

Synergistic Antimicrobial Effects of Cefabronchin[®]

Isabel Stephany-Brassesco¹, Stefan Bereswill², Markus M. Heimesaat^{2*†} and Matthias F. Melzig^{1†}

¹Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

²Institute of Microbiology, Infectious Diseases and Immunology, Charité - University Medicine Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

Received: 02 May 2019; accepted: 29 May 2019

Antibiotic resistance of *Streptococcus pneumoniae* has risen to worrying levels in the past few decades worldwide, and subsequently, effective treatment of respiratory tract infections has become even more challenging. While the need to develop new strategies to combat bacterial infections is urgent, novel antibiotic compounds are no longer a priority of the pharmaceutical industry. However, resistance-modifying agents can alleviate the spread of antibiotic resistance and render existing antibiotics effective again. In the present study, we aimed to determine the combinatory antimicrobial effects of the commercial herbal product Cefabronchin[®] and antibiotic compounds, such as amoxicillin and clarithromycin, on 6 clinical isolates of *S. pneumoniae*. Therefore, the minimal inhibitory concentration (MIC) of each agent before and after adding Cefabronchin[®] at different concentrations was determined by applying the checkerboard method. Sub-inhibitory concentrations of the added Cefabronchin[®] were found to reduce the MIC down to between 3.4% and 29.2% of the amoxicillin MIC and down to between 10.4% and 45.8% of the clarithromycin MIC in all 6 strains. In conclusion, this study provides evidence for the improved antimicrobial effects of commonly used antibiotics in combination with Cefabronchin[®] in order to combat infections with antibiotic-resistant *S. pneumoniae* strains.

Keywords: *Streptococcus pneumoniae*, antibiotic resistance modifiers, β -lactam resistance, macrolide resistance, natural products

Introduction

Treatment of bacterial infections is becoming increasingly challenging around the world due to the undetermined ability of antibiotics to combat emerging multidrug-resistant bacteria. Selection and dissemination of antibiotic-resistant bacterial strains have resulted from decades of misuse of antibiotics both in the farming industry and medical practice [1]. According to the European Commission, approximately 25,000 patients die in Europe each year as a result of infections caused by antibiotic-resistant bacteria, while the Center for Disease Control and Prevention (CDC) reports an estimate of 2-million infections by antibiotic-resistant bacteria and at least 23,000 annual fatal cases in the USA [2]. Resistance has emerged towards all classes of known antibiotics so far, with agents exhibiting rapid cross-resistance to others within the same class [3]. Even with a clear increase in incidence and severity of infections caused by multidrug-resistant bacterial strains, current market conditions fail to incentivize major pharmaceutical companies towards antimicrobial research and development.

Peptidoglycan is comprised of glycan chains of alternating amino sugars (*N*-acetylglucosamine and *N*-acetylmuramic acid) that are cross-linked by peptide chains. Antibiotics with a β -lactam ring, such as amoxicillin, affect the DD-transpeptidase, also termed penicillin binding protein (PBP), subsequently inhibiting the formation of the cross links and therefore of new cell walls. Pneumococcal resistance to peni-

cillin is mediated by changes in the affinity of acylation of PBPs [4]. There are 5 high molecular mass and only one low molecular mass PBPs that have been identified in *Streptococcus pneumoniae* so far, namely, 1a, 1b, 2a, 2b, 2x, and 3, respectively. Alterations in 2x, 2b, and 1a reduce the binding capacities to the target site [5].

