Research, Society and Development, v. 9, n. 7, e264974089, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i7.4089

Cordeiro, LV, Figueiredo, PTR, Sousa, AP, Andrade Jr, FP, Souza, HDS, Araújo, DL, Sobreira, ALC & Lima, EO. (2020). Association of carvacrol with ceftazidime and cefepime against Klebsiella pneumoniae. ***Research, Society and Development,*** *9*(7):1-10, e264974089.

Associação do carvacrol com ceftazidima e cefepima contra *Klebsiella pneumoniae*

Association of carvacrol with ceftazidime and cefepime against *Klebsiella pneumoniae*

Asociación de carvacrol con ceftazidima y cefepima contra *Klebsiella pneumoniae*

Recebido: 29/04/2020 | Revisado: 01/05/2020 | Aceito: 05/05/2020 | Publicado: 12/05/2020

Laísa Vilar Cordeiro
ORCID: http://orcid.org/0000-0002-8884-7331
Universidade Federal da Paraíba, Brasil
E-mail: laisavilar@gmail.com
Pedro Thiago Ramalho de Figueiredo
ORCID: http://orcid.org/0000-0001-6132-6248
Universidade Federal da Paraíba, Brasil
E-mail: pedrotrfigueiredo@gmail.com
Aleson Pereira de Sousa
ORCID: http://orcid.org/0000-0002-3430-477X
E-mail: aleson_155@hotmail.com
Francisco Patricio de Andrade Júnior
ORCID: http://orcid.org/0000-0003-0681-8439
Universidade Federal da Paraíba, Brasil
E-mail: juniorfarmacia.ufcg@outlook.com
Helivaldo Diógenes da Silva Souza
ORCID: http://orcid.org/0000-0002-1708-4066
Universidade Federal da Paraíba, Brasil
E-mail: helivaldog3@gmail.com
Daniel Lopes Araújo
ORCID: http://orcid.org/0000-0002-1625-0368
Centro Universitário de Patos, Brasil
E-mail: daniel124.dl718@gmail.com
Ana Laura de Cabral Sobreira
ORCID: http://orcid.org/0000-0002-2091-0437
Universidade Federal da Paraíba, Brasil

E-mail: lauracabralas@gmail.com

Edeltrudes de Oliveira Lima

ORCID: http://orcid.org/0000-0002-9547-0886

Universidade Federal da Paraíba, Brasil

E-mail: edelolima@yahoo.com.br

Resumo

Klebsiella pneumoniae é um importante patógeno oportunista responsável por taxas consideráveis de infecções nosocomiais. Além disso, esta espécie é uma importante fonte de resistência a antibióticos, apresentando grande capacidade de adquirir plasmídeos com genes que lhes conferem resistência a múltiplos antibacterianos, o que torna necessário o desenvolvimento de alternativas terapêuticas. A combinação de fitoconstituintes e drogas clássicas demonstra ser benéfica. Com base nisso, este estudo teve como objetivo investigar o uso potencial de carvacrol em combinação com os antibacterianos usados classicamente na prática clínica ceftazidima e cefepima, a fim de otimizar o tratamento dessas infecções. A associação foi realizada pelo método *checkerboard*. O resultado observado para 100% das cepas analisadas foi um efeito aditivo na combinação de carvacrol com ceftazidima e carvacrol com cefepima. Assim, o carvacrol tem potencial uso como adjuvante no tratamento de infecções causadas por *K. pneumoniae* em combinação com ceftazidima e cefepima, devido ao efeito aditivo mostrado na combinação com ambos os fármacos. Alterações estruturais podem ser feitas para melhorar esse efeito observado. Esta pesquisa abre perspectivas para mais estudos que visam analisar os efeitos da combinação de carvacrol com outras drogas antibacterianas.

Palavras-chave: Fitoconstituintes; Antibacterianos; Método *checkerboard*.

