Accepted Manuscript

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Please cite this article as: Rodríguez-Cerrato V, del Prado G, Huelves L, Naves P, Ruiz V, García E, Ponte C, Soriano F, Comparative efficacy of novobiocin and amoxicillin in experimental sepsis caused by β-lactam-susceptible and highly resistant pneumococci, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2010.02.007

Comparative efficacy of novobiocin and amoxicillin in experimental sepsis caused by β-lactam-susceptible and highly resistant pneumococci

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ABSTRACT

Therapeutic alternatives are needed against infections caused by highly multidrug-resistant *Streptococcus pneumoniae*. Novobiocin, an old antibiotic, was tested in vitro and in a murine sepsis model against one amoxicillin-susceptible and three amoxicillin-resistant strains [minimum inhibitory concentrations (MICs) 8–64 mg/L]. Novobiocin MICs for all strains were 0.25–0.5 mg/L. In sepsis, novobiocin and amoxicillin were evaluated at 25, 50, 100 and 200 mg/kg given at 1, 5, 24 and 48 h post bacterial challenge. The most effective regimens in animals infected with the amoxicillin-susceptible strain were 200 mg/kg novobiocin and 25 mg/kg amoxicillin, achieving 100% survival and undetectable organisms in the peritoneum. Among mice infected with amoxicillin-resistant *S. pneumoniae*, 200 mg/kg novobiocin gave the highest protection (90–100% survivors), followed by 200 mg/kg amoxicillin (60–100%), 100 mg/kg novobiocin (50–87.5%) and 50 mg/kg amoxicillin (14.3–25%). The killing effect of antibiotics in the peritoneum (mean $\Delta\log_{10}$ colony-forming units/mL between treated and control mice) was as follows: 200 mg/kg novobiocin (-6.6) > 200 mg/kg amoxicillin (-5.6) > 100 mg/kg novobiocin (-3.7) > 50 mg/kg amoxicillin (-0.7). Total plasma and ultrafiltrate pharmacokinetics of novobiocin (200 mg/kg, single dose) in non-infected mice showed, respectively, half-lives of 151 min and 215 min, area under the concentration–time curves (AUCs) of 945.0 mg h/L and 136.6 mg h/L and maximal concentrations of 147 mg/L and 18 mg/L. Novobiocin may be a promising agent for therapy of highly β-lactam-resistant pneumococcal infections.

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1. Introduction

The emergence and steady spread of antibiotic-resistant bacterial pathogens are global public health concerns. In particular, the rapid rise of antibiotic resistance among *Streptococcus pneumoniae* has complicated the therapy of pneumococcal infections both in adults and children [1–4]. Therapeutic strategies for combating antibiotic-resistant pneumococcal infections are currently being explored, including the possibility of reviving old active drugs. Based on previous reports showing remarkable in vitro activity of novobiocin against *S. pneumoniae* [5,6], we have re-evaluated in vitro and in vivo the antipneumococcal activity of this out-of-the-market antibiotic, which was licensed until recently as an antibiotic for human use in many countries such as the USA and in Europe (i.e. Albamycin® 200 mg capsules for oral administration; Pharmacia-Upjohn).

Novobiocin, an aminocoumarin natural product elaborated by streptomycetes, was widely used as an antibacterial agent in the 1950s and 1960s. Novobiocin is an inhibitor of bacterial DNA gyrase. Like other coumarin products, it binds more tightly to gyrase (as reflected by equilibrium dissociation constants in the range of 10 nM [7]) than do typical quinolones, and novobiocin also causes inhibition of ATP binding [8]. Novobiocin was used for the treatment of a variety of infections caused by susceptible organisms, including pneumococcal pneumonia [9]. However, several reports published in the 1950s regarding the emergence of resistance and side effects [10–12], along with the development of other antibiotics (i.e. penicillinase-resistant penicillins), likely determined the lack of usage of this otherwise potent and inexpensive antibiotic.

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The aims of this study were to investigate the in vitro activity, pharmacokinetics and effectiveness of novobiocin therapy using a murine sepsis model caused by amoxicillin-susceptible and -resistant *S. pneumoniae* strains.