Macrolides constitute a class of bacteriostatic antibiotics that inhibit bacterial protein synthesis by reversibly binding to the 50S ribosome subunit in domain V of the 23S rRNA of susceptible bacteria. Macrolides do not inhibit the formation of peptide bonds, but rather cause premature dissociation of peptides during translation [6]. In *S. pneumoniae* and *S. pyogenes* strains, resistance to macrolides can be due to binding site modifications that occur through the presence of a methylase, encoded by *erm* class genes, which pushes a conformational change by adding one or two methyl residues to an adenine residue in the V domain (peptidyl transferase center) of 23S rRNA [7]. This phenotype has been defined as the macrolide (M), lincosamide (L), and streptogramin B (S_B) resistance and can be inducible or constitutive [8]. A macrolide-specific efflux mechanism encoded by *mef* has been described as the *S. pneumoniae* M phenotype, characterized by resistance to 14- and 15-membered macrolides and susceptibility to 16-membered macrolides, lincosamides, and streptogramin B, distinct from the efflux system long established for erythromycin-resistant staphylococci [9]. The efflux mechanism is the main form of resistance to macrolides in North America, while target site modifications are predominant in Europe and Asia. However, some countries like Germany, Norway, and Austria have reported incidence of the efflux mechanism similar to those in North America, and isolates that are positive for both phenotypes are appearing more frequently worldwide [10–12].

*Author for correspondence: Charité - University Medicine Berlin, CC5, Department of Microbiology, Infectious Diseases and Immunology, Campus Benjamin Franklin, FEM, Garystr. 5, D-14195 Berlin, Germany; Phone: +49-30-450524318; E-mail: markus.heimesaat@charite.de.

†These authors contributed equally.

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S. pneumoniae is linked to both upper respiratory tract illnesses (URTI) and pneumonia. While URITs are often associated with cough and purulent sputum production and are a frequent and major cause of morbidity in vulnerable populations, most cases are not life-threatening and can be either self-limiting or easily treated. However, they can be causes for concern to parents or caretakers and are a prevalent reason for outpatient visits [17]. Despite cough and cold medications being marketed to both adults and children, effectiveness in pediatric populations has been specially challenged in various studies [18, 19].

Knowledge of the distinct pathogens involved in community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP) is a prerequisite for the antibiotic choice for treatment. CAP can be handled by hospitalization or in an outpatient scheme, and this, along with age, gender, and location, influences the clinical outcomes and mortality rates. HAP is defined as a nosocomial pneumonia that arises after at least 48 h in the hospital, in patients who have not been on a ventilator for 48 h preceding the infection, and can occur either in or out of the intensive care unit (ICU) [20]. Mortality in HAP is related to comorbidities of the affected patient and the severity of illness, which in turn are determined by the respective bacterial strain(s) and initial empiric and follow-up therapy. VAP is often associated with higher mortality rates and has different bacteriology than HAP, and as lower respiratory tract cultures are collected more often than in HAP, there is less need for empiric antibiotic choice [20].

Determining the etiology of pneumonia, community- or hospital-acquired, has been proven challenging. Obtaining samples from the lung is difficult and invasive, and biopsies are only performed in a minority of cases. Most bacteria linked to pneumonia are commonly found among the commensal flora of the upper respiratory tract, so how relevant cultures from sputum or nasopharyngeal secretion are has yet to be determined. Blood cultures yield positive results only in 4 to 24% of hospitalized patients and in less than 1% of outpatients [21, 22]. The most frequent pathogen associated with CAP is *S. pneumoniae*, while infections by *Haemophilus influenzae*, *Legionella* species, *Staphylococcus* species, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Chlamydia* species, *Coxiella burnetii*, and various viruses are also reported as causal agents with variant importance depending on the country of isolation [23]. Treatment for CAP strongly depends on the initial assessment of the severity of illness, which can be determined using the CURB-65 criteria or the Pneumonia Severity Index (PSI) [24, 25]. In general, previously healthy patients with no risk factors for drug-resistant *S. pneumoniae* should be treated with amoxicillin or a macrolide, while patients that have comorbidities (such as heart, lung, liver, or renal disease, diabetes mellitus, or other immunosuppressive conditions, for instance), that have been treated with antibiotics within the previous 3 months, or have risk factors for infection with drug-resistant *S. pneumoniae*, should obtain a respiratory fluoroquinolone or a combination of a β -lactam and a macrolide. A respiratory fluoroquinolone or a β -lactam plus a macrolide are recommended treatment options for hospitalized non-ICU patients, whereas ICU patients should receive a β -lactam antibiotic plus either azithromycin or a fluoroquinolone in the case of severe pneumonia [25, 26]. *S. pneumoniae* is involved in HAP and VAP as well, but also methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Stenotrophomonas maltophilia* are important causal agents [20]. In general, HAP should be treated with piperacillin-tazobactam, cefepime, levofloxacin, or a carbapenem. For the treatment of

