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HEALTH SCIENCES

Effect of 2-chloro-*N*-(4-fluoro-3-nitrophenyl) acetamide in combination with antibacterial drugs against *Klebsiella pneumoniae*

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Abstract: *Klebsiella pneumoniae* is a species of Gram-negative bacteria related to a wide range of infections and high rates of drug resistance. The combined use of antibacterial agents is one of the strategies that has been analyzed in recent years as part of the alternatives in the treatment of drug-resistant infections. Recently, the antibacterial activity of of 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide has been demonstrated against *K. pneumoniae*, also indicating that this acetamide did not show significant cytotoxic potential in preliminary tests. Thus, it becomes an interesting substance for future studies that explore its antimicrobial capacity, including investigating its association with antibacterial drugs. Based on this, this research aimed to analyze the effects of the association of 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide (CFA) with ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem against *K. pneumoniae* strains. The results showed additivity when the substance was combined with ciprofloxacin and cefepime, indifference when associated with ceftazidime and synergistic effect when combined with meropenem and imipenem. Thus, the acetamide was able to optimize the effects of antibacterial drugs, reducing the concentrations necessary to cause bacterial death. These data indicate a potential future clinical use of these combinations, and further studies are needed to analyze this viability.

Key words: acetamide, drug association, *Klebsiella pneumoniae,* 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic Gram-negative pathogen that causes several types of diseases, such as: pneumonia, endophthalmitis, liver abscess, urinary tract infections, cystitis, endocarditis, septicemia and infections associated with surgical procedures (Navon-Venezia et al. 2017, Effah et al. 2020). This species is part of the "ESKAPE" pathogens group, an acronym using the initials of the scientific names of the six species considered most virulent and pathogenic to humans: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. (Pendleton et al. 2013).

It is estimated that *K. pneumoniae* causes about one third of all infections caused by Gram-negative bacteria. In addition, this is a bacterial species that has several resistance mechanisms to most of the last-line antibiotics that are usually used, presenting a great capacity to acquire plasmids with genes that give them

resistance to multiple antibacterials (Navon-Venezia et al. 2017, Effah et al. 2020).

This microorganism has the ability to quickly react to selective environmental pressure modifications. One of the main mechanisms of drug-resistant *K. pneumoniae* is through enzymes that inactivate antibacterial agents and prevent their action. The dissemination of these resistant determinants has been recognized as a major challenge in the treatment of bacterial infections worldwide. Drug-resistant infections are a public health problem, but also an economic issue, resulting in high financial costs for health systems (Dadgostar 2019, Oliveira et al. 2020).

The combination of issues related to increasingly restricted pharmacological options for the treatment of drug-resistant bacterial infections and the high rates of infections caused by *K. pneumoniae* makes it necessary to research and develop new antibacterial agents, which is one of the main countermeasures listed in the World Health Organization (WHO) global action plan (WHO 2017) to combat microbial resistance.

Combating antimicrobial resistance requires multiple therapeutic strategies, including the discovery of new molecular supports, improvements in antimicrobial administration and drug design strategies based on synthetic chemistry (Belousoff et al. 2019, Murtaza et al. 2019). Obtaining synthetic drugs from acetamides is a promising strategy, because this group is easily synthesized and its derivatives show a wide range of biological properties, especially antibacterial (Katke et al. 2011, Patel et al. 2012, 2013, Murtaza et al. 2019). Several studies present synthetic derivatives as potential agents against bacteria (Fuloria et al. 2009, Katke et al. 2011, Pradidphol et al. 2012, Kaplancikli et al. 2012, Murtaza et al. 2019, Cordeiro et al. 2020).

The combined use of antibacterial agents is one of the strategies that has been analyzed in recent years as part of the alternatives in the treatment of drug-resistant infections. In clinical practice, the treatment of infections caused by Gram-negative bacteria includes the combination of antibiotics, which usually consist of a β-lactam and an aminoglycoside or fluoroquinolone (Tamma et al. 2012). In addition, the potential of synergistic interactions of phytochemicals with antibacterial agents against resistant bacteria has been demonstrated (Ayaz et al. 2019).

Recently, Cordeiro et al. (2020) showed the first report of the antibacterial activity of 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide on *K. pneumoniae*, also indicating that this acetamide did not present significant cytotoxic potential in preliminary tests. Thus, it becomes an interesting substance for studies that explore its antimicrobial capacity, including investigating its association with antibacterial drugs. Based on this, this research aimed to investigate the effects of the association of 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide (CFA) with ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem against *K. pneumoniae* strains.

