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(RESEARCH ARTICLE)

Antibacterial effects of peonol in combination with amoxicillin against *Streptococcus pneumonia* clinical isolates

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Abstract

Drug resistance is a crisis among major pathogens such as *Streptococcus pneumonia (S. pneuomonia)*. The aim of this study was assessment of synergistic effects of amoxicillin and paeonol checkerboard assay on *S. pneumonia*. *S. pneumonia* isolates were resistant to several antibiotics. By combining peonol with amoxicillin, the MIC for amoxicillin alone decreased significantly compared to that of stand-alone amoxicillin. For example, the MIC for amoxicillin when combined with peonol ranged from 0.015 to 0.5 µg/mL, which corresponds to an approximate 8-16 fold decrease of amoxicillin MIC in some samples. Likewise, the average MIC of peonol decreased from approximately 2 mg/mL to an MIC of 0.125 mg/mL when combined with amoxicillin. The results calculated by FIC indices for all bacterial isolates that were exposed to both compounds demonstrated that 15 (75%) of bacteria tested indicated synergy (0.5≤FIC); thus indicating the development of strong synergistic effects between peonol and amoxicillin, while 5 (25%) indicated addition (1≥FIC>0.5) and no strain showed indifference (>1 FIC). No strains showed antagonistic interactions. Therefore, paeonol had good synergistic effect with amoxicillin.

Keywords: *Streptococcus pneumonia;* Synergistic effects; Peonol; Amoxicillin

1. Introduction

Pneumococcus (*Streptococcus pneumoniae*) is a bacterium which causes a range of diseases and continues to be a significant issue for patient health worldwide [1]. Pneumococcus causes multiple serious diseases that include pneumonia, meningitis, bacteremia, and ear infections (otitis media) [2]. The overall burden of disease is made worse by the increasing resistance of these bacteria to many types of antibiotics; particularly penicillin and other beta-lactams. This increasing resistance creates challenges for physicians when trying to adequately treat patients infected with pneumococcus and points to the need for alternative/adjunctive means of augmenting the effectiveness of conventional antimicrobial therapy. The emergence of many strains of pneumococcus that are resistant to multiple drugs has created urgency for new and more effective methods for treating pneumococcal infections [3].

A promising method of moving forward is to consider using phytochemicals (i.e., plant-derived compounds) demonstrating antimicrobial effects in conjunction with traditional antibiotics [4]. These phytochemicals, when used with traditional antibiotics, may work together to induce a synergistic effect by enhancing bacterial killing, reducing the amount of antibiotic needed for elimination, and decreasing the likelihood of developing resistant strains [5]. One such phytochemical is paeonol, which is a phenolic compound derived from a number of different plant sources, most commonly *Paeonia suffruticosa*. Paeonol has been shown to have multiple biological activities, including anti-inflammatory, antioxidant, and antimicrobial activity. While research into the antimicrobial activity of paeonol has not been as thorough as for traditional antibiotics, emerging evidence suggests it may damage bacterial cell membranes and otherwise disrupt the pathogenic process in bacteria [6, 7].

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Amoxicillin is an aminopenicillin antibiotic that continues to play an important role in treating infections caused by *S. pneumoniae*. Bacterial cell walls are protected and maintained by a structure called peptidoglycan, Amoxicillin works by inhibiting the synthesis and development of the bacterial cell wall. Due to the development of strains of *S. pneumoniae* that are resistant to amoxicillin, studies have recently focused on finding ways to restore the activity of amoxicillin using combination therapies [8].

A new approach to restoring or improving the effectiveness of amoxicillin is through synergy between naturally occurring compounds and amoxicillin, such as paenol and other herbal extracts and natural compounds. Synergy occurs when the combined effects of two agents exceed the individual effects. Synergy allows for reduced doses of both components and a reduction in side effects from the combination of both agents [9, 10]. Synergistic interactions between Paeonol and Amoxicillin have been studied extensively in vitro (in laboratory cultures), but an understanding of these interactions at the microbiological level and their potential for use as combination therapy is limited, particularly against clinical isolates of *S. pneumoniae*. Therefore, this study will use clinical isolates of *S. pneumoniae* to evaluate the synergistic antimicrobial effects of paeonol and amoxicillin, by measuring and comparing their antimicrobial activity when used together. This study will also provide an opportunity to further evaluate the potential for using paeonol as an adjunct therapy to enhance the effectiveness of amoxicillin and/or similar antibiotics, in light of the increased resistance to current antibiotics. The findings of this study may be used to develop new ways to treat infections caused by *S. pneumoniae*, improve how well current antibiotics work, and develop other ways to treat drug-resistant *S. pneumoniae* infections.