VAP, 3 groups of antibiotics should be considered, namely, compounds active against Gram-positive bacteria including MRSA (vancomycin and linezolid), active against Gram-negative bacteria and with antipseudomonal β -lactam-based agents (piperacillin-tazobactam, cefepime, ceftazidime, carbapenems and aztreonam), and active against Gram-negative bacteria and with antipseudomonal non- β -lactam-based agents (ciprofloxacin, levofloxacin, amikacin, gentamicin, and colistin) [27].

It is worth noting that, while antibiotic therapy is often initiated empirically before the etiology of pneumonia is known from cultures, the high incidence of multidrug-resistant bacteria enhances the probability of inappropriate initial antibiotic selection [28]. The decrease in *S. pneumoniae* susceptibility towards first line antibiotics, particularly β -lactams and macrolides, prompts the development of ways to enhance antimicrobial susceptibility of these agents. Using combinations of antibiotics to treat bacterial infections is a strategy employed either to lower the doses to be administered, therefore reducing their toxic effects, or to improve clinical outcomes with strains that are susceptible to one or more antibiotics, enhancing the activity and achieving synergistic effects. Synergy is defined as a significantly greater activity provided by two agents combined than that provided by the sum of each agent alone [29].

Cefabronchin[®] is a traditional herbal medicine that has been approved for commercial distribution in Germany for a long time. According to the medication information leaflet insert, Cefabronchin[®] is “for use in adults and adolescents above 12 years old. Traditionally used to aid mucus solution and reduce irritation in the respiratory tract in case of cough due to cold”. Cefabronchin[®] contains a combination of extracts from plants such as *Thymus vulgaris*, *Cetraria islandica*, *Saponaria officinalis*, *Pimpinella saxifraga*, *Eucalyptus globulus*, *Foeniculum vulgare*, and *Illicium verum*. Thymol is a monocyclic phenolic compound present in *Thymus vulgaris* essential oil and extract, and its properties are extensively used in the food, chemical, and medical industries [13]. The mechanism by which thymol acts against bacteria has been attributed to toxic effects on the membrane structure and functions. Considering its lipophilic properties, thymol can partition into the lipid parts of the membranes, expanding it and decreasing its fluidity, modifying its permeability and disturbing its proteins and transport processes [14]. Recent saponins have been linked to anti-inflammatory activity by suppressing the expression of COX-2, iNOS, and pro-inflammatory cytokines in mouse models [15]. Some saponin studies, for instance, revealed high antimicrobial activity against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis* [16]. This prompted us to uncover potential synergistic antimicrobial effects of Cefabronchin[®] and antibiotics such as amoxicillin and clarithromycin on clinical drug-resistant *S. pneumoniae* isolates in the present study.

Materials and Methods

The antibiotics used were purchased from SIGMA ALDRICH (amoxicillin batch no.: 097M4848V/clarithromycin batch no.: 087M4081V).

The manufacturer declares the following composition of Cefabronchin[®]:

- Thyme fluid extract (1:2–3). Medium: ammonia solution 10% m/m, glycerol 85% v/v, ethanol 90% v/v, purified water..... 4.95 g
- Excerpt from a mixture of 5 g Iceland moss, red soaproot, bibernell root, eucalyptus leaves, and bitter fennel fruit (1:1:1:1:1) and 1 g star aniseed fruit.
Medium: ethanol 30% v/v 2.08 g

• Ethanol 30% v/v liqueur wine (contains grape juice, wine alcohol, glucose fructose, simple syrup, and potassium hydrogen sulfite).