MATERIALS AND METHODS

Substances

The substance 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide (CFA) was obtained and characterized according to processes described by Peixoto et al. (2016) and Cordeiro et al. (2020). Briefly, a mixture of 4-fluoro-3-nitroaniline (3.12 g, 20 mmol) and Et_3N (3.3 mL, 24 mmol) solubilized in 20 mL of $CHCl_3$ contained in a 50 mL flask was cooled to a temperature of 0°C in an ice bath. Then, 2-chloroacetyl chloride (2.71 g, 24 mmol) solubilized in 5 mL of $CHCl_3$ was slowly added to the reaction mixture. At the

end of the addition, the ice bath is removed and the reaction is stirred for 20 hours at room temperature. The reaction mixture was followed by TLC (1:1 hexane/ethyl acetate). At the end of the reaction, the reaction mixture was extracted. The organic phase was washed with water (3 x 25 mL), dried over anhydrous sodium sulfate and then concentrated under reduced pressure to provide a precipitate. The solid was recrystallized from an ethanol/water mixture to give a brown solid in 80% yield.

The antibacterial drugs ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem used were obtained commercially from the Merck /Sigma-Aldrich® laboratory.

Both CFA and conventional antibacterials were solubilized in dimethyl sulfoxide (DMSO) at 5% and Tween-80 at 2%, to obtain emulsions in the concentrations necessary for use in the tests.

Strains

The effect of CFA in association with conventional antibacterials was evaluated on *K. pneumoniae* strains.

The clinical isolates strains of *K. pneumoniae* used in this study belong to the MICOTECA of the Laboratório de Pesquisa em Atividade Antibacteriana e Antifúngica da Universidade Federal da Paraíba, Brasil, which are: KP-26, KP-56, KP-83, KP- 176 and KP-260. In addition, the American Type Culture Collection strain ATCC-700603 was used as a control.

For use in the assays, bacterial suspensions were prepared in 0.9% saline solution, from fresh cultures, and adjusted to the McFarland standard 0.5 scale.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination

To perform the antibacterial drug association test, it is first necessary to determine the Minimum Inhibitory Concentrations (MICs) of the isolated drugs against *K. pneumoniae*. Thus, the necessary information is obtained to later calculate the Fractional Inhibitory Concentration Index (FICI), when the drugs are combined, classifying the effect of the association.

To determine MICs, the analysis was carried out using the broth microdilution technique in a 96-well plate to obtain different concentrations of the substances (CLSI 2015). At the same time, sterility, cell viability and interference controls of vehicles used in the preparation of emulsions of substances (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 ± 2 °C.

The Minimum Bactericidal Concentration (MBC) provides additional data on the classification of the action of a given isolated substance against the strains analyzed, determining whether the effect is bactericidal or bacteriostatic.

Thus, after the MIC reading, the MBC determination test was performed. For this, aliquots were removed from the wells where there was no visible growth (suprainhibitory concentrations) and inoculating in new plates containing only culture broth (CLSI 1999, Silva et al. 2020). All controls were performed. MBC is defined as the lowest concentration capable of causing complete inhibition of bacterial growth after 24 hours at 35 ± 2 °C. Both tests were performed in triplicate.

Drug association test

To check the effect of the association of CFA with the antibacterial drugs: ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem against *K. pneumoniae* strains, the checkerboard association method was performed. Through this method, different concentrations of 2-chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide (8xMIC, 4xMIC, 2xMIC, MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC) were combined with different concentrations of antibacterial drugs (8xMIC, 4xMIC, 2xMIC, MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC).

To perform this test, culture broth was added to the wells of sterile microplates containing 96 wells, with a U-shaped bottom. Then, different concentrations (8x MIC, 4x MIC, 2x MIC, MIC, 1/2 MIC, 1/4 MIC and 1/8 MIC) of CFA and the antibacterial drug were added to the microplate horizontally and vertically, respectively (Wu et al. 2017, Silva et al. 2020).

In this way, an entire microplate has different concentrations of combining acetamide with just one conventional antibacterial. This process was performed for the association with ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem, always analyzing in triplicate. Finally, bacterial inoculums (1×10^7 CFU / mL) were added to each well. The plates were incubated at 35 ± 2°C for 24-48 hours and then bacterial growth was observed.

The effects produced between the combination of CFA and conventional antibiotics was determined by the Fractional Inhibitory Concentration Index (FICI). From this index, is possible to define the type of interaction: synergistic, additive, indifferent or antagonistic.