Abstract

Klebsiella pneumoniae is an important opportunistic pathogen responsible for considerable rates of nosocomial infections. In addition, this species is an important source of antibiotic resistance, presenting a great capacity to acquire plasmids with genes that give them resistance to multiple antibacterials, which makes it necessary to develop therapeutic alternatives. The combination of phytochemicals and classic drugs has been shown to be beneficial. Based on this, this study aimed to investigate the potential use of carvacrol in combination with the antibacterials classically used in clinical practice ceftazidime and cefepime, in order to optimize the treatment of these infections. The association was carried

out using the checkerboard method. The observed result for 100% of the strains analyzed was an additive effect in the combination of carvacrol with ceftazidime and carvacrol with cefepime. Thus, carvacrol has potential use as an adjunct in the treatment of infections caused by *K. pneumoniae* in combination with ceftazidime and cefepime, due to the additive effect shown in the combination with both drugs. Structural changes can be made to improve this observed effect. This research opens perspectives for more studies that aim to analyze the effects of the combination of carvacrol with other antibacterial drugs.

Keywords: Phytochemicals; Antibacterials; Checkerboard method.

Resumen

Klebsiella pneumoniae es un importante patógeno oportunista responsable de tasas considerables de infecciones nosocomiales. Además, esta especie es una fuente importante de resistencia a los antibióticos, ya que muestra una gran capacidad para adquirir plásmidos con genes que les dan resistencia a múltiples antibacterianos, lo que hace necesario desarrollar alternativas terapéuticas. La combinación de fitoquímicos y drogas clásicas demuestra ser beneficiosa. En base a esto, este estudio tuvo como objetivo investigar el uso potencial de carvacrol en combinación con los antibacterianos utilizados clásicamente en la práctica clínica ceftazidima y cefepima, con el fin de optimizar el tratamiento de estas infecciones. La asociación se realizó utilizando el método de tablero de ajedrez. El resultado observado para el 100% de las cepas analizadas fue un efecto aditivo en la combinación de carvacrol con ceftazidima y carvacrol con cefepima. Por lo tanto, el carvacrol tiene un uso potencial como complemento en el tratamiento de infecciones causadas por *K. pneumoniae* en combinación con ceftazidima y cefepima, debido al efecto aditivo que se muestra en la combinación con ambos medicamentos. Se pueden hacer cambios estructurales para mejorar este efecto observado. Esta investigación abre perspectivas para futuros estudios que tienen como objetivo analizar los efectos de combinar carvacrol con otros medicamentos antibacterianos.

Palabras clave: Fitoquímicos; Antibacterianos; Método *checkerboard*.

1. Introduction

Klebsiella pneumoniae is an important opportunistic pathogen responsible for considerable rates of nosocomial infections. It is related to cases of extra intestinal infections, including urinary tract infections, cystitis, pneumonia, infections resulting from surgical procedures, endocarditis and septicemia. This pathogen is also involved in serious community

infections like pneumonia necrotizing, pyogenic liver abscesses and endophthalmitis endogenous (Navon-Venezia et al., 2017).

In addition, this species is an important source of antibiotic resistance, presenting a great capacity to acquire plasmids with genes that give them resistance to multiple antibacterials. This agent is gaining more notoriety, since the increase in the number of serious infections and the growing scarcity of effective drugs, become a challenge in the treatment of these diseases (Paczosa & Mecsas, 2016).

The World Health Organization (WHO) released in 2017 a list of the most important bacteria for which there is an urgent need for develop new treatments. The experts used as basis for the construction of this document criteria such as mortality, prevalence of resistance and transmissibility. The list was divided into three levels of need for antibiotic development: critical, high and medium. The critical group comprises Gram-negative bacteria, including *Klebsiella pneumoniae* resistant to multiple drugs (WHO, 2017). Health authorities around the world are looking for solutions to this crisis and emphasize that we urgently need to know more about antibiotics in order to develop new alternatives for the treatment of these infections (Piddock, 2017).