Thirty-six droplets equal 1 g.

(Batches no.: 1700794 and 1700793)

The clinical *S. pneumoniae* isolates were provided by Labor Berlin (Microbiological Diagnostics Department at the Charité – University Medicine Berlin).

Minimal inhibitory concentrations (MICs) for all three agents were determined using the serial microdilution method. Each MIC determination plate provided 7 repetitions, and 6 plates were incubated for each agent. The combinatory antimicrobial effect of agents (Cefabronchin® + respective antibiotic compound) as compared to the MIC of each agent alone was determined using the microdilution synergy test model (checkerboard method), which consists of a matrix of wells that contain a decreasing concentration of each agent in each axis. Six repetition plates were incubated for each matrix.

By using the MIC of the antimicrobial combination, it is possible to calculate the fractional inhibitory concentration (FIC) index, which is a mathematical expression used to represent the interaction and determine the effect: synergistic, additive, indifferent, or antagonistic [29, 30]. The theoretical definition of synergy is an FIC index value of <1. An additive effect happens when the effect of the combination is equal to that of the sum of either agent, i.e., an FIC index value between 1 and 2. An indifferent effect is indicated when the combination of antibacterial products results in a similar effect of the most active one. Antagonism is defined as an increase in the MIC when in combination, and a FIC index value ≥ 2 .

$$\text{FIC index} = \text{FIC}_x + \text{FIC}_y = \frac{X}{\text{MIC}_x} + \frac{Y}{\text{MIC}_y}$$

Where *X* is the concentration of drug *x*, and *Y* is the concentration of drug *y* in the same well; MIC_x and MIC_y are the minimal inhibitory concentrations of the corresponding drug.

Statistical Analyses. Means, standard deviations (SD), and significance levels were determined using the Wilcoxon Signed Rank Test (GraphPad Prism version 5.01) as indicated. Two-sided probability (*p*) values ≤ 0.05 were considered significant. Experiments were reproduced 6 times.

Ethics. Bacterial strains were derived from clinical samples that had been sent for routine diagnostic analyses at Charité - University Medicine Berlin. Thus, the patients had given prior informed consent. Furthermore, bacterial strains were supplied in an anonymous fashion, hence, no correlation to patients' data could be derived. Therefore, the study was exempted from an additional ethical approval by the local institutions.

Table 1. MICs for 6 *S. pneumoniae* strains

Strain	Amoxicillin MIC (µg/mL)	Clarithromycin MIC (µg/mL)	Cefabronchin® MIC (% v/v)
RE20869	16	2	3.7 ± 0.8
RE20891	4	2	2.3 ± 0.8
RE21286	80	160	7.3 ± 1.6
BK79062	8	100	11.5 ± 2.6
29	64	100	4.7 ± 1.6
17	16	160	18.7 ± 6.5

The lowest concentration of antibiotic that resulted in no growth for that particular strain after 24 h of incubation at 37 °C is shown. Each MIC determination plate has 7 repetitions for any given concentration, and 6 plates were incubated for each strain. There was no variance between measurements, and therefore, no SD is shown for the antibiotics.

Table 2. Fractional inhibitory concentration (FIC) indexes for the Cefabronchin® + antibiotic combinations and combinatory effect determination

Strains	FIC index Cefabronchin®–amoxicillin	FIC index Cefabronchin®–clarithromycin
RE20869	0.47 ± 0.0 (S)	0.44 ± 0.09 (S)
RE20891	0.47 ± 0.09 (S)	0.53 ± 0.02 (S)
RE21286	0.25 ± 0.05 (S)	0.64 ± 0.29 (S)
BK79062	0.31 ± 0.01 (S)	0.77 ± 0.09 (S)
29	0.47 ± 0.0 (S)	0.25 ± 0.05 (S)
17	0.51 ± 0.10 (S)	1.18 ± 0.19 (A–I)

A FIC index was calculated for each well with the lowest concentration of antibiotic that resulted in no growth for each concentration of Cefabronchin® tested. The value shown is the mean ± SD of all the FIC indexes in every checkerboard (6 repetitions were performed). The calculation of FIC indexes was done considering the MIC values as the whole dilution concentration closest to the mean value. S: synergistic FIC index <1; A–I: additive–indifferent FIC index 1–2; A: Antagonistic FIC index > 2.