The FICI was calculated by the sum of fractional inhibitory concentrations (FIC), where FIC_A = (MIC of CFA in combination)/ (MIC of CFA alone) and FIC_B = (MIC of conventional antibiotic in combination)/ (MIC of conventional antibiotic 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, Silva et al. 2020).

RESULTS AND DISCUSSION

The MICs results of each substance isolated against the *K. pneumoniae* strains used in this study are shown in Table I.

The CFA showed a MIC of 512 µg/mL against all strains analyzed in this study, which is a concentration several times higher than the MICs found for conventional antibacterials (Table I).

K. pneumoniae strains were inhibited in the presence of 1 µg/mL ciprofloxacin (CIP) concentration, except for the KP-56 strain, for which the MIC was 8 µg/mL. The cefepime (CEF) showed MIC of 1 µg/mL against four of the six strains analyzed and MIC 16 µg/mL against KP-56. The action of ceftazidime (CAZ) on *K. pneumoniae* resulted in MIC of 1 to 2 µg/mL for most strains tested, despite having demonstrated MIC of 32 µg/mL on ATCC-700603 and KP-56. The carbapenems

Table I. Minimum Inhibitory Concentrations (MICs) of 2-chloro-*N*-(4-fluro-3-nitrophenyl)acetamide (CFA), ciprofloxacin (CIP), cefepime (CEF), ceftazidime (CAZ), meropenem (MER) and imipenem (IMI) on *Klebsiella pneumoniae* strains.

Strains	MIC (µg/mL)					
	CFA	CIP	CEF	CAZ	MER	IMI
ATCC-700603	512	1	1	32	0,5	0,25
KP-26	512	1	0,5	1	0,5	0,25
KP-56	512	8	16	32	1,0	0,5
KP-83	512	1	1	1	0,5	0,25
KP-176	512	1	1	1	0,5	0,25
KP-260	512	1	1	2	1,0	0,5

meropenem (MER) and imipenem (IMI) showed similar MIC results on the strains analyzed, with values ranging between 0.25 and 1.0 µg/mL (Table I).

The high MIC values of ceftazidime (CAZ) over the standard strain ATCC-700603 occur because it is a strain characterized as producing ESBL (Extended-spectrum β-lactamases). These enzymes are capable of hydrolyzing oxy-β-lactams such as ceftazidime, which promotes their resistance to antibiotics. This strain is recommended as a standard to be used in tests for antimicrobial activity, especially for those using *K. pneumoniae*. The MIC values of the antibacterials analyzed in this study on ATCC-700603 are in accordance with those established by CLSI (2018). As all MIC determinations were made under the same conditions for standard strains and isolated clinical strains, this indicates the reliability of the other results.

Among the clinical isolates used in this study, the KP-56 strain shows resistance to cephalosporin and ceftazidime, according to the intervals defined by CLSI (2018). This strain probably expresses one or more types of β-lactamases such as ESBLs or AmpC, which hydrolyze such antibacterials and make them resistant to their action (Ruppé et al. 2015), although further studies are needed to investigate what mechanisms are involved. β-lactamases are the main mechanism of resistance to β-lactams in *Enterobacteriaceae* and *K. pneumoniae* is known to be one of the most important sources of resistance to antibiotics, with high transmissibility and ease of resistance acquisition, this species has shown increased rates of resistance to broad-spectrum cephalosporins worldwide (Ruppé et al. 2015, Navon-Venezia et al. 2017).

In addition, it is possible to observe that KP-56 was also resistant to ciprofloxacin (CLSI 2018), and several mechanisms may be involved in this type of resistance, such as changes in the structure of target enzymes, expression of efflux pumps and reduced permeability of the cell by decreasing the amount of porins expressed in the bacterial membrane (Hooper & Jacoby 2015).

Thus, the KP-56 strain, although it is a strain of community origin, is resistant to more than one class of antibacterial. Community infections due to multidrug-resistant *Enterobacteriaceae* are a global reality and their spread has been considered a reason for alert in several countries (Vasoo et al. 2015, Duin & Paterson 2016).

The Minimum Bactericidal Concentrations of CFA and the antibacterials used in this study are expressed in Table II.

The CFA showed the same MBC values for all strains tested, which coincide with the respective MICs. To classify a substance as a bactericidal agent, the ratio between MBC and MIC must be less than or equal to 4 (CLSI 1999, Pankey & Sabath 2004). Thus, the results indicate that this acetamide is bactericidal from the MIC. This same classification was found for ciprofloxacin, meropenem and imipenem, which have the ratio between MBC and MIC being less than or equal to 4.