In this context, plants are one of the most important sources of new substances used as medicinal agents, because they have a wide variety of active components, produced from their metabolism, which are able to inhibit the growth of pathogens (Harvey et al., 2015). The phytochemicals present in several essential oils extracted from plants have activity against Gram-positive and Gram-negative bacteria, and many of them are effective against resistant strains. The association between phytochemicals and synthetic antibacterials has been shown to be effective even against pathogens resistant to conventional drugs. Furthermore, it is possible to reduce the concentration of medicines used, which brings more therapeutic safety to the patient (Nazzaro et al., 2013).

One of the most studied phytochemicals is carvacrol, a monoterpene found in essential oils like oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and other plants. This compound has a wide range of biological activities substances already reported in the literature, as an antioxidant, antitumor and antimicrobial. Carvacrol has a strong antibacterial activity, both against Gram-positive and against Gram-negative. Its activity in relation to several strains, such as: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Escherichia coli* and *Klebsiella pneumonia*. Studies have highlighted the possibility of its use in treatments (Sharifi-Rad et al., 2017; Marinelli et al., 2018).

Based on the above, this study aimed to investigate the potential use of carvacrol in combination with the antibacterials classically used in clinical practice ceftazidime and cefepime, in order to optimize the treatment of these infections.

2. Methodology

2.1 Test conditions

All tests were performed in vitro, following the protocols recommended for each experiment, as well as the recommended methodologies for scientific research (Pereira et al., 2018).

2.2 Substances

The carvacrol, ceftazidime and cefepime were purchased from the Sigma-Aldrich / Merck® laboratory and solubilized with dimethyl sulfoxide (DMSO) and Tween 80 to obtain emulsions of each substance in different concentrations to be used in the tests.

2.3 Microorganisms

All microorganisms used in this study come from clinical isolates and belong to the MICOTECA collection of the "Laboratório de Atividade Antibacteriana e Antifúngica de Produtos Naturais e Sintéticos Bioativos" from the "Departamento de Ciências Farmacêuticas (DCF)" of the "Universidade Federal da Paraíba (UFPB)". The strains were maintained on nutrient agar (NA) at 4 °C. To prepare the inoculum, the colonies obtained from *K. pneumoniae* cultures were suspended in sterile 0.9 % saline solution and adjusted according to the standard 0.5 on the Mc Farland scale to obtain 10^6 CFU/mL (CLSI, 2015).

2.4 Minimum Inhibitory Concentration (MIC)

The MIC determination was performed based on the standard recommendations (CLSI, 2015), using the broth microdilution technique in a 96-well plate to obtain different concentrations of the substances. At the same time, the sterility controls of the culture medium, viability of the strains and interference of the vehicles (DMSO and Tween-80) were

also performed. MIC is defined as the lowest concentration capable of causing complete inhibition of bacterial growth after 24 hours at 35±2ºC. The test was performed in triplicate.

2.5 Association test

The association of carvacrol with ceftazidime and cefepime was performed using the checkerboard method. Thus, different concentrations of carvacrol (8xMIC, 4xMIC, 2xMIC, MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC) were combined with different concentrations of antibacterials (8xMIC, 4xMIC, 2xMIC, MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC) and then microbial inoculum was added. All controls were performed in parallel. The reading of the experiment was done after incubation at 35 ± 2ºC for 24 hours to observe the presence or not of the visible bacterial growth (Wu et al., 2017).

The effect produced between the combination was determined by the fractional inhibitory concentration index (FICI). This index was calculated by the sum of fractional inhibitory concentrations (FIC), where FIC_A = (MIC of substance A in combination)/(MIC of substance A alone) and FIC_B = (MIC of substance B in combination)/(MIC of substance B alone), thus $FICI = FIC_A + FIC_B$. The association was defined as synergistic for FICI ≤ 0.5, as additive for 0.5 <FICI <1, as indifferent for 1 ≤ FICI <4, and as antagonistic for FICI ≥ 4 (Wu et al., 2017). In this way it is possible to determine the result of the combination of two agents and, therefore, have a perspective for future clinical uses.