2. Material and methods

2.1. Bacterial strains and antibiotics

Four clinical strains of *S. pneumoniae* were used in this study, for which the serotype, origin and penicillin minimum inhibitory concentration (MIC) were as follows: strain AR33118, serotype 3, blood, MIC = 0.015 mg/L; strain MJD3693, 19F, cerebrospinal fluid, MIC = 2 mg/L; strain SPC2162, 19A, blood, MIC = 16 mg/L; and strain SPC2552, 23F, respiratory tract, MIC = 32 mg/L). Novobiocin sodium salt (N6160; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used for in vitro and in vivo studies. Amoxicillin trihydrate of known potency (GlaxoSmithKline S.A., Madrid, Spain) and amoxicillin sodium salt (Clamoxyl® 1 g; GlaxoSmithKline S.A.) were used for in vitro and in vivo studies, respectively.

2.2. Minimum inhibitory concentrations and time–kill analysis

MICs of novobiocin and amoxicillin for the various strains were determined by the microdilution method approved by the Clinical and Laboratory Standards Institute (CLSI) [13] using cation-adjusted Mueller–Hinton II broth (Becton, Dickinson and Co., Le Pont-de-Claix, France) supplemented with 4% lysed horse blood (CA-MHB-LHB). Modal values of three separate determinations were considered.

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Time–kill assays were performed by exposing cultures of the various strains to novobiocin or amoxicillin at 2×, 8× or 32× MIC, except for the strain that was most resistant to penicillin (strain SPC2552) for which the amoxicillin concentration of 32× MIC was not tested owing to poor solubility of the powder. Bacteria were grown in broth to early log phase and then inoculated tubes with CA-MHB-LHB were incubated at 35 °C in normal atmosphere at a final titre of 2 × 10^6 colony-forming units (CFU)/mL. Samples of 100 µL were removed at the time of inoculation (time zero) and after 2 h and 5 h, diluted and plated on blood agar plates for colony counting. The killing effects of antibiotics were thus compared by determining the change in $\log_{10}$ CFU/mL at 2 h and 5 h compared with counts in the control tubes at each time point, and such differences in bacterial titres were expressed as negative values. Antibiotic carryover was minimised by dilution. Each test was performed in duplicate.

2.3. Mouse sepsis model

Experimental animals were adult female Swiss mice weighing 30 ± 2 g (Centro de Investigaciones Biológicas, Madrid, Spain). Animals were housed in regulation cages with free access to food and water.

For animal inoculation, pneumococcal suspensions were prepared immediately before use by suspending colonies from fresh overnight cultures on 5% blood agar plates in sterile Todd–Hewitt broth (THB), adjusted to an optical density giving a concentration of ca. 5 × 10^8 CFU/mL. For the penicillin-susceptible

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strain, the suspension was further diluted into THB to a final inoculum ranging from 4.8×10^2 to 1.3×10^4 CFU/mL. For the three penicillin-resistant strains and immediately before inoculation, the bacterial suspensions were diluted 1:10 in molten nutrient agar, which was used as adjuvant for inoculation of the mice, yielding a final agar concentration of 1.5% (w/v) and a final inoculum ranging from 4.2×10^7 to 1.4×10^8 CFU/mL. Sepsis with each strain was induced by intraperitoneal (i.p.) injection of 0.5 mL of inoculum into non-anaesthetised mice 1 h before therapy with antibiotics or apyrogen sterile distilled water (control). These inocula were 80–100% lethal within 1–3 days and provided a consistently high number of bacteria in the peritoneal cavity. The inoculum size was confirmed by determination of the bacterial titre in each experiment. The potential lethal effect of agar alone was examined by i.p. injection of 0.5 mL of 1.5% (w/v) agar–THB solution to three animals. None of these animals appeared ill or died within the 72 h period of observation.

2.4. Treatment protocols

Treatment of mice was performed as a subcutaneous (s.c.) injection in a volume of 0.1 mL per dose in the neck region. For the efficacy studies, four doses of antibiotics were given at 1, 5, 24 and 48 h after infection, and survival was recorded daily throughout the study period, which ended 72 h after inoculation. At the end of the study, animals were euthanised by CO_2 overdose, and peritoneal lavage fluid specimens were collected and processed for CFU determination. Peritoneal lavage was also performed post-mortem in animals that were found dead before finishing the experiment. The results of peritoneal bacterial load were expressed as mean ± standard deviation (S.D.) $\log_{10}$

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CFU/mL. Differences in the number of CFU/mL between treated and control animals were calculated to compare the effectiveness of the treatment regimens. The lower limit of organism quantification in these studies was 10 CFU/mL.