Results and Discussion

Antibiotic MICs. The variability of the amoxicillin, clarithromycin, and Cefabronchin® MICs between tested *S. pneumoniae* strains is shown in Table 1, respectively. Amoxicillin MICs ranged from 4 µg/mL to 80 µg/mL, while clarithromycin MICs varied from 2 µg/mL to 160 µg/mL. According to the European Committee on Antimicrobial Susceptibility Testing, all these strains would be considered “resistant” to both antibiotics. Bactericidal activity is relevant for pathogen eradication and optimization of clinical outcomes, so the likelihood of selection of antibiotic-resistant strains is reduced as potency of the antibiotic increases [31].

Cefabronchin® MICs. While Cefabronchin® MICs appear to be relatively low, it is impossible to rank the tested strains within a sensitive-to-resistant spectrum due to the lack of consensus regarding defined MIC breakpoints for herbal extracts. MICs for Cefabronchin® ranged between 2–4% v/v and 16–32% v/v. The most sensitive *S. pneumoniae* strains to amoxicillin and clarithromycin were also the most sensitive ones towards Cefabronchin®. Conversely, the Cefabronchin® MIC for strain RE21286, which showed high resistance against the used antibiotic compounds, was only one dilution step higher (around 8%, v/v). The highest Cefabronchin® MIC of 16% was for strain 17, among those tested.

It is worth noting that while individual herbal extracts that comprise Cefabronchin® are expected to have a weaker antimicrobial activity against *S. pneumoniae*, the combined product might be more effective due to synergistic effects resulting from individual compounds within the formulation.

Inhibitory Concentration of Antibiotics in Combination as a Percentage of the Antibiotic MIC. A FIC index was calculated for all clinical isolates in each of the Cefabronchin®–amoxicillin and Cefabronchin®–clarithromycin combinations tested. Each concentration of Cefabronchin® that was below the Cefabronchin® MIC resulted in a FIC index, and the mean values were calculated. All FIC indexes <1 indicate synergistic effects as shown in Table 2.

The effects exerted by the different Cefabronchin® concentrations on the amoxicillin MIC are shown in Table 3. The last Cefabronchin® concentration shown in the tables corresponds to the determined Cefabronchin® MIC, and unless the combination results in an antagonistic effect, no bacterial growth is expected at this concentration, and therefore, 0% of the amoxicillin MIC is required. Three dilution steps below the Cefabronchin® MIC (MIC/2³), the amoxicillin concentration was lowered to between 3.38% and 29.17% of the amoxicillin MIC alone. The most prominent effect was observed with

Table 3. Influence of Cefabronchin® on the amoxicillin MIC against 6 strains of *S. pneumoniae*

Strain	Cefabronchin® Concentration (% v/v)											
	0	0.5	1	1.56	2	3.13	4	6.25	8	12.5	16	32
RE20869	100 ± 0	5.99 ± 5.1*	4.04 ± 4.3	N/T	2.47 ± 1.0	N/T	0 ± 0*	N/T	N/T	N/T	N/T	N/T
RE20891	100 ± 0	3.38 ± 2.3*	1.56 ± 0.9	N/T	0.26 ± 0.4	N/T	0 ± 0	N/T	N/T	N/T	N/T	N/T
RE21286	100 ± 0	N/T	5.21 ± 1.6*	N/T	1.04 ± 0.4*	N/T	0.26 ± 0.4	N/T	0 ± 0	N/T	N/T	N/T
BK79062	100 ± 0	N/T	N/T	4.67 ± 2.4*	N/T	1.04 ± 0.8	N/T	0 ± 0	N/T	0 ± 0	N/T	N/T
29	100 ± 0	29.17 ± 10.2*	21.35 ± 8.9	N/T	2.60 ± 0.8*	N/T	0 ± 0*	N/T	N/T	N/T	N/T	N/T
17	100 ± 0	N/T	N/T	N/T	N/T	N/T	4.16 ± 1.6*	N/T	2.34 ± 0.9	N/T	0.39 ± 0.4*	0 ± 0