3. Results and Discussion

Initially, the Minimum Inhibitory Concentrations (MICs) of carvacrol, ceftazidime and cefepime were determined (Table 1).

Table 1 - Minimum Inhibitory Concentration (MIC) of carvacrol, ceftazidime and cefepime against *K. pneumoniae.*

***K. pneumoniae* strains**	**MIC**		
	Carvacrol	**Ceftazidime**	**Cefepime**
LM-25	256 µg/mL	1 µg/mL	0.5 µg/mL
LM-83	256 µg/mL	1 µg/mL	1 µg/mL
LM-173	256 µg/mL	0.5 µg/mL	0.5 µg/mL
LM-260	256 µg/mL	2 µg/mL	1 µg/mL
LM-326	256 µg/mL	1 µg/mL	1 µg/mL

Source: Prepared by the authors, 2020.

As seen in Table 1, carvacrol showed MIC of 256 µg/mL against all strains tested. For ceftazidime, the MIC varied between 0.5 and 2 µg/mL, and for cefepime, the values obtained were in the range of 0.5 to 1 µg/mL (Table 1). Ceftazidime and cefepime had different MICs, but all strains were considered sensitive to both antibacterials, according to the criteria established by CLSI (2018).

The criteria used to classify the antimicrobial activity of natural products determine that: strong activity (MIC up to 500 µg/mL), moderate activity (MIC between 600 and 1500 µg/mL) and weak activity (MIC greater than 1600 µg/mL) (PEIXOTO et al., 2016). Although the MIC values of carvacrol are much higher than the MIC values of classic antibacterials, carvacrol can still be classified as a product with high antibacterial potential for *K. pneumoniae*. These results reinforce the existing data in the literature on the antibacterial potential of carvacrol (Sharifi-Rad et al., 2017; Marinelli et al., 2018) and corroborates with a study by Raei et al. (2017) using *K. pneumoniae* strains, which also identified a strong antibacterial activity of carvacrol, with MICs ranging from 125 and 250 µg/mL, even on producing *K. pneumoniae* strains of resistance enzymes.

The results of the association of carvacrol with ceftazidime and carvacrol with cefepime are shown in Table 2.

Table 2 - Association effect of carvacrol with ceftazidime and cefepime against *K. pneumoniae.*

***K. pneumoniae* strains/ Drugs**	**FICI**	**Effect**
LM-25		
Ceftazidime + Carvacrol	0.75	Additivity
Cefepime + Carvacrol	0.62	Additivity
LM-83		
Ceftazidime + Carvacrol	0.75	Additivity
Cefepime + Carvacrol	0.56	Additivity
LM-173		
Ceftazidime + Carvacrol	0.75	Additivity
Cefepime + Carvacrol	0.62	Additivity
LM-260		
Ceftazidime + Carvacrol	0.75	Additivity
Cefepime + Carvacrol	0.56	Additivity
LM-326		
Ceftazidime + Carvacrol	0.75	Additivity
Cefepime + Carvacrol	0.75	Additivity

Source: Prepared by the authors, 2020.

As seen in table 2, FICI values obtained for all strains fit in the range between 0.5 and 1. The association was defined as synergistic for FICI ≤ 0.5, as additive for 0.5 <FICI <1, as indifferent for 1 ≤ FICI <4, and as antagonistic for FICI ≥ 4 (Wu et al., 2017). Thus, additive effects were observed between all the combinations performed in different strains.

The combination of phytochemicals with conventional antibacterial drugs can be effective in combating resistant strains, since both molecules can act on different targets and potentiate the antimicrobial effect. In addition, the additive effect resulting from the observed combinations makes it possible to use smaller amounts of classic antibacterial drugs and carvacrol, reducing the side and toxic effects related to these drugs (Nazzaro et al., 2013).