Two types of experiments were performed to evaluate the effectiveness of antibiotic therapy, and the most effective antimicrobial regimen was defined as the regimen that achieved the highest survival rate and the most potent antibacterial effect in the peritoneal fluid.

2.4.1. Drug effectiveness in sepsis caused by a penicillin-susceptible strain

Mice with sepsis induced by the penicillin-susceptible strain (AR33118) received novobiocin (25, 50, 100 or 200 mg/kg) or amoxicillin (25 mg/kg). A group of mice received apyrogen sterile distilled water as control for the lethality and the peritoneal bacterial load of the infection. Mostly, five to seven mice were used for each regimen in these dose-ranging experiments.

2.4.2. Drug effectiveness in sepsis caused by penicillin-resistant strains

Following infection with each of the three penicillin-resistant *S. pneumoniae* strains, mice were treated with doses of novobiocin at 100 mg/kg or 200 mg/kg or amoxicillin at 50 mg/kg or 200 mg/kg. A group of mice that received apyrogen sterile distilled water was tested as control. Each regimen was given to mice in groups of at least seven animals (range, seven to ten animals). Survival was noted daily and peritoneal bacterial loads were determined as above.

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2.5. Pharmacokinetics and tolerance of novobiocin

A single-dose plasma pharmacokinetic (PK) study of novobiocin was performed in non-infected mice given s.c. doses of novobiocin at 200 mg/kg. For each time point, three mice were sampled by cardiac puncture (immediately after euthanasia with CO_2 overdose) at 15, 30, 60, 120, 240, 360, 540 and 720 min after injection with heparin-rinsed syringes. Blood samples were centrifuged at $5000 \times g$ for 10 min. The plasma obtained was divided into two parts and one of them was centrifuged in tubes with filters with a cut-off of ca. 30 kDa (Centrifree® YM-30; Millipore, Carrigtwohill, Ireland) in a fixed-angle rotor at $3000 \times g$ for 12 min. Both samples were stored at $-20\ ^{\circ}$C until analysis.

Novobiocin concentrations were determined by an agar diffusion microbiological assay using *Kocuria rhizophila* (previously designated as *Micrococcus luteus*) ATCC 9341 as the test organism [14]. The antibiotic concentration in both parts (total plasma and protein-free ultrafiltrate) was calculated using standard curves derived from solutions prepared with different antibiotic concentrations dissolved, respectively, in pooled mouse plasma and 0.9% NaCl. Analyses were performed in duplicate and the intraday and interday variation was <10%.

PK analysis of novobiocin in total plasma and plasma ultrafiltrate was performed using TopFit V2 software (Karl Thomae, Boehringer–Ingelheim, Ingelheim, Germany) and a non-compartmental model. The area under the concentration–time curve (AUC) of novobiocin from 0 min (time of administration) to 720 min was calculated using the linear trapezoidal rule.

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Four non-infected animals were injected subcutaneously with four doses of 200 mg/kg novobiocin at 1, 5, 24 and 48 h to monitor tolerance. Weight, aspect and behaviour of mice were examined daily for 1 week and weekly thereafter for 4 weeks. In addition, the aspect (once a week) and survival of mice were observed for 4 months.

2.6. Data analysis

Survival rates were compared by Fisher's exact test. Bacterial counts in peritoneal lavage of the different groups were presented as mean $\pm$ S.D. and 95% confidence interval. Comparison of peritoneal bacterial counts between two groups was performed by Mann–Whitney test, and comparisons among three or more groups were examined with Kruskal–Wallis test followed by Dunn's multiple-comparisons test among groups when they were significantly different. Culture-negative samples (<10 CFU/mL) were included in the calculation of means assuming a value at the detection limit (1.0 $\log_{10}$ CFU/mL). A *P*-value of <0.05 was considered significant.

3. Results

3.1. Minimum inhibitory concentrations and time–kill curves

Novobiocin and amoxicillin MICs, respectively, of the study organisms were as follows: strain AR33118, 0.25 mg/L and 0.015 mg/L; strain MJD3693, 0.5 mg/L and 8 mg/L; strain SPC2162, 0.25 mg/L and 16 mg/L; and strain SPC2552, 0.5 mg/L and 64 mg/L.