Table II. Minimum Bactericidal Concentrations (MBCs) of 2-chloro-*N*-(4-fluro-3-nitrophenyl)acetamide (CFA), ciprofloxacin (CIP), cefepime (CEF), ceftazidime (CAZ), meropenem (MER) and imipenem (IMI) on *Klebsiella pneumoniae* strains.

Strains	MBC (µg/mL)					
	CFA	CIP	CEF	CAZ	MER	IMI
ATCC-700603	512	2	>8	256	2	1
KP-26	512	2	>4	8	2	1
KP-56	512	16	16	256	4	2
KP-83	512	2	>8	8	2	1
KP-176	512	2	>8	8	2	1
KP-260	512	2	>8	16	4	2

On the other hand, the antibacterials ceftazidime and cefepime showed bacteriostatic activities on all the strains analyzed, demonstrating an MBC/MIC ratio greater than 4.

Based on the previous results, it is evident that both CFA and conventional antibacterials showed some level of activity against *K. pneumoniae* when used alone. Starting from this information, the effect of the association of acetamide with each antibacterial drug was investigated, and the results are shown in Table III.

The effect of drug association takes into account the Minimum Inhibitory Concentrations obtained from the isolated substances and the MICs obtained when these substances are put together to act against the tested strains. The combination effect is classified 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, Silva et al. 2020).

The combination of CFA with ciprofloxacin resulted in additivity against all strains, except KP-56, for which there was indifference in the combination of substances. The combination of acetamide and cefepime also resulted in an additive effect on all strains analyzed in this study. The ceftazidime drug did not demonstrate any interaction to potentiate the antibacterial effect when combined with CFA, as the effect on all strains was indifferent. In contrast, the best combinations analyzed in this study were found in the association of acetamide with the carbapenems meropenem and imipenem, with synergism of action for 100% of the strains tested.

Thus, with the exception of ceftazidime, the association of CFA with the antibacterial drugs conventionally used to treat infections caused by *K. pneumoniae* suggests a potentiation of the antibacterial effect of antibiotics, through additivity and synergism.

Additivity is defined when the result of the association is similar to the sum of the effects

Table III. Effects of 2-chloro-*N*-(4-fluro-3-nitrophenyl) acetamide (CFA) association with different antibacterial drugs: ciprofloxacin, cefepime, ceftazidime, meropenem and imipenem against *K. pneumoniae* strains. Synergism: FICI ≤ 0.5, additivity: 0.5 < FICI < 1, indifference: 1 ≤ FICI < 4 and antagonism: FICI ≥ 4. FICI = Fractional Inhibitory Concentration Index.

Strains and drugs	FICI	Effect
ATCC-700603		
Ciprofloxacin	0.75	Additivity
Cefepime	0.56	Additivity
Ceftazidime	2.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism
KP-26		
Ciprofloxacin	0.75	Additivity
Cefepime	0.56	Additivity
Ceftazidime	1.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism
KP-56		
Ciprofloxacin	1.00	Indifference
Cefepime	0.75	Additivity
Ceftazidime	2.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism
KP-83		
Ciprofloxacin	0.75	Additivity
Cefepime	0.56	Additivity
Ceftazidime	1.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism
KP-176		
Ciprofloxacin	0.75	Additivity
Cefepime	0.56	Additivity
Ceftazidime	1.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism
KP-260		
Ciprofloxacin	0.75	Additivity
Cefepime	0.56	Additivity
Ceftazidime	2.06	Indifference
Meropenem	0.50	Synergism
Imipenem	0.50	Synergism

of the substances individually, although lower concentrations of both molecules are necessary to obtain an antimicrobial effect. Synergism, on the other hand, occurs when the association results in a greater effect than that observed for individual substances (EUCAST 2000).

As explained by Díaz-Reval et al. (2008), synergism can occur because each substance is contributing to cell death through different mechanisms of action. There is also the possibility that one of the substances acts as an adjuvant: Whether preventing the degradation of the other drug or even promoting its accumulation and retention inside the bacterial cell. In addition, it can also inhibit repair mechanisms or bacterial tolerance to primary drugs and, because of this, lead to an increase in the final antibacterial effect (Cottarel & Wierzbowski 2007).