2. Methods

2.1. Study Design and Bacterial isolates

The design of the study consisted of an experiment to determine the effect of two antibacterial agents (peonol and amoxicillin) on the activity of 20 *S. pneumoniae* clinical isolates collected from patients who had pneumonia between January and June 2025 at a Al-Hussein Teaching Hospital-Al-Muthanna. The identification of the clinical isolates was done using standard laboratory tests (colony morphology, optochin susceptibility, bile solubility tests). All clinical isolates were kept frozen (-80°C) in brain-heart infusion(BHI) broth mixed with glycerol until tested.

2.2. Preparation of Agents

Peonol was obtained in pure form from a supplier, and samples of stock solutions of peonol were prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL and stored at -20°C until use. The pharmaceutical-grade formulation of amoxicillin was purchased and reconstituted with sterile water to obtain a stock solution of 1 mg/mL. Working solutions of amoxicillin were made each time prior to the test.

2.3. Antimicrobial Susceptibility Testing

The initial susceptibility of isolates to amoxicillin was established utilizing the disk method of diffusion according to Clinical Laboratory Standards Institute (CLSI) protocols. In summary, bacterial samples were diluted to 0.5 McFarland standards and spread onto 5% sheep blood-supplemented Mueller-Hinton agar plates similar to above. Amoxicillin disks containing 25 ug were introduced after which plates were placed at 37 C in the presence of 5% $CO2$ for a period of 24 hours. Subsequently, the diameters of the zones of inhibition were measured and categorized as susceptible, intermediate or resistant by CLSI breakpoints.

2.4. Assessing Potential Synergistic Effects

The checkerboard microdilution method was implemented to investigate the possibility of synergistic effects occurring in this study. Bacterial dilutions were created at a concentration of 1 × 106 CFU/mL. Bacteria were plated into 96-well microtiter plates that included serially diluted two-fold mixtures of peonol and amoxicillin. Peonol was tested at different concentrations ranging from 0.125 milligrams per millilitre (mg/ml) to 4 mg/ml, while amoxicillin was tested at concentrations running from 0.0625 to 2 micrograms per millilitre (ug/ml). Following preparation, the plates were incubated for 24 hours at 37 C in the presence of 5% CO2. Bacterial growth was visually determined and followed up using a reduction assay with the resazurin dye.

For all isolates, the minimum inhibitory concentration (MICs) for both agents tested individually as well as in combination was reported, while the fractional inhibitory concentration (FIC) index for each isolate was subsequently calculated as:

FIC of peonol = MIC of peonol in combination / MIC of peonol alone

FIC of amoxicillin = MIC of amoxicillin in combination / MIC of amoxicillin alone

FIC index = FIC of peonol + FIC of amoxicillin

FIC index interpretation: ≤0.5 synergy, >0.5-1 additive/combined, >1-4 indifference, >4 antagonism.

2.5. Statistical Analysis

Data were analysed using SPSS version 25. The proportion of isolates exhibiting synergy was quantified and paired t-tests were applied to assess the significance of reduction in MICs of tested combinations vs single agents at a level of $p < 0.05$.

3. Results

3.1. Baseline Susceptibility Profiles

Of the 20 clinical isolates of *S. pneumoniae* evaluated, a total of 12 (60%) were fully susceptible to amoxicillin, 3 (15%) were classified as intermediates, while 5 (25%) were assessed as resistant per CLSI guidelines. The range of amoxicillin MICs for the resistant isolates was ≥2 μg/mL, whereas the MIC range of amoxicillin for all other isolates, as per CLSI guidelines, was between 0.03-4 μg/mL. The amoxicillin MICs for the isolates tested individually with peonol ranged from 0.5-4 mg/mL, with a mean of 2 mg/mL (shown in Figure 1).