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Time–kill data for the four *S. pneumoniae* strains are presented in Table 1. Baseline inocula ranged from 6.03 $\log_{10}$ CFU/mL to 6.75 $\log_{10}$ CFU/mL (time zero). Novobiocin exposure resulted in a >2 $\log_{10}$ difference in viable counts versus the controls for all strains and all concentrations tested by 5 h, except for strains AR33118 and SPC2162 at 2× MIC. The antibacterial effect of amoxicillin was more potent and rapid than that of novobiocin, achieving at 2× MIC a >2 $\log_{10}$ difference by 2 h and >4 $\log_{10}$ difference by 5 h, but this effect was somewhat lower for the most resistant strain (SPC2552). As shown in Table 1, for novobiocin there was an increase in killing by increasing concentrations on MIC basis as well as on time basis by comparing 2 h vs. 5 h.

3.2. Therapeutic efficacy in sepsis caused by amoxicillin-susceptible and -resistant strains

The in vivo efficacy of novobiocin against amoxicillin-susceptible and -resistant *S. pneumoniae* strains in a mouse sepsis model is shown in Fig. 1. With the treatment schedule consisting of four s.c. doses at 1, 5, 24 and 48 h after infection, both novobiocin 200 mg/kg and amoxicillin 25 mg/kg were associated with a 100% survival rate of mice infected with the amoxicillin-susceptible strain AR33118 ($P < 0.01$ vs. untreated control group). For this strain, novobiocin 100 mg/kg achieved a 71% survival rate.

With the amoxicillin-resistant strains, novobiocin 200 mg/kg achieved the highest protection (90–100% survivors), followed by amoxicillin 200 mg/kg (60–100%), and these differences were statistically significant ($P < 0.01$) compared

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with the controls, except for the group infected with strain SPC2162 and treated with amoxicillin 200 mg/kg, which had a 60% survival rate. Novobiocin 100 mg/kg protected 50–87.5% of the animals, whereas amoxicillin 50 mg/kg protected only 14.3–25% of the animals, with the lowest survival rate in animals infected with the most resistant strain (SPC2552). Notably, against this strain the 200 mg/kg amoxicillin regimen gave protection (87.5% survivors) similar to that showed by novobiocin 100 mg/kg.

Table 2 shows the peritoneal bacterial counts after therapy with novobiocin and amoxicillin regimens in mice with sepsis induced by four pneumococcal strains that were survivors and were humanely sacrificed for sampling at 72 h after infection or animals that died before 72 h. All control animals that died had >8 $\log_{10}$ CFU/mL in the peritoneum (mean value 8.7 $\log_{10}$ CFU/mL, range 8.2–9.1 $\log_{10}$ CFU/mL).

All mice infected with the amoxicillin-susceptible strain (AR33118) and treated with both novobiocin 200 mg/kg and amoxicillin 25 mg/kg survived, and the bacterial load in the peritoneum was undetectable, whereas the mean decline in peritoneal bacterial counts of survivor mice treated with novobiocin 100 mg/kg compared with those of the control group was only 1.7 log units.

Bacterial clearance (undetectable CFU/mL) in peritoneal lavage fluid after sacrifice of survivor mice infected with the amoxicillin-resistant strains was detected as follows: (a) among mice infected with strain MJD3693, in five of nine animals treated with the high-dose novobiocin regimen, in two of five mice

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treated with the low-dose novobiocin regimen, and in seven of eight mice treated with the high-dose amoxicillin regimen; (b) among mice infected with strain SPC2162, in six of nine mice treated with novobiocin 200 mg/kg, in two of four mice treated with novobiocin 100 mg/kg, and in three of six mice treated with amoxicillin 200 mg/kg; and (c) among mice infected with strain SPC2552, in seven of eight mice receiving novobiocin 200 mg/kg, in two of seven mice receiving novobiocin 100 mg/kg, and in all seven mice receiving amoxicillin 200 mg/kg. Overall, the killing effect of the different antibiotic regimens in the peritoneum in mice infected with the amoxicillin-resistant strains could be ranked as follows (mean $\Delta\log_{10}$ CFU/mL between treated and untreated control mice after 72 h): novobiocin 200 mg/kg (-6.6) > amoxicillin 200 mg/kg (-5.6) > novobiocin 100 mg/kg (-3.7) > amoxicillin 50 mg/kg (-0.7).

3.3. Novobiocin pharmacokinetics and tolerance

Single-dose PK studies performed in non-infected mice that received single s.c. doses of 200 mg/kg novobiocin demonstrated the following PK values for total plasma and ultrafiltrate, respectively: elimination half-life, 151 min and 215 min; AUC 945.0 mg h/L and 136.6 mg h/L; and maximal concentration 147 mg/L and 18 mg/L. The level of protein binding in mouse plasma was 85%.