In the combination of carvacrol with ceftazidime and cefepime, it was possible to observe an additive effect. In other words, both products, in concentrations that would be subinhibitory if used alone, had an inhibitory effect on the growth of *K. pneumoniae* when applied together. Such fact may prove to be interesting for clinical practice, as the association may reduce the concentration antibiotic needed to be administered to the user and thus contributing to mitigate possible side effects due to the medication, as well as assist in combating resistant infections. Thus, it is relevant that future studies will be performed to better investigate the benefits of this association.

4. Final Considerations

This article provides contributions on the association of carvacrol with antibacterials commonly used against *K. pneumoniae*. Carvacrol has potential use as an adjunct in the treatment of infections caused by *K. pneumoniae* in combination with ceftazidime and cefepime, due to the additive effect shown in the combination with both drugs.

Structural changes can be made to improve this observed effect. This research opens perspectives for more studies that aim to analyze the effects of the combination of carvacrol with other antibacterial drugs.

References

Clinical Laboratory Standards Institute (CLSI). (2015). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved standard M07-A10. Pennsylvania, United States of America: National Committee for Clinical Laboratory Standards, 2015.

Clinical Laboratory Standards Institute (CLSI). (2018). *Performance Standards for Antimicrobial Susceptibility Testing. 28th ed*. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.

Harvey, AL, Edrada-Ebel, R & Quinn, RJ. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery, 14*(2), 111-129.

Marinelli, L, Di Stefano, A & Cacciatore, I. (2018). Carvacrol and its derivatives as antibacterial agents. *Phytochemistry Reviews, 17*(4), 903-921.

Navon-Venezia, S, Kondratyeva, K & Carattoli, A. (2017). *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Reviews, 41*(3), 252-275.

Nazzaro, F, Fratianni, F, De Martino, L, Coppola, R & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals, 6*(12), 1451-1474.

Paczosa, MK, & Mecsas, J. (2016). *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews, 80*(3), 629-661.

Pereira, AS, Shitsuka, DM, Parreira, FJ & Shitsuka, R. (2018). *Metodologia da pesquisa científica* [e-book]. Santa Maria. Ed. UAB/NTE/UFSM. Disponível em: https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf. (Accessed 09 May 2020).

Piddock, LJ. (2017). Understanding drug resistance will improve the treatment of bacterial infections. *Nature Reviews Microbiology, 15*(11), 639-640.

Raei, P, Pourlak, T, Memar, MY, Alizadeh, N, Aghamali, M, Zeinalzadeh, E, Asgharzadeh, M & Kafil, HS. (2017). Thymol and carvacrol strongly inhibit biofilm formation and growth of carbapenemase-producing Gram negative bacilli. *Cellular and Molecular Biology, 63*, 108-112.

Sharifi-Rad, M, Varoni, EM, Iriti, M, Martorell, M, Setzer, WN, del Mar Contreras, M, Salehi, B, Soltani-Nejad, A, Rajabi, S, Tajbakhsh M & Sharifi-Rad, J. (2018). Carvacrol and human health: A comprehensive review. *Phytotherapy Research*, *32*(9), 1675-1687.

World Health Organization (WHO). 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017. http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/. (Accessed 04 March 2020).

Wu, X, Li, Z, Li, X, Tian, Y, Fan, Y, Yu, C, Zhou, B, Liu, Y, Xiang, R & Yang, L. (2017). Synergistic effects of antimicrobial peptide DP7 combined with antibiotics against multidrug-resistant bacteria. *Drug design, development and therapy*, *11*, 939.

Percentage of contribution of each author in the manuscript

Laísa Vilar Cordeiro - 20%

Pedro Thiago Ramalho de Figueiredo - 10%

Aleson Pereira de Sousa - 10%

Francisco Patricio de Andrade Júnior - 10%

Helivaldo Diógenes da Silva Souza - 10%

Daniel Lopes Araújo – 10%

Ana Laura de Cabral Sobreira - 10%

Edeltrudes de Oliveira Lima - 20%