Each value represents the mean ± SD of the relative concentration of amoxicillin, in combination with different concentrations of Cefabronchin®, needed to inhibit growth of each particular strain after a 24-h incubation period at 37 °C. The Cefabronchin® concentrations tested depended on the Cefabronchin® MIC for each strain. N/T: concentration not tested for that strain. The MIC of amoxicillin alone was considered 100%. Each experiment was performed 6 times and *p*-values were calculated using Wilcoxon Signed Ranked Test.

**p*-value < 0.05 when compared to the previous concentration tested.

strain “RE20891” and the weakest with strain “29”. The difference between lack of Cefabronchin® and added Cefabronchin® MIC/2³ was significant in all the tested *S. pneumoniae* strains (*p* < 0.05). However, increasing the concentration and adding Cefabronchin® MIC/2² did not result in significant differences as compared to one additional dilution step (MIC/2³) in any of the strains except “RE21286”. This result indicates that the addition of low Cefabronchin® concentrations is sufficient to yield a significant reduction of the amoxicillin MIC that is required to inhibit growth in the tested strains and that increasing Cefabronchin® concentrations does not necessarily impact the inhibitory outcome in a significant manner.

Overall, FIC indexes calculated for the combination of Cefabronchin® and clarithromycin indicate a synergistic interaction against the strains tested. Only against strain “17”, the combination of agents revealed an off-trend effect, with the FIC index indicating indifference.

The influence of the Cefabronchin® concentrations tested in combination with clarithromycin, expressed as a percentage of the clarithromycin MIC necessary to inhibit growth, is shown in Table 4. While synergy was observed on 5 of the 6 clinical isolates evaluated, only in 3 did the lowest concentration of Cefabronchin®, 0.25%, have a significant effect on the clarithromycin MIC (RE20869, RE20891, and 29) (*p* < 0.05). However, on those strains, Cefabronchin® concentrations of 0.50% and 1% showed no significant difference compared to 0.25%. The effect of 2% of Cefabronchin®, which incidentally corresponds to the MIC/2 of “RE20869” and the MIC of “RE20891”, was significantly stronger than that of 1% (*p* < 0.05). This observation indicates that the synergy mechanism might be saturable, and that while adding small concentrations of Cefabronchin® lower the clarithromycin MIC significantly, increasing the said concentration is not necessarily relevant.

The lowest concentration of Cefabronchin® to which strains “RE21286”, “BK79062”, and “17” was exposed resulted in no significant increase in the effect when compared to only exposing them to clarithromycin. With strain “RE21286” however, adding 2% of Cefabronchin® (MIC/22) did result in a meaningful decrease in the clarithromycin MIC. For strains “BK79062”, the second highest concentration resulted in a considerable reduction of the clarithromycin MIC.

Essential oils in general are thought to target the bacterial cell wall and cytoplasmic membrane, causing permeabilization and thus allowing leakage of ions, breakdown of the proton pump, and depletion of ATP available [32]. Lack of knowledge of the exact composition of Cefabronchin® makes it difficult to speculate, but if this is, at least in part, the mechanism of action by which it inhibits *S. pneumoniae* bacterial growth, synergy with clarithromycin might be explained by an increase of intracellular clarithromycin concentrations due to a less restrictive cytoplasmic membrane.

As antibiotic-resistant strains become more and more prevalent, the relevance of commonly used antibiotics such as amoxicillin and clarithromycin needs to be recalibrated. Multi-drug therapy is less vulnerable to adaptive resistance due to biological systems being less able to compensate effects from two or more substances or from one substance that acts on multiple targets simultaneously [33]. A shift in the way chemotherapy has been approached in the last few years indicates that multidrug therapies are going to replace mono-substance therapies in the near future as the standing paradigm [34].