Long-term evaluation revealed appropriate tolerance to novobiocin at the tested regimen, as assessed by observing the weight, aspect and behaviour of the treated mice for 4 weeks, as well as the aspect and survival for 4 months.

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4. Discussion

Multidrug-resistant and highly antibiotic-resistant pneumococci have emerged as significant pathogens causing community-acquired infections worldwide [15]. However, following the 2008 CLSI parenteral penicillin breakpoints for non-meningitis pneumococcal isolates (intermediate, 4 mg/L; resistant, $\geq$8 mg/L) [13], there are few up-to-date published studies dealing with highly β-lactam-resistant pneumococci (penicillin MIC $\geq$ 8 mg/L), especially regarding their optimal antibiotic therapy [16–18]. Among therapeutic strategies for combating pneumococcal infections caused by highly β-lactam-resistant strains, here we have explored the use of novobiocin, an old drug, which we hypothesise could be re-introduced to the antibiotic armamentarium specifically for therapy of pneumococcal infections caused by strains with high-level resistance to the clinically used antibiotics.

Novobiocin was extensively used before the development of broad-spectrum agents such as β-lactam and quinolone agents. Because novobiocin displays potent activity against staphylococci, including meticillin-resistant *Staphylococcus aureus* (MRSA), pneumococci, enterococci and many other Gram-positive organisms [5,6], during the 1980s the suitability of clinical re-evaluation of this drug was suggested [6]. Clinical trials conducted in the 1990s demonstrated the efficacy of novobiocin-containing combinations for eradicating the carrier state of MRSA [19,20]. Indeed, novobiocin combined with rifampicin prevented the development of resistance in MRSA to either drug even more efficiently than the combination of co-trimoxazole and rifampicin [20]. Remarkably, among severely ill patients with haematological disorders, the

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combination of novobiocin with doxycycline or rifampicin was effective for the treatment of vancomycin-resistant *Enterococcus faecium* bacteraemia [21]. Regarding the side effects associated with the use of novobiocin alone or in combination, it should be stressed that many patients [19,20,22] and healthy volunteers [23,24] received this drug, mostly at 500 mg every 12 h for a variable period of time, without reporting relevant toxicity, and it has been suggested that some of the side effects could be attributed to impurities in the formulation that have since been removed [20]. Besides, novobiocin has also been administered as a modulator of alkylating agent cytotoxicity combined with cyclophosphamide, with the maximum tolerated dose being 6 g/day of novobiocin [25].

The in vitro results using time–kill curves show that the antibacterial effect of amoxicillin is more potent and rapid than that of novobiocin but the concentrations of amoxicillin used (16–512 mg/L) for the amoxicillin-resistant strains were much higher than those of novobiocin (0.5–16 mg/L).

In the mouse sepsis model induced by an amoxicillin-susceptible pneumococcal strain, the highest dosage of novobiocin (200 mg/kg) was as effective as amoxicillin 25 mg/kg in terms of survival and killing of the peritoneal bacterial load. Results with the amoxicillin-resistant strains show that dosages of 200 mg/kg novobiocin or amoxicillin were needed to cure most animals. Nevertheless, survival in animals inoculated with strain SPC2162 was, respectively, 100% and 60% when treated with 200 mg/kg of both novobiocin and amoxicillin.

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The doses selected for amoxicillin administration were based on the results

from a previous study by our group [26] which showed that the maximum

amoxicillin concentrations attained in mouse serum after single doses of

amoxicillin of 200 mg/kg and 50 mg/kg were 300 mg/L and 75 mg/L,

respectively. However, such high amoxicillin concentrations cannot be achieved

in patients following oral administration of usual dosages. In fact, peak levels of

amoxicillin of 7.5 mg/L and 4 mg/L in the blood of patients occur 2 h after

ingestion of 500 mg and 250 mg doses taken fasting, respectively [27,28], so

only a high dose of this drug administered parenterally could achieve a peak

serum concentration closed to 100 mg/L. On the other hand, a PK study carried

out in humans, as determined by HPLC, showed that after ingestion of

novobiocin at 500 mg every 12 h the mean peak serum concentration was 62.5

$\pm$ 13.4 mg/L with an AUC of 407 mg h/L and a half-life of ca. 6 h [23], but even

higher concentrations can be achieved following parenteral administration. For

a highly protein-bound drug such as novobiocin, free drug concentrations must

be determined, and our results show that maximum survival of animals was

achieved with a free peak plasma concentration of ca. 18 mg/L. Our results also

show that novobiocin could achieve sufficient concentrations to eradicate highly

β-lactam-resistant pneumococcal strains, whereas amoxicillin does not.