The combination of therapies has proven to be a highly effective alternative for the treatment of infections by resistant bacteria (Palaniappan & Holley 2010, Toledo et al. 2015, Ayaz et al. 2019). Evidence suggests that the combination of therapeutic regimes may even reduce the emergence of resistant microorganisms (Drusano et al. 2010, Ayaz et al. 2019).

Another interesting advantage of the drug association is the possibility of using lower concentrations of each substance individually and, in spite of that, obtaining greater effects than using the substances alone (Ayaz et al. 2019, Sun et al. 2016). Due to this, it would be possible to also reduce the side effects resulting from the administration of these medications. The results indicate that CFA in combination with conventional antibacterials increased the antimicrobial effect, in an additive or synergistic way, suggesting that there was a reduction in the viability of the strains using a lower concentration of these substances.

It is difficult and expensive to screen for or develop new drugs, so it is important to find new methods to reduce the development of antibiotic resistance by pathogenic organisms, especially by *K. pneumoniae*, which is associated with high levels of resistance and wide epidemiological distribution (Cottarel & Wierzbowski 2007)

An emerging option to combat such pathogens is the combination therapy. The strategy of combining substances against bacteria has been extensively studied in recent years and shows promising results, mainly in the association of products of natural origin with conventional antibiotics or with another natural substance (Palaniappan & Holley 2010, Ayaz et al. 2019).

The CFA is a synthetic substance, about which there are still no reports in the literature of its association with other drugs. Although a weak antibacterial activity has been observed when used alone, this molecule has potential to be used in combination with other antibacterials such as ciprofloxacin, cefepime, meropenem and imipenem.

As demonstrated by Cordeiro et al. (2020), this acetamide presents favorable cytotoxicity and mutagenicity results for future *in vivo* studies to be carried out. In addition, *in silico* analysis suggest a pharmacokinetic profile with good parameters for oral use. The CFA can also serve as a basis for future structural changes that aim to improve its biological activity. Such factors make it interesting that more studies will be performed, in order to investigate the feasibility of the association of this substance with conventional antibacterials in clinical practice.

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REFERENCES

AYAZ M, ULLAH F, SADIQ A, ULLAH F, OVAIS M, AHMED J & DEVKOTA H. 2019. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. Chem Biol Interact 308: 294-303.

BELOUSOFF MJ, VENUGOPAL H, WRIGHT A, SEONER S, STUART I, STUBENRAUCH C, BAMERT RS, LUPTON DW & LITHGOW T. 2019. CryoEM-guided development of antibiotics for drug-resistant bacteria. Chem Med Chem 14: 527-531.

CLSI – CLINICAL AND LABORATORY STANDARDS INSTITUTE. 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26-A.

CLSI – CLINICAL LABORATORY STANDARDS INSTITUTE. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A10.

CLSI – CLINICAL LABORATORY STANDARDS INSTITUTE. 2018. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed, CLSI supplement M100.

CORDEIRO L ET AL. 2020. Potential of 2-Chloro-*N*-(4-fluoro-3-nitrophenyl)acetamide Against *Klebsiella pneumonia* and In Vitro Toxicity Analysis. Molecules 25: 3959.

COTTAREL G & WIERZBOWSKI J. 2007. Combination drugs, an emerging option for antibacterial therapy. Trends Biotechnol 25: 547-555.

DADGOSTAR P. 2019. Antimicrobial resistance: implications and costs. Infect Drug Resist 12: 3903.

DÍAZ-REVAL MI, GALVÁN-OROZCO R, LÓPEZ-MUÑOZ FJ & CARRILLO-MUNGUÍA N. 2008. Sinergismo de la cafeína sobre los efectos antinociceptivos del metamizol. Cir y Cir 76: 241-246.

DRUSANO GL, SGAMBATI N & EICHAS A. 2010. The combination of rifampin plus moxifloxacin is synergistic for suppression of resistance but antagonistic for cell kill of *Mycobacterium tuberculosis* as determined in a hollow-fiber infection model. MBio 1: 1-8.

DUIN D & PATERSON D. 2016. Multidrug Resistant Bacteria in the Community: Trends and Lessons Learned. Infect Dis Clin North America 30: 377-390.

EFFAH CY, SUN T, LIU S & WU Y. 2020. *Klebsiella pneumoniae*: an increasing threat to public health. Ann Clin Microbiol Antimicrob 19: 1-9.

EUCAST – EUROPEAN COMMITTEE FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING. 2000. Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Clin Microbiol Infect 6: 503-508.

