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Antagonistic, synergistic, and additive antibacterial interaction between ciprofloxacin and amoxicillin against *Staphylococcus aureus*

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Abstract

The aim of this in vitro study was to evaluate the interaction between ciprofloxacin and amoxicillin against beta-lactamase-producing *Staphylococcus aureus*. Concentration-dependent curves for each individual drug were carried out to obtain the mean inhibitory concentration in the agar well diffusion assay. Then, different ratios of the ciprofloxacin–amoxicillin combination (0.5:0.5, 0.8:0.2, 0.2:0.8, 0.9:0.1, 0.1:0.9, 0.95:0.05, and 0.05:0.95) were assessed. Data were analyzed using the isobolographic analysis and interaction index. The isobolographic evaluation shows that the 0.9:0.1 and 0.95:0.05 ratios of the ciprofloxacin–amoxicillin combination produced a synergistic antimicrobial interaction, the 0.8:0.2, 0.2:0.8, 0.1:0.9, and 0.05:0.95 proportions showed an additive antibacterial effect, and the 0.5:0.5 proportion induced antagonistic antimicrobial effects. The interaction index showed similar outcomes to the isobolographic analysis. In conclusion, the data of this study mainly show antimicrobial additive results of the ciprofloxacin–amoxicillin combination against beta-lactamase-producing *S. aureus*.

KEYWORDS
amoxicillin, antimicrobial interaction, ciprofloxacin, *Staphylococcus aureus*

Abbreviations: S. aureus, Staphylococcus aureus; USA, United States of America; WHO, World Health Organization; DNA, Deoxyribonucleic acid; Cat., Catalogue; ATCC, American Type Culture Collection; MIC, Mean inhibitory concentration.

Eduardo Gómez-Sánchez and Mario Alberto Isiordia-Espinoza contributed equally, and both could be considered the first authors.

1 | INTRODUCTION

Staphylococcus aureus produces several types of infections of different severity. Annually, approximately 20 000 patients die from *S. aureus* bacteremia in the United States [1]. Other *S. aureus* infections are not life threatening; however, they represent a considerable expense for health services such as skin infections, wounds [2], and bone and joint infections [3]. According to David et al, *S. aureus* is the most frequent antimicrobial drug-resistant human pathogen [4].

The World Health Organization (WHO) has urged research for the generation of new antimicrobials due to the large and growing number of antibiotic-resistant microorganisms [5]. Within this list of microorganisms, *S. aureus*—resistant to methicillin [6] and vancomycin [7]—appear as a high-priority microorganism (Level 2) [5]. *S. aureus* has a high antimicrobial drug resistance and a high presence in hospital infections [8].

In its statement, the WHO recommended increasing research for the development of new drugs that can be used effectively to treat infections by multi-resistant microorganisms [5]. Antibiotic combinations to treat clinical infections in humans are "common" [4]. However, there is no scientific evidence that supports the clinical efficacy use of antimicrobial drug combinations [4]. For this reason, the aim of this in vitro study was to evaluate the interaction between ciprofloxacin, a fluoroquinolone with a mechanism of action that inhibits the enzyme bacterial DNA topoisomerases type 2, DNA gyrase, and topoisomerase IV [8], and amoxicillin, a synthetic penicillin-binding to penicillin-binding protein 1A [9], against *S. aureus* using isobolographic analysis and interaction index.

2 | MATERIALS AND METHODS

2.1 | Type of study

This was an in vitro study performed in the Instituto de Investigación en Ciencias Médicas of the Departamento de Clínicas of the División de Ciencias Biomédicas of the Centro Universitario de los Altos of the Universidad de Guadalajara. The protocol was submitted and approved by the Colegio Departamental de Clínicas (Acta junio 21, 2021–2022). The experiments were carried out according to the Handbook of Microbiology Laboratory Practices [10, 11].

2.2 | Drugs and bacteriological strain

Ciprofloxacin (Cat. 17850) and amoxicillin (Cat. A8523) were obtained from Sigma-Aldrich (St. Louis, MO). Nutrient agar (Bioxon) was purchased from Becton Dickinson México (BD). *S. aureus* subsp. *aureus* derived from ATCC 11632—a beta-lactamase-producing bacteria—was acquired from Microbiologics, Inc.