Lastly, to our knowledge there is insufficient experimental evidence on the

development of novobiocin resistance in pneumococci to preclude its use for

the therapy of multidrug-resistant pneumococcal infections. Very few studies

have approached this problem in *S. pneumoniae*. An in vitro study by Muñoz et

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al. [29] described that a specific amino acid change (Ser-127→Leu) in the DNA gyrase B subunit of *S. pneumoniae* increases novobiocin resistance, with MICs of 1 mg/L to 128 mg/L for the novobiocin-susceptible and -resistant strain, respectively.

In conclusion, until novel antibacterial agents are discovered, our in vitro and in vivo data suggest that novobiocin may be a therapeutic alternative in pneumococcal infections caused by highly β-lactam-resistant strains.

Funding

Competing interests

None declared.

Ethical approval

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Animal studies were approved by the Animal Experimentation Ethics Committee of the Fundación Jiménez Díaz (Madrid, Spain). All animal studies were conducted in accordance with regulations regarding the care and use of laboratory animals in the European Union.

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References

[1] Feikin D, Schuchat A, Kolczak M, Barrett NL, Harrison LH, Lefkowitz L, et al. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995–97. Am J Public Health 2000;90:223–9.

[2] MacGowan AP; BSAC Working Parties on Resistance Surveillance. Clinical implications of antimicrobial resistance for therapy. J Antimicrob Chemother 2008;62(Suppl 2):ii105–14.

[3] Tleyjeh IM, Tlaygeh HM, Hejal R, Montori VM, Baddour LM. The impact of penicillin resistance on short-term mortality in hospitalized adults with pneumococcal pneumonia: a systematic review and meta-analysis. Clin Infect Dis 2006;42:788–97.

[4] World Health Organization. Pneumococcal vaccines. Wkly Epidemiol Rec 2003;78:110–9.

[5] Gombert ME, Aulicino TM. Susceptibility of multiply antibiotic-resistant pneumococci to the new quinoline antibiotics, nalidixic acid, coumermycin, and novobiocin. Antimicrob Agents Chemother 1984;26:933–4.

[6] Jones RN. Should novobiocin be clinically re-evaluated? Diagn Microbiol Infect Dis 1989;12:363–5.

[7] Schmutz E, Mühlenweg A, Li S-M, Heide L. Resistance genes of aminocoumarin producers: two type II topoisomerase genes confer resistance against coumermycin A_1 and clorobiocin. Antimicrob Agents Chemother 2003;47:869–77.

[8] Sugino A, Higgins NP, Brown PO, Peebles CL, Cozzarelli NR. Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc Natl Acad Sci USA 1978;75:4838–42.

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[9] Limson BM, Romansky MJ. Novobiocin, a new antibiotic; laboratory and clinical evaluation of thirty patients with bacterial pneumonia. Antibiotic Med Clin Ther 1956;2:277–81.

[10] Bridges RA, Berendes H, Good RA. Serious reactions to novobiocin. J Pediatr 1957;50:579–85.

[11] Cox RP, Foltz EL, Raymond S, Drewyer R. Novobiocin jaundice. N Engl J Med 1959;261:139–41.

[12] Nichols RL, Finland M. Novobiocin; a limited bacteriologic and clinical study of its use in forty-five patients. Antibiotic Med Clin Ther 1956;2:241–57.

[13] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing.* Eighteenth informational supplement Document M100-S18. Wayne, PA: CLSI; 2008.

[14] Tang JS, Gillevet PM. Reclassification of ATCC 9341 from *Micrococcus luteus* to *Kocuria rhizophila*. Int J Syst Evol Microbiol 2003;53:995–7.

[15] Richter SS, Heilmann KP, Dohrn CL, Riahi F, Beekmann SE, Doern GV. Changing epidemiology of antimicrobial-resistant *Streptococcus pneumoniae* in the United States, 2004–2005. Clin Infect Dis 2009;48:e23–33.

[16] McGee L, Biek D, Ge Y, Klugman M, du Plessis M, Smith AM, et al. In vitro evaluation of the antimicrobial activity of ceftaroline against cephalosporin-resistant isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2009;53:552–6.