FULORIA NK, SINGH V, YAR MS & ALI M. 2009. Synthesis, characterization and antimicrobial evaluation of novel imines and thiazolidinones. Acta Pol Pharm 66: 141-146.

HOOPER DC & JACOBY GA. 2015. Mechanisms of drug resistance: quinolone resistance. Ann N Y Acad Sci 1354: 12-31.

KAPLANCIKLI ZA, YURTTAŞ L, TURAN-ZITOUNI G, ÖZDEMIR A, ÖZIC R & ULUSOYLAR-YILDIRIM Ş. 2012. Synthesis, antimicrobial activity and cytotoxicity of some new carbazole derivatives. J Enzyme Inhib Med Chem 27: 868-874.

KATKE SA, AMRUTKAR SV, BHOR RJ & KHAIRNAR MV. 2011. Synthesis of biologically active 2-chloro-N-alkyl/aryl acetamide derivatives. Int J Pharm Sci Res 2: 148-156.

MURTAZA S, ALTAF AA, HAMAYUN M, IFTIKHAR K, TAHIR MN, TARIQ J & FAIZ K. 2019. Synthesis, antibacterial activity and docking studies of chloroacetamide derivatives. Eur J Chem 10: 358-366.

NAVON-VENEZIA S, KONDRATYEVA K & CARATTOLI A. 2017. *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 41: 252-275.

OLIVEIRA DMP ET AL. 2020. Antimicrobial Resistance in ESKAPE Pathogens. Clin Microbiol Rev 33: e00181-19.

PALANIAPPAN K & HOLLEY RA. 2010. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. Int J Food Microbiol 140: 164-168.

PANKEY GA & SABATH LD. 2004. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect Dis 38: 864-870.

PATEL RV, KUMARI P, RAJANI DP & CHIKHALIA KH. 2013. Synthesis of coumarin-based 1, 3, 4-oxadiazol-2ylthio-N-phenyl/benzothiazolyl acetamides as antimicrobial and antituberculosis agents. Med Chem Res 22: 195-210.

PATEL RV, PATEL PK, KUMARI P, RAJANI DP & CHIKHALIA KH. 2012. Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-N-phenyl (benzothiazolyl) acetamides as antibacterial, antifungal and antituberculosis agents. Eur J Med Chem 53: 41-51.

PEIXOTO IN, SOUZA HDS, LIRA BF, SILVA DF, LIMA EO, BARBOSA-FILHO JM & ATHAYDE-FILHO PF. 2016. Synthesis and Antifungal Activity against Candida Strains of Mesoionic System Derived from1,3-Thyazolium-5-thiolate. J Braz Chem Soc 27: 1807-1813.

PENDLETON JN, GORMAN SP & GILMORE BF. 2013. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 11: 297-308.

PRADIDPHOL N, KONGKATHIP N, SITTIKUL P, BOONYALAI N & KONGKATHIP B. 2012. First synthesis and anticancer activity of novel naphthoquinone amides. Eur J Med Chem 49: 253-270.

RUPPÉ E, WOERTHER P & BARBIER F. 2015. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Ann Intensive Care 5: 21.

SILVA D ET AL. 2020. (R)-(+)-β-Citronellol and (S)-(−)-β-Citronellol in Combination with Amphotericin B against *Candida* spp. Int J Mol Sci 21: 1785.

SUN W, SANDERSON PE & ZHENG W. 2016. Drug combination therapy increases successful drug repositioning. Drug Discov Today 21: 1189-1195.

TAMMA PD, COSGROVE SE & MARAGAKIS LL. 2012. Combination therapy for treatment of infections with gram-negative bacteria. Clin Microbial Rev 25: 450-470.

TOLEDO PV, ARANHA AA, AREND LN, RIBEIRO V, ZAVASCKI AP & TUON FF. 2015. Activity of antimicrobial combinations against KPC-2-producing *Klebsiella pneumoniae* in a ratmodel and time-kill assay. Antimicrob Agents Chemother 59: 4301-4304.

VASOO S, BARRETO JN & TOSH PK. 2015. Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician. Mayo Clin Proc 90: 395-403.

WHO – WORLD HEALTH ORGANIZATION. 2017. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Available online: http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/. (Accessed on 4 June 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 Des Dev Ther 11: 939-946.

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LVC, HDSS, APS, FPAJ, PTRF, RFO and EOL idealized the study, performed the experiments and analyzed the results. LVC, HDSS, PFAF, AAOF and EOL wrote and revised the manuscript. PFAF, AAOF and EOL were responsible for acquiring resources, supervising the study and reviewing the work.