2.3 | Agar well diffusion test

The samples containing the bacteria were thawed, cultured in nutrient agar, and incubated for 24 h at 37°C. The next day, the experiments were carried out. Biomass was taken with a microbiological loop from the Petri dish cultured and placed in 10 ml of saline to obtain turbidity of 0.5 on the McFarland scale.

S. aureus cultures were made on a table with a stainless steel surface, which was disinfected before starting the experiments with 70° ethyl alcohol, and subsequently, three burners were placed to obtain an aseptic area.

The agar well diffusion assays were performed on nutrient agar. Briefly, a standard sterile cotton swab was massively cultured on the entire surface of the nutrient agar in each Petri box. Subsequently, with a 5-mm punch, which was sterilized directly over a burner flame, five wells were made. A central well in which the saline solution was placed and in the peripheral wells ciprofloxacin, amoxicillin, or the drug combination was placed. The concentrations were placed in the wells following the hands of the clock. The highest concentration of each individual drug, or the combination, was placed at 12 h, and then subsequent concentrations were placed at 3, 6, and 9 h [12, 13].

2.4 | Experimental design

Ciprofloxacin 0.05, 0.1, 0.3, and 0.5 mg/ml and amoxicillin 1, 3, 5, and 10 mg/ml were used to obtain a concentration-dependent curve for each individual drug. The saline solution was used as a vehicle. Antibiograms were performed by placing 20 μl of the vehicle in the central well with a micropipette, and similarly, the four concentrations of the individual drugs were placed in the wells as explained above. The inhibition halos were measured with a Vernier digital caliper at 24 h. Using these concentration-dependent curves, the mean inhibitory concentration (MIC) of each antimicrobial was determined. Then, different ratios of the ciprofloxacin–amoxicillin combination—0.5:0.5, 0.8:0.2, 0.2:0.8, 0.9:0.1, 0.1:0.9, 0.95:0.05, and 0.05:0.95—were calculated as explained previously [14], and each of these proportions was tested by evaluating four subsequent concentrations (Table 1).

The inactivation of the liquid samples was done by sterilization, and that of the bacterial cultures in Petri dishes was done by exposure to ultraviolet light.

TABLE 1 Concentrations (mg/ml) of each proportion of the ciprofloxacin–amoxicillin combination

Proportion	Ciprofloxacin	Amoxicillin
0.5:0.5 (1:1)		
MIC/1	0.03	0.2
MIC/2	0.015	0.1
MIC/4	0.008	0.05
MIC/8	0.004	0.025
0.8:0.2 (5:1)		
MIC/1	0.046	0.07
MIC/2	0.023	0.035
MIC/4	0.011	0.018
MIC/8	0.006	0.009
0.2:0.8 (1:5)		
MIC/1	0.012	0.3
MIC/2	0.006	0.15
MIC/4	0.003	0.075
MIC/8	0.001	0.0375
0.9:0.1 (10:1)		
MIC/1	0.052	0.032
MIC/2	0.026	0.016
MIC/4	0.013	0.008
MIC/8	0.006	0.004
0.1:0.9 (1:10)		
MIC/1	0.006	0.31
MIC/2	0.003	0.16
MIC/4	0.001	0.08
MIC/8	0.0007	0.04
0.95:0.05 (20:1)		
MIC/1	0.06	0.020
MIC/2	0.03	0.010
MIC/4	0.015	0.005
MIC/8	0.007	0.002
0.05:0.95 (1:20)		
MIC/1	0.003	0.33
MIC/2	0.0015	0.17
MIC/4	0.001	0.08
MIC/8	0.0005	0.04

2.5 | Data analysis

The % antibacterial activity was calculated in comparison with ciprofloxacin 10 mg/ml—concentration tested with the highest antimicrobial effect. The interaction between ciprofloxacin and amoxicillin was determined using isobolographic analysis [15–17] and the interaction index [18] according to the Tallarida statistical method. Isobolographic analysis takes on that the drug mixture is done with equipotent doses of each individual drug [14–17]. Therefore, using the dose–response curves of each individual agent, the dose producing 50% of the effect (MIC value) can be obtained [15–17]. Theoretical and experimental MIC were compared with Student's *t*-test. A value of $P < 0.05$ was considered a statistical difference.