20

[17]   Sá Figueiredo AM, Austrian R, Urbaskova P, Teixeira A, Tomasz A. Novel penicillin-resistant clones of *Streptococcus pneumoniae* in the Czech Republic and in Slovakia. Microb Drug Resist 1995;1:71–8.

[18]   Soriano F, Cafini F, Aguilar L, Tarragó D, Alou L, Giménez MJ, et al. Breakthrough in penicillin resistance? *Streptococcus pneumoniae* isolates with penicillin/cefotaxime MICs of 16 mg/L and their genotypic and geographical relatedness. J Antimicrob Chemother 2008;62:1234–40.

[19]   Arathoon EG, Hamilton JR, Hench CE, Stevens DA. Efficacy of short courses of oral novobiocin–rifampin in eradicating carrier state of methicillin-resistant *Staphylococcus aureus* and in vitro killing studies of clinical isolates. Antimicrob Agents Chemother 1990;34:1655–9.

[20]   Walsh TJ, Standiford HC, Reboli AC, John JF, Mulligan ME, Ribner BS, et al. Randomized double-blinded trial of rifampin with either novobiocin or trimethoprim–sulfamethoxazole against methicillin-resistant *Staphylococcus aureus* colonization: prevention of antimicrobial resistance and effect of host factors on outcome. Antimicrob Agents Chemother 1993;37:1334–42.

[21]   Montecalvo MA, Horowitz H, Wormser GP, Seiter K, Carbonaro CA. Effect of novobiocin-containing antimicrobial regimens on infection and colonization with vancomycin-resistant *Enterococcus faecium*. Antimicrob Agents Chemother 1995;39:794.

[22]   Shane SH. Novobiocin, a new antibiotic. Can Med Assoc J 1956;75:51–2.

[23]   Drusano GL, Townsend RJ, Walsh TJ, Forrest A, Antal EJ, Standiford HC. Steady-state serum pharmacokinetics of novobiocin and rifampin alone and in combination. Antimicrob Agents Chemother 1986;30:42–5.

21

[24]   Welch H, Lewis CN, Putnam LE, Randall WA. A study of the sensitizing potential of novobiocin. Antibiotic Med Clin Ther 1956;3:27–32.

[25]   Eder JP, Wheeler CA, Teicher BA, Schnipper LE. A phase I clinical trial of novobiocin, a modulator of alkylating agent cytotoxicity. Cancer Res 1991;51:510–3.

[26]   Soriano F, Ponte C, Nieto E, Parra A. Correlation of in-vitro activity and pharmacokinetic parameters with in-vivo effect of amoxycillin, co-amoxiclav and cefotaxime in a murine model of pneumococcal pneumonia. J Antimicrob Chemother 1996;38:227–36.

[27]   Mandell GL, Sande MA. Penicillins, cephalosporins, and other β-lactam antibiotics. In: Hardman J, Limbird L, Gilman A, editors. *Goodman and Gilman's The pharmacological basis of therapeutics*. 7th ed. New York, NY: Macmillan Publishing Company; 1985. p. 1115–49.

[28]   Neu HC. Penicillins. In: Mandell GL, Douglas RG Jr, Bennett JE, editors. *Principles and practice of infectious diseases*, Vol. 1. 3rd ed. New York, NY: Churchill Livingstone; 1985. p. 230–50.

[29]   Muñoz R, Bustamante M, de la Campa AG. Ser-127-to-Leu substitution in the DNA gyrase B subunit of *Streptococcus pneumoniae* is implicated in novobiocin resistance. J Bacteriol 1995;177:4166–70.

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Fig. 1. Survival of mice with sepsis infected with four different *Streptococcus pneumoniae* strains: •, non-treated controls; ∆, novobiocin 25 mg/kg; ∎, novobiocin 50 mg/kg; ☐, novobiocin 100 mg/kg; ▲, novobiocin 200 mg/kg; +, amoxicillin 25 mg/kg; ○, amoxicillin 50 mg/kg; ×, amoxicillin 200 mg/kg.