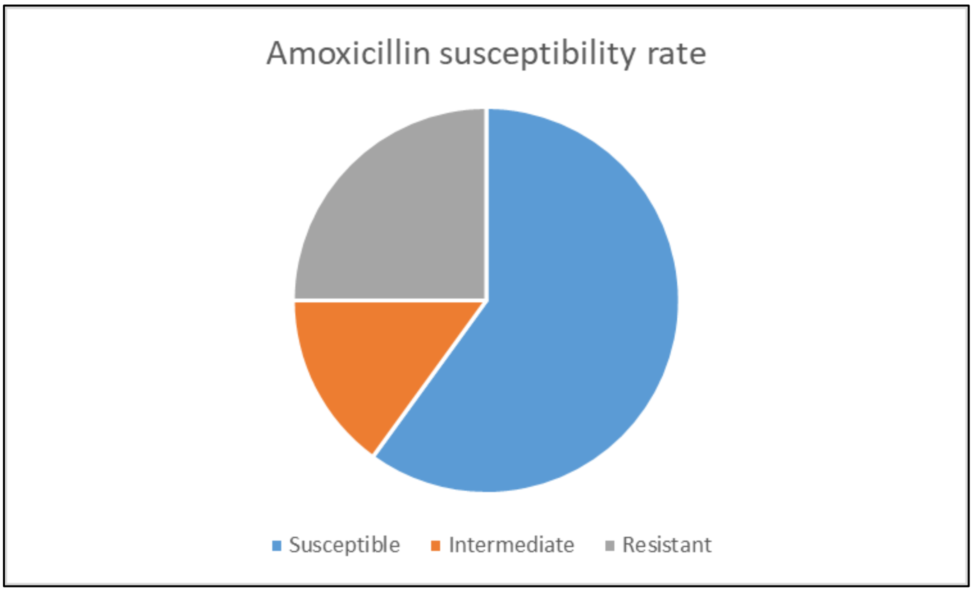

Figure 1 Susceptibility of *S. pneumonia* isolates to amoxicillin

3.2. The checkerboard assay

The checkerboard assay was used to assess the interaction outcomes of the combination of peonol with amoxicillin. By combining peonol with amoxicillin, the MIC for amoxicillin alone decreased significantly compared to that of stand-alone amoxicillin. For example, the MIC for amoxicillin when combined with peonol ranged from 0.015 to 0.5 μg/mL, which corresponds to an approximate 8-16 fold decrease of amoxicillin MIC in some samples. Likewise, the average MIC of peonol decreased from approximately 2 mg/mL to an MIC of 0.125 mg/mL when combined with amoxicillin.

The results calculated by FIC indices for all bacterial isolates that were exposed to both compounds demonstrated that 15 (75%) of bacteria tested indicated synergy (0.5≤FIC); thus indicating the development of strong synergistic effects between peonol and amoxicillin, while 5 (25%) indicated addition (1≥FIC>0.5) and no strain showed indifference (>1 FIC). No strains showed antagonistic interactions (Figure 2).