3 | RESULTS

3.1 | Antibacterial effect

Ciprofloxacin and amoxicillin induced a concentration-dependent antibacterial activity against *S. aureus*. The antibacterial effects of ciprofloxacin were between 54% and 82%, while amoxicillin ranged from 61% to 76%. Moreover, the ciprofloxacin–amoxicillin mixture produced a concentration-dependent antimicrobial effect versus *S. aureus*. The maximum antimicrobial effects of the ciprofloxacin–amoxicillin combination in ratios 0.5:0.5, 0.8:0.2, 0.2:0.8, 0.9:0.1, 0.1:0.9, 0.95:0.05, and 0.05:0.95 were 54%, 65%, 56%, 77%, 71%, 87%, and 59%, respectively (Figure 1).

3.2 | Isobolographic analysis and interaction index

The isobolographic assessment of the ratios of the ciprofloxacin–amoxicillin combination against *S. aureus* showed antagonistic (Figure 2a), additive (Figure 2b,c,e,g), and synergistic (Figure 2d,f) antibacterial interactions. The interaction index (Table 2) showed similar outcomes to the isobolograms. Moreover, the MIC of the 0.9:0.1 and 0.95:0.05 ratios of the combination showed a statistical difference between the theoretical value and the experimental value (Table 2).

4 | DISCUSSION

This in vitro study evaluated the antibacterial interaction of the ciprofloxacin–amoxicillin combination versus *S. aureus* using the agar well diffusion technique, isobolographic statistical method, and the interaction index. The agar well diffusion method is widely used and accepted to determine the antimicrobial effect of drugs and natural extracts [12, 13, 19–22]. *S. aureus* showed high susceptibility to ciprofloxacin. Tested small concentrations of this fluoroquinolone induced high antibacterial activity. However, high concentrations (5 and 10 mg/ml) of amoxicillin showed limited antibacterial action against this same bacterium when compared to ciprofloxacin. The limited antibacterial activity of amoxicillin could be explained by the production of beta-lactamase by *S. aureus*.

This basic study shows the importance of evaluating different proportions of a drug–drug combination. The proportions of the ciprofloxacin–amoxicillin