Conclusion

Bactericidal activity is pivotal for pathogenic eradication and optimization of clinical outcomes, and so the likelihood of selection of antibiotic-resistant strains is reduced as potency

Table 4. Influence of Cefabronchin® on the clarithromycin MIC against six strains of *S. pneumoniae*

Strain	Cefabronchin® Concentration (% v/v)												
	0	0.25	0.5	1	1.56	2	3.13	4	6.25	8	12.5	16	32
RE20869	100 ± 0	20.83 ± 6.4*	19.79 ± 8.3	16.67 ± 9.4	N/T	0 ± 0*	N/T	N/T	N/T	N/T	N/T	N/T	N/T
RE20891	100 ± 0	45.83 ± 10.2*	43.75 ± 15.3	31.25 ± 15.3	N/T	0 ± 0*	N/T	N/T	N/T	N/T	N/T	N/T	N/T
RE21286	100 ± 0	N/T	N/T	83.33 ± 25.8	N/T	16.67 ± 16.6*	N/T	3.39 ± 1.5*	N/T	0 ± 0*	N/T	N/T	N/T
BK79062	100 ± 0	N/T	N/T	N/T	83.33 ± 25.8	N/T	50.0 ± 0	N/T	10.42 ± 3.2*	N/T	0.52 ± 1.2*	N/T	N/T
29	100 ± 0	16.67 ± 6.4*	10.42 ± 3.2	4.17 ± 1.6	N/T	0 ± 0*	N/T	N/T	N/T	N/T	N/T	N/T	N/T
17	100 ± 0	N/T	N/T	N/T	N/T	N/T	N/T	91.67 ± 20.4	N/T	58.33 ± 20.4	N/T	29.17 ± 10.2*	0 ± 0*

Each value represents the mean ± SD of the relative concentration of clarithromycin, in combination with different concentrations of Cefabronchin®, needed to inhibit growth of each particular strain after a 24 h incubation period at 37 °C. The Cefabronchin® concentrations tested depended on the Cefabronchin® MIC for each strain. N/T: concentration not tested for that strain. The MIC of clarithromycin alone was considered 100%. Each experiment was performed six times and *p* values were calculated using Wilcoxon Signed Ranked Test. An asterisk (*) indicates a *p* value < 0.05 when compared to the previous concentration tested.

of the respective antibiotic compound increases [31]. However, as antibiotic-resistant strains become more and more prevalent, the relevance of commonly used antibiotics like amoxicillin and clarithromycin needs to be reconsidered. Creative ways to repurpose these antibiotics, like combining them with natural products, are one of the strategies suggested to be able to treat these infections while facing the lack of antimicrobial development in the pharmaceutical industry.

The ingredients of Cefabronchin® act synergistically within themselves and the antibiotics tested (amoxicillin and clarithromycin) against *S. pneumoniae*, presumably by modifying the resistance mechanisms expressed by the pathogen. The resistance phenotype of the studied strains was not investigated, but knowing the resistance mechanism by which any given strain fights when exposed to antibiotics is required to understand the mechanism of action that enables Cefabronchin® and its components to modify the said resistance.

Funding Sources

This work was supported by the research groups involved. S.B. and M.M.H. received grant support from the German Federal Ministries of Education and Research (BMBF) in frame of the zoonoses research consortium PAC-Campylobacter (IP7/ 01K11725D).

Authors' Contributions

I.S.B. performed experiments, analyzed data, and wrote the paper. S.B. provided advice in the experimental design, critically discussed the results, and edited the paper. M.M.H. edited the paper. M.F.M. designed the experiments, provided advice in the experimental design, critically discussed the results, and edited the paper.

Conflict of Interest

S.B. and M.M.H. are Editorial Board members.

Acknowledgements. We thank Gernot Reifenber for excellent technical assistance.

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