regression of the dose–effect data from each product fitted to Hill’s model provided the primary PD parameters maximum effect (E_{max}), effective dose killing 50 % of the E_{max} (ED_{50}), and slope (N), as well as the secondary PD parameters bacteriostatic dose (BD) and the doses required to kill the first (1LD_{50}) and second (2LD_{50}) log of bacteria (SigmaPlot 12.3). To test the therapeutic equivalence, the magnitudes of these parameters were compared (each generic vs. the innovator) by curve fitting analysis (GraphPad Prism 6.05) as was described thoroughly elsewhere [13]. The quality of the nonlinear
regressions was assessed by the adjusted coefficient of determination (adj.R²), the standard error of estimate (Sₑₑₑ), the fulfillment of the assumptions of normality and homoscedasticity (constant variance), and the absence of multicollinearity (variance inflation factor). Accepting a 5% chance for a type I error (α-error) and expecting residuals’ standard deviations ≤0.5 log, the treatment of 10 animals per product to compare nine generic products with the innovator confers 87% power to reject the null hypothesis (H₀: generics = innovator product) if the magnitude of the difference on antibacterial efficacy is >1 log₁₀ CFU/g.

Abbreviations
AAC(6’)-I: aminoglycoside 6’-N-acetyltransferase type I; AAC(3’)-II: aminoglycoside 3’-N-acetyltransferase type II; Adj. R²: adjusted coefficient of determination; ATCC: American Type Culture Collection; AUC: area under the curve concentration-time; BD: bacteriostatic dose; Cmax: the maximum concentration; CFA: curve fitting analysis; CFU: colony forming unit; CLSI: Clinical and Laboratory Standard Institute; EMIC: minimal inhibitory concentration; Emax: maximum effect; ED₅₀: effective dose to kill 50% of E₀₀₀₀; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; N: Slope; NLR: non-linear regression; Sₑₑₑ: standard error of the estimate; SE: standard error; TLKD and 2LKD: 1- and 2-log kill dose, respectively.

Authors’ contributions
AFZ contributed during the experimental process, took care of the animals, performed the analysis and interpretation of data and wrote the first manuscript. CAR took care of the animals, helped during in vitro experimentation, and reviewed the first manuscript. MA performed the experiments and took care of the animals. OV conceived, directed, and designed the study, obtained funding, edited all drafts and rewrote the final version of the paper. All authors read and approved the final manuscript.

Author details
1 GRIPÉ [Grupo Investigador de Problemas en Enfermedades Infecciosas], Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia. 2 Department of Pharmaceutics and Toxicology at Medical School, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia. 3 Department of Internal Medicine, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia. 4 Infectious Diseases Unit, Hospital Universitario San Vicente Fundación, Medellín, Colombia.

Acknowledgements
This work was supported by the University of Antioquia UdeA and Sistema General de Regalías of Colombia (BPIN: 201300100183)—Ruta N—Centro de Informacion y Estudio de Medicamentos y Tóxicos (CIEMTO). The authors wish to express their gratitude to Beatriz E. Salazar and Wilson Galvis for their help during the experimental execution.

Competing interests
Zuluaga has received honoraries for unrelated lectures from Allergan, Amgen, Lilly, Mundipharma, Novonordisk, Pfizer, Roche and Sanofi. Rodriguez has received honoraries for unrelated lectures from Roche and Amgen. Agudelo and Vesga have no conflicts of interest to declare. None of these companies or any other pharmaceutical company were involved in the design, execution, or publication of this study.

Availability of supporting data
The data supporting the results of this article are included within the article. There are no additional files.

Received: 2 June 2015 Accepted: 21 September 2015
Published online: 07 October 2015