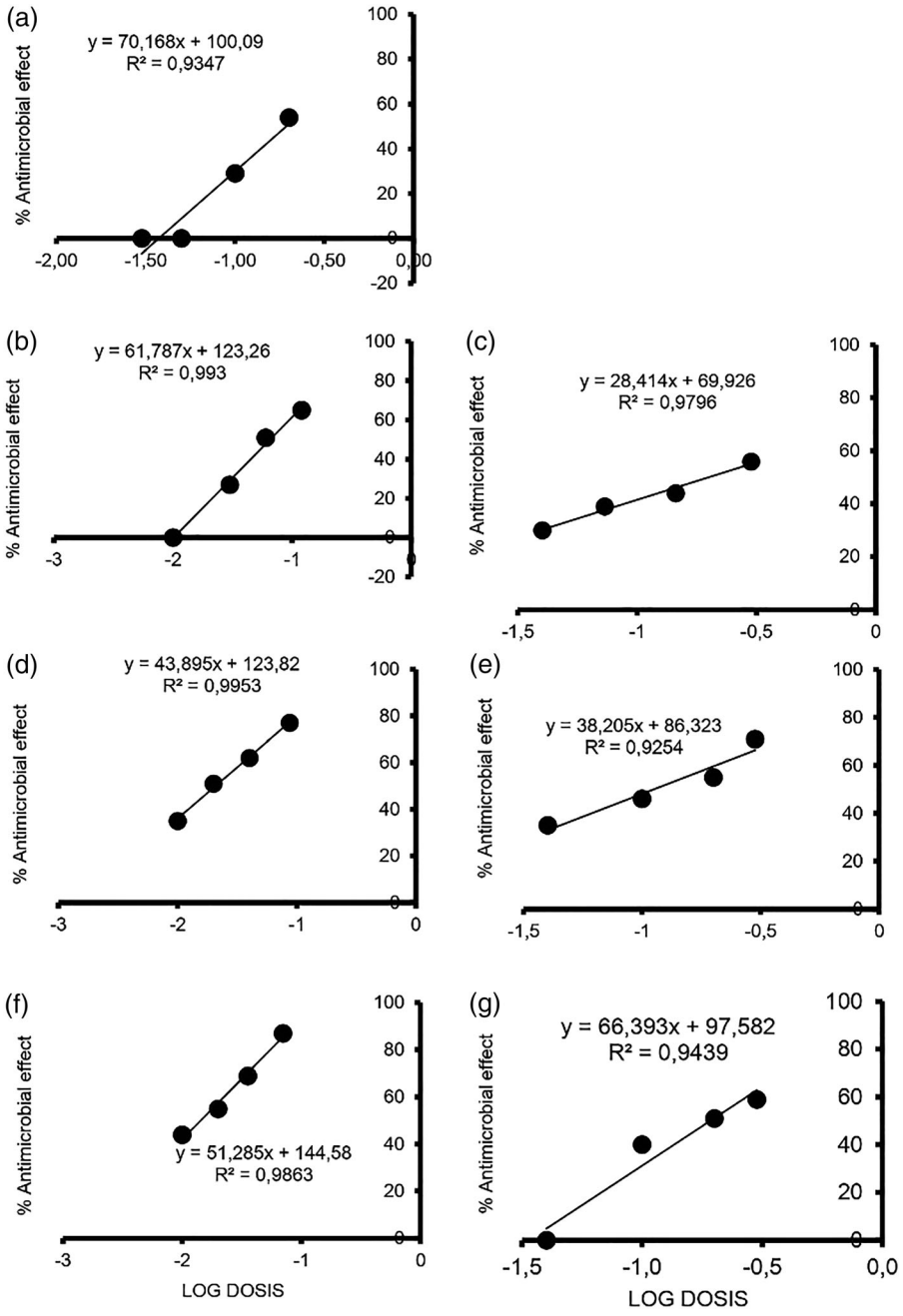

FIGURE 1 Concentration-dependent curves of the antibacterial activity of the ciprofloxacin-amoxicillin combination against *Staphylococcus aureus*. Antibacterial effect of the ratios 0.5:0.5 (a), 0.8:0.2 (b), 0.2:0.8 (c), 0.9:0.1 (d), 0.1:0.9 (e), 0.95:0.05 (f), and 0.05:0.95 (g) was determined. Data are means ± SEM.

combination to determine the antimicrobial effect against *S. aureus* showed different types of interaction as described in Section 3 [23]. It is important to note that the drug ratios with the high amoxicillin content had the poorest antimicrobial effects. In this sense, it is possible to explain these findings by the use of a beta-lactamase-producing *S. aureus* in the experiments of this study.

The synergistic antimicrobial effect observed with the 0.9:0.1 and 0.95:0.05 ratios could be due to the different mechanisms of action of amoxicillin and ciprofloxacin. Amoxicillin, by lethally affecting the bacterial cell wall (inhibition of the structural organization of peptidoglycans by inhibition of the penicillin-binding enzyme) [9, 24], could have facilitated the entry of ciprofloxacin into the bacteria so that it exerts its antimicrobial effect (inhibition of both Type II topoisomerase [DNA gyrase] and Type IV topoisomerase) [25], thus enhancing the antimicrobial effect [26].