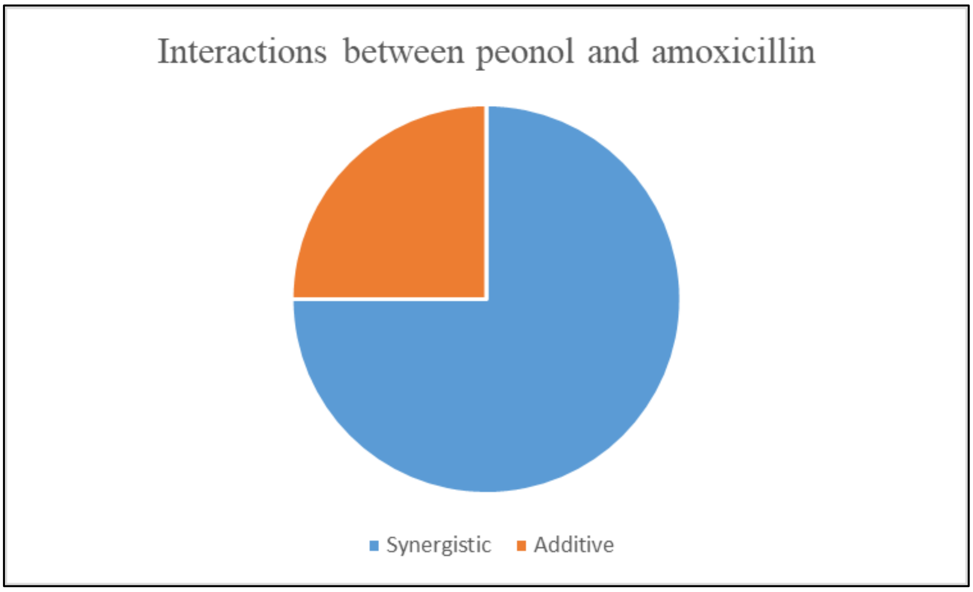

Figure 2 Synergistic and additive effects of peonol with amoxicillin

3.3. Effects on Resistant Isolates

Three of the five amoxicillin-resistant isolates evaluated in this study were identified as having synergistic interactions with paeonol, resulting in a 60% reduction in the dose needed to reach an effective concentration against *S. pneumoniae* (MIC < 0.5 µg/ml), bringing it into the susceptible range. The remaining two resistant isolates did not show synergism with paeonol but rather an additive or indifferent effect. The paired t-test was used to analyze the differences in the concentration at which the drug will kill bacteria (MIC) between amoxicillin alone and when combined with paeonol (P = < 0.001). The mean MIC for amoxicillin alone was 1.5 µg/mL and decreased to 0.2 mg/mL when paired with paeonol. Furthermore, both the additive effects of amarol and that of combined with paeonol were statistically significant.

4. Discussion

The rise in antibiotic-resistant strains of *S. pneumoniae* is a major issue in public health and therefore requires new approaches to restore some of the lost activity associated with the use of traditional antibiotic therapies. The combination of antibiotics with natural products provides an opportunity to investigate potential synergisms to maximize the inhibition of bacterial growth, reduce the required therapeutic dose of each agent and decrease the likelihood of developing antibiotic resistance in the future [11, 12]. In this regard, the combination of paeonol (a naturally-occurring phenolic compound found in plants, including chamomile and passionflower) with amoxicillin represents a potential area of further research on the possibility of enhancing the activity of existing antibacterial agents through the use of additional compounds with different modes of action and their ability to influence the development of antimicrobial resistance. Paeonol has demonstrated a broad spectrum of biological activities. Its antimicrobial activity, although moderate when used alone, is attributed to its capacity to interfere with bacterial membrane integrity, inhibit enzymatic functions, and modulate oxidative stress responses within bacterial cells [13]. Previous studies have shown that paeonol can inhibit the growth of various bacterial pathogens, including Gram-positive and Gram-negative bacteria [14, 15], although its efficacy against *S. pneumoniae* remains less well-characterized. Nonetheless, its ability to disrupt bacterial cell membranes may increase permeability, thereby facilitating the entry of antibiotics like amoxicillin into bacterial cells.

In this study, five (25%) of *S. pneumonia* isolates were amoxicillin-resistant. Most of them exhibited susceptibility in synergistic and also additive effects in checkerboard by paeonol compound.

Paeonol combined with amoxicillin can work synergistically through multiple mechanisms. First, paeonol causes damage to the bacterial membrane, allowing for greater penetration of amoxicillin into the bacterial cell and, thus, greater access to PBPs. Second, it may affect bacterial stress response pathways, causing bacteria to be more sensitive to amoxicillin. In addition, amoxicillin's ability to inhibit cell wall synthesis will allow for greater bactericidal effects of paeonol due to exposure of intracellular targets.

Some flavonoids and phenolic natural products have been demonstrated to restore the activity of antibiotics against resistant strains through the inhibition of efflux pumps and the disruption of cellular membranes [16, 17]. Therefore, it is plausible that similar principles may apply to paeonol, specifically with respect to the combination of paeonol with amoxicillin against clinical isolates of S. pneumonia. In evaluations of synergistic actions, common methods include checkerboard assay, time-kill determination and FICIs. FICI values of ≤0.5 usually show synergy, whereas FICI values of between 0.5 and 1.0 illustrate a treatment regimen producing an additive effect; FICI values >4 show an antagonistic effect. Research has previously demonstrated that the use of checkerboard assays, time-kill studies and FICIs for assessing the effects of combination therapies (from natural compounds and an antibiotic) have produced positive results.

The identification of a synergistic effect between paeonol and amoxicillin has possible important implications for clinical medicine. By reducing the amount of amoxicillin needed to achieve a therapeutic effect through combination with a natural agent, the negative side effects and toxicities associated with amoxicillin can potentially be reduced. Furthermore, the combination of amoxicillin and paeonol will allow for the in vitro restoration of amoxicillin activity against resistant bacteria, due to the ability of paeonol to overcome bacterial resistance mechanisms. In addition, the use of natural agents in conjunction with traditional antibiotics such as amoxicillin may lead to decreased development of new antibiotic resistance and contribute to the development of sustainable anti-microbial strategies.

4.1. Limitations

Although in vitro findings provide valuable data, clinical application of these findings must be confirmed with the use of validated human clinical trials and in vivo animal studies. The bioavailability and potential toxicity of paeonol need to be thoroughly assessed as well as the pharmacokinetic properties. Furthermore, investigation into the molecular mechanisms of the observed synergy will lead to improved target combinations for synergistic therapies.