References
1. Dettelbach HR. A time to speak out on bioequivalence and therapeutic equivalence. J Clin Pharmacol. 1986;26(5):307–8.
2. Rheinstein PH. Therapeutic inequivalence. Drug Saf. 1990;5(Suppl 1):114–9.
3. Frank RG. The ongoing regulation of generic drugs. N Engl J Med. 2007;357(20):1993–6. doi:10.1056/NEJMpa078193.
4. Zarowitz BJ. The generic imperative. Geriatr Nurs. 2008;29(4):223–6. doi:10.1016/j.gerinurse.2008.06.003.
5. Howland RH. Evaluating the bioavailability and bioequivalence of generic medications. J Psychosoc Nurs Ment Health Serv. 2010;48(11):113–6. doi:10.3928/02799369-20091204-07.
6. Arnold ME. Implications of differences in bioanalytical regulations between Canada, USA and South America. Bioanalysis. 2011;3(3):253–8. doi:10.4155/bio.10.187.
7. Carpenter D, Tobbell DA. Bioequivalence: the regulatory career of a pharmaceutical concept. Bull Hist Med. 2011;85(1):93–131. doi:10.1353/bhm.2011.0024.
8. Zuluaga AF, Agudelo M, Rodriguez CA, Vesga O. Application of micro- biological assay to determine pharmaceutical equivalence of generic intravenous antibiotics. BMC Clin Pharmacol. 2009;9:1.
9. Glantz LH, Anna GJ. Impossible? Outlawing state safety laws for generic drugs. N Engl J Med. 2011;365(8):681–3. doi:10.1056/NEJMp1107832.
10. Mastoraki E, Michalopoulous A, Krias A, Mouchthouri E, Falagas M, Karatzas D, et al. Incidence of postoperative infections in patients undergoing coronary artery bypass grafting surgery receiving antimicrobial prophylaxis with original and generic cefuroxime. J Infect. 2008;56(1):35–9.
11. Zuluaga AF, Rodriguez CA, Agudelo M, Vesga O. About the validation of animal models to study the pharmacodynamics of generic anti- microbiotics. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014;59(4):549–61. doi:10.1093/cid/ciu306.
12. Agudelo M, Rodriguez CA, Zuluaga AF, Vesga O. Relevance of various animal models of human infections to establish therapeutic equivalence of a generic product of piperacillin/tazobactam. Int J Antimicrob Agents. 2015;45(2):161–7. doi:10.1016/j.ijantimicag.2014.10.014.
13. Vesga O, Agudelo M, Salazar BE, Rodriguez CA, Zuluaga AF. Generic vancomycin products fail in vivo despite being pharmaceutical equivalents of the innovator. Antimicrob Agents Chemother. 2010;54(8):3271–9. doi:10.1128/AAC.01044-09.
14. Rodriguez CA, Zuluaga AF, Salazar BE, Agudelo M, Vesga O. Experimental comparison of 11 generic products (GP) of oxacillin (DXA) with the original compound (OC) in terms of concentration of active principle (CAP), in vitro activity, and in vivo efficacy, using the neutropenic murine thigh infection model (NMTIM), Abstract A-1305. 54th interscience conference on antimicrobial agents and chemotherapy (ICAAC), Washington, DC: American Society for Microbiology, 2004.
15. Rodriguez CA, Agudelo M, Zuluaga AF, Vesga O. In vitro and in vivo comparison of the anti-staphylococcal efficacy of generic products and the innovator of oxacillin. BMC Infect Dis. 2010;10:153.
16. Zuluaga AF, Agudelo M, Cardeno JJ, Rodriguez CA, Vesga O. Determination of therapeutic equivalence of generic products of gentamicin in the neutropenic mouse thigh infection model. PLos One. 2010;5(5):e10744.
17. Agudelo M, Rodriguez CA, Pelaez CA, Vesga O. Even apparently insignific- ant chemical deviations among bioequivalent generic antibiotics can lead to therapeutic nonequivalence: the case of meropenem. Antimicrob Agents Chemother. 2014;58(2):1005–18. doi:10.1128/AAC.00350-13.
18. Salazar BE, Zuluaga AF, Rodriguez CA, Agudelo M, Vesga O. Experimental comparison of 7 generic products of lincomycin with the original compound in terms of concentration of active principle, in vitro activity, and in vivo efficacy, using the neutropenic murine thigh infection model. Abstract A-1879. 44th interscience conference on antimicrobial agents and chemotherapy (ICAAC), Washington, DC: American Society for Microbiology; 2004.
19. Zuluaga AF, Salazar BE, Loaiza S, Agudelo M, Vesga O. Therapeutic equiv- alence with the original compound of 8 generic products of ampicillin determined in the neutropenic murine thigh infection model. Abstract E-2033. 44th interscience conference on antimicrobial agents and chemotherapy, Washington, DC: American Society for Microbiology, 2004.
20. Agudelo M, Zuluaga AF, Rodriguez CA, Salazar BE, Vesga O. Determination of therapeutic equivalence for 5 generic products of penicillin G using the neutropenic murine thigh infection model. Abstract A-1877. 44th interscience conference on antimicrobial agents and chemotherapy. Washington, DC: American Society for Microbiology, 2004.