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Table 1

Time–kill experiments of four *Streptococcus pneumoniae* strains after exposure to novobiocin or amoxicillin at 2, 8 and 32 times the

minimum inhibitory concentration (MIC)

Strain (MIC [a])	Time (h)	Differences in $\log_{10}$ CFU/mL [b]					
		Novobiocin			Amoxicillin		
		2× MIC	8× MIC	32× MIC	2× MIC	8× MIC	32× MIC
AR33118 (0.25, 0.015)	2	−0.68 ± 0.21	−1.36 ± 0.16	−1.45 ± 0.04	−3.96 ± 0.12	−4.18 ± 0.20	−3.88 ± 0.11
	5	−1.29 ± 0.05	−2.71 ± 0.51	−3.04 ± 0.15	−4.33 ± 1.42	−4.36 ± 0.32	−5.80 ± 1.01
MJD3693 (0.5, 8)	2	−1.31 ± 0.06	−1.58 ± 0.16	−1.72 ± 0.08	−2.58 ± 0.16	−3.16 ± 0.03	−3.18 ± 0.01
	5	−2.89 ± 0.05	−3.35 ± 0.1	−3.50 ± 0.01	−4.73 ± 0.09	−4.68 ± 0.14	−4.68 ± 0.35
SPC2162 (0.25, 16)	2	−1.20 ± 0.16	−1.53 ± 0.14	−1.75 ± 0.10	−2.46 ± 0.00	−2.68 ± 0.09	−2.17 ± 0.15
	5	−1.96 ± 0.01	−3.13 ± 0.10	−3.74 ± 0.25	−4.64 ± 0.33	−4.66 ± 0.04	−3.62 ± 0.03
SPC2552 (0.5, 64)	2	−1.55 ± 0.14	−1.90 ± 0.5	−2.36 ± 0.23	−2.55 ± 0.28	−1.85 ± 0.02	NT
	5	−2.98 ± 0.33	−3.59 ± 0.03	−4.17 ± 0.12	−4.10 ± 0.67	−3.64 ± 0.21	NT

CFU, colony-forming units; NT, not tested.

[a] Novobiocin and amoxicillin MICs (mg/L), respectively.

[b] Differences in bacterial colony counts between those found in antibiotic-containing tubes and those shown in control tubes at 2 h

and 5 h (inocula ranged from 6.03 $\log_{10}$ CFU/mL to 6.75 $\log_{10}$ CFU/mL at time zero).

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Table 2

Bacterial counts (mean ± standard deviation) in peritoneal fluid and survival after therapy with novobiocin and amoxicillin regimens

in mice with sepsis induced by four *Streptococcus pneumoniae* strains [a]

Drug regimen [b]	Log_{10} CFU/mL in survivor/log_{10} CFU/mL in dead animals (no. of survivor/no. of dead mice) [c]			
	Strain AR33118	Strain MJD3693	Strain SPC2162	Strain SPC2552
Control	None [d]/9.1 ± 0.4 (0/6)	3.3/9.1 ± 0.7 (1/9)	3.4 ± 1.7/8.2 ± 0.8 (2/8)	None [d]/8.3 ± 0.6 (0/8)
Novobiocin				
25 mg/kg	None [d]/8.9 ± 1.1 (0/3)	NT	NT	NT
50 mg/kg	None [d]/8.2 ± 1.1 (0/6)	NT	NT	NT
100 mg/kg	7.4 ± 1.5/8.5 ± 0.7 (5/2)	5.6 ± 4.3/7.2 ± 0.2 (5/3)	3.5 ± 2.4/6.7 ± 2.8 (4/4)	2.9 ± 1.5 */5.7 (7/1)
200 mg/kg	<1.0 */None [d] (5/0)	2.0 ± 1.3 */6.2 (9/1)	1.8 ± 1.7 */None [d] (9/0)	1.5 ± 1.4 */None [d] (8/0)
Amoxicillin				
25 mg/kg	<1.0 */None [d] (4/0)	NT	NT	NT
50 mg/kg	NT	5.7 ± 3.5/9.2 ± 0.8 (2/6)	5.2 ± 4.8/8.0 ± 0.5 (2/6)	<1.0/8.9 ± 0.0 (1/6)
200 mg/kg	NT	1.4 ± 1.2 */None [d] (8/0)	3.7 ± 3.1/8.2 ± 0.8 (6/4)	<1.0 */7.3 (7/1)

NT, not tested.

[a] The respective novobiocin/amoxicillin MICs of strains AR33118, MJD3693, SPC2162 and SPC2552 were 0.25/0.015, 0.5/8,

0.25/16 and 0.5/64 mg/L.

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^b Doses were given at 1, 5, 24 and 48 h after infection.

^c Evaluation was performed at 72 h after the start of therapy or before if the animal was found dead.

^d No bacterial counts are shown because there were no animals in these groups.

* P < 0.05 versus controls.

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FIG. 1.

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