5. Conclusion

In summary, Investigating the potential synergistic interactions of paeonol and amoxicillin to treat *S. pneumoniae* clinical isolates provides a new approach to increasing the effectiveness of antimicrobial therapies. It also suggests that using of traditional antibiotics together with naturally occurring phytochemicals may offer an alternative method for the treatment of multidrug-resistant bacterial infections in addition to prolonging the life span of current antibiotic treatments. Therefore, it is imperative to continue researching and investigating natural compound-antibiotic combinations in response to the increasing global threat of antimicrobial resistance. Additionally, our investigation revealed that the combination of paeonol and amoxicillin exhibited robust synergy against S. *pneumoniae* clinical isolates, many of which are resistant to amoxicillin, as evidenced by reduced MIC values, which indicate that paeonol enhances the ability of amoxicillin to kill bacteria. Furthermore, these data support future studies using natural compound-antibiotic combinations to develop new therapies for treating MDR bacterial infections.

Compliance with ethical standards

Acknowledgments

This study was designed and performed by the authors.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

After agreements of the ethics committee, informed consent was obtained from all participants of the study.

References

[1] Narciso, A.R., et al., *Streptococcus pneumoniae epidemiology, pathogenesis and control.* Nature Reviews Microbiology, 2025. **23**(4): p. 256-271.

[2] Kruckow, K.L., et al., *Acute organ injury and long-term sequelae of severe pneumococcal infections.* Pneumonia, 2023. **15**(1): p. 5.

[3] Mercadante, S., et al., *The thousand faces of invasive group A streptococcal infections: update on epidemiology, symptoms, and therapy.* Children, 2024. **11**(4): p. 383.

[4] Abu Tamam, A.N., et al., *Herbal medicine as an adjunct in the treatment of periodontal diseases-A systematic review.* The Open Dentistry Journal, 2024. **18**(1).

[5] Leak, J.A., *Herbal medicine: Is it an alternative or an unknown? A brief review of popular herbals used by patients in a pain and symptom management practice setting.* Current review of pain, 1999. **3**(3): p. 226-236.

[6] Zhang, L., D.-c. Li, and L.-f. Liu, *Paeonol: pharmacological effects and mechanisms of action.* International Immunopharmacology, 2019. **72**: p. 413-421.

[7] Chan, E.W.C., *Paeoniflorin and paeonol from Paeonia species are promising compounds with anticancer and other pharmacological properties.* Journal of Applied Pharmaceutical Science, 2025. **15**(6): p. 001-009.

[8] Gibson, P.S. and J.-W. Veening, *Gaps in the wall: understanding cell wall biology to tackle amoxicillin resistance in Streptococcus pneumoniae.* Current opinion in microbiology, 2023. **72**: p. 102261.

[9] Bellio, P., et al., *New and simplified method for drug combination studies by checkerboard assay.* MethodsX, 2021. **8**: p. 101543.

[10] Cokol-Cakmak, M., et al., *Guided screen for synergistic three-drug combinations.* PloS one, 2020. **15**(7): p. e0235929.

[11] Hemaiswarya, S., A.K. Kruthiventi, and M. Doble, *Synergism between natural products and antibiotics against infectious diseases.* Phytomedicine, 2008. **15**(8): p. 639-652.

[12] Herman, A. and A.P. Herman, *Herbal products and their active constituents used alone and in combination with antibiotics against multidrug-resistant bacteria.* Planta Medica, 2023. **89**(02): p. 168-182.

[13] Wang, J., et al., *Paeonol derivatives and pharmacological activities: a review of recent progress.* Mini Reviews in Medicinal Chemistry, 2020. **20**(6): p. 466-482.

[14] Pandya, J., et al., *Synthesis, crystal feature and spectral characterization of paeonol derived Schiff base ligands and their Cu (II) complexes with antimicrobial activity.* Journal of the Indian Chemical Society, 2022. **99**(4): p. 100403.

[15] Zeng, Q., et al., *Effect of paeonol against bacterial growth, biofilm formation and dispersal of Staphylococcus aureus and Listeria monocytogenes in vitro.* Biofouling, 2022. **38**(2): p. 173-185.

[16] Górniak, I., R. Bartoszewski, and J. Króliczewski, *Comprehensive review of antimicrobial activities of plant flavonoids.* Phytochemistry reviews, 2019. **18**(1): p. 241-272.

[17] Liu, Y., et al., *Flavonoids as promising natural compounds for combating bacterial infections.* International Journal of Molecular Sciences, 2025. **26**(6): p. 2455.