21. Rodriguez CA, Agudelo M, Zuluaga AF, Vesga O. Generic vancomycin enriches resistant subpopulations of Staphylococcus aureus after exposure in the neutropenic mouse thigh infection model. Antimicrob Agents Chemother. 2011. doi:10.1128/AAC.03633-14.

22. Rodriguez CA, Agudelo M, Zuluaga AF, Vesga O. Generic vancomycin enriches resistant subpopulations of Staphylococcus aureus after exposure in the neutropenic mouse thigh infection model. Antimicrob Agents Chemother. 2011;59(1):53–8. doi:10.1128/AAC.03633-14.

23. Gonzalez LS III, Spencer JP. Aminoglycosides: a practical review. Am Fam Physician. 1998;58(8):1811–20.

24. Jones RN, Fritschre TR, Moet GJ. In vitro potency evaluations of various piperacillin-tazobactam generic products compared with the contemporary branded (Zosyn, Wyeth) formulation. Diagn Microbiol Infect Dis. 2008;61(1):76–9.

25. Meredith P. Bioequivalence and other unresolved issues in generic drug substitution. Clin Ther. 2003;25(11):2875–90.

26. Blossom DB, Kallen AJ, Patel PR, Elward A, Robinson L, Gao G, et al. Outbreak of adverse reactions associated with contaminated heparin. N Engl J Med. 2008;359(25):2674–84.

27. Norrby SR, Nord CE, Finch R. Lack of development of new antimicrobial drugs: a potential serious threat to public health. Lancet Infect Dis. 2003;5(2):115–9. doi:10.1016/S1473-3099(03)01283-1.

28. Alonis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res. 2005;36(6):697–705. doi:10.1016/j.arcmed.2005.06.009.

29. Touati PL, Bousquet-Melou A. Rebuttal to the reaction of the EGGVP to the review article ‘the consequences of generic marketing on antibiotic consumption and the spread of microbial resistance: the need for new antibiotics’. J Vet Pharmacol Ther. 2014;37(6):618–23. doi:10.1111/jvpt.12166.

30. Miller GH, Sabatelli FJ, Hare RS, Glupczynski Y, Mackey P, Shlaes D, et al. The most frequent aminoglycoside resistance mechanisms—changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. Clin Infect Dis Off Publ Infect Dis Soc Am. 1997;24(Suppl 1):S46–S62.

31. Rodriguez CA, Agudelo M, Gonzalez M, Zuluaga AF, Vesga O, editors. Generic piperacillin-tazobactam (TZP) enriches resistant subpopulations of Escherichia coli after exposure in the neutropenic mouse thigh infection model (NMTIM). 53 interscience conference on antimicrobial agents and chemotherapy. American Society for Microbiology; 2013.

32. Bau R, Tsyba I. Crystal structure of amikacin. Tetrahedron. 1999;55(52):14839–46. doi:10.1016/S0040-4020(99)00983-7.

33. Amikacin Sulfate. 2010. http://www.drugs.com/monograph/amikacin-sulfate.html. Accessed 12 Dec 2010.

34. Mangia A. Acylation of kanamycin a. Google Patents; 1998.

35. Schiffman DO. Evaluation of amikacin sulfate (Amikin). A new aminoglycoside antibiotic. JAMA. 1977;238(14):1547–50.

36. Clinical, Laboratory Standards I. Performance standards for antimicrobial susceptibility testing, approved standard M100-S19. Wayne: Clinical and Laboratory Standards Institute (CLSI); 2009.

37. Glantz SA. Primer of biostatistics. 7th ed. New York: McGraw-Hill; 2012. p. 489.

38. Zuluaga A, Salazar B, Galvis W, Loaiza S, Agudelo M, Vesga O. Foundation of the first functional MPF animal facility in Colombia. Iatreia [Spanish]. 2003;16:115–31.

39. Zuluaga AF, Salazar BE, Rodriguez CA, Zapata AX, Agudelo M, Vesga O. Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. BMC Infect Dis. 2003;6(11):1–10.

40. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 2006;65:55.

41. Craig WA, Redington J, Ebert SC. Pharmacodynamics of amikacin in vitro and in mouse thigh and lung infections. J Antimicrob Chemother. 1991;27(Suppl C):29–40.