Vancomycin is the standard antimicrobial treatment for methicillin-resistant *S. aureus* infections. However, this antimicrobial monotherapy has many drawbacks, which have been described in detail in previous scientific reports. Therefore, the combination of antimicrobials against *S. aureus* is an interesting alternative [27]. Numerous in vitro studies have been carried out to

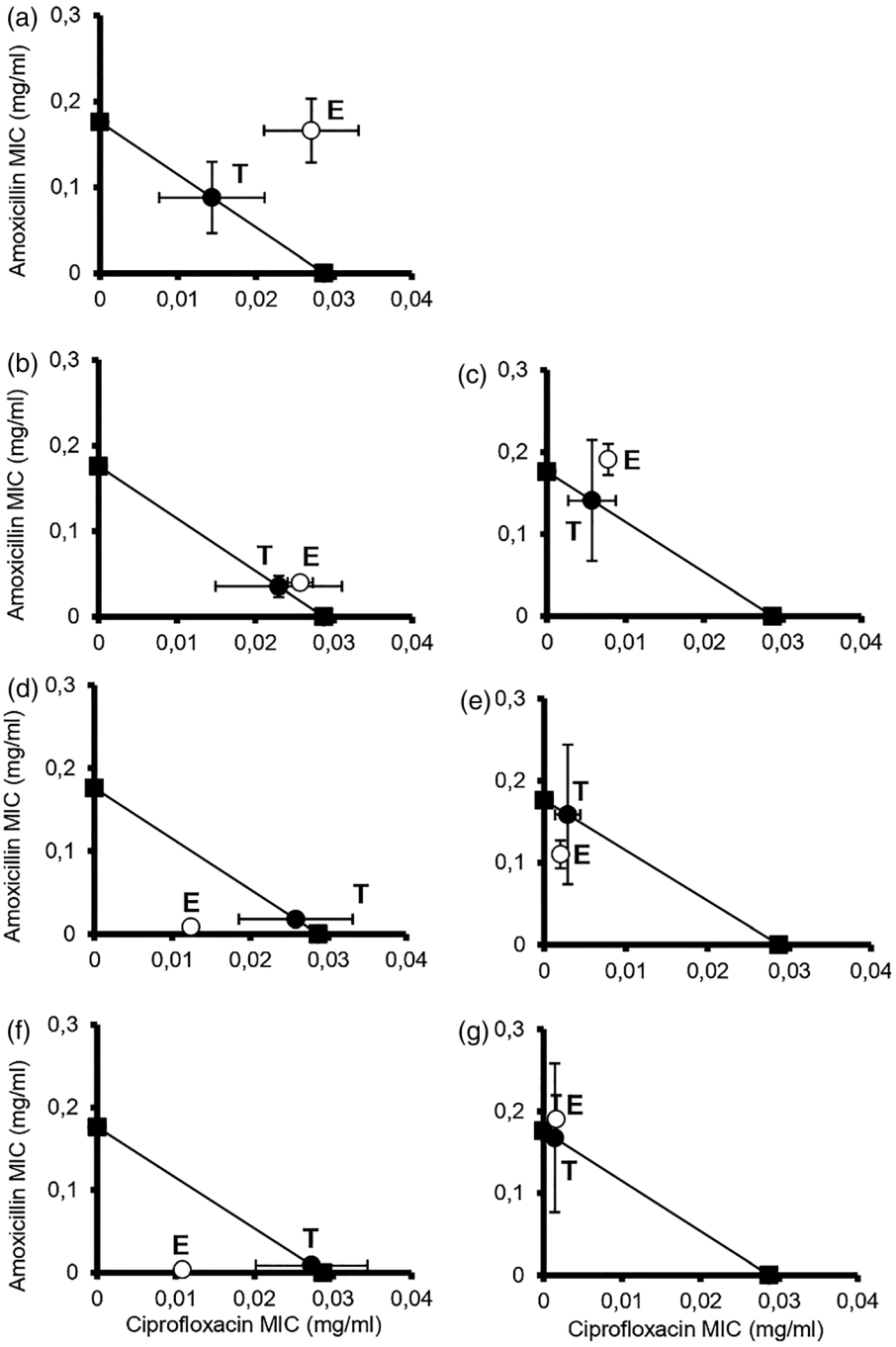

FIGURE 2 Isobolograms showing the type of antibacterial interaction of the proportions 0.5:0.5 (a), 0.8:0.2 (b), 0.2:0.8 (c), 0.9:0.1 (d), 0.1:0.9 (e), 0.95:0.05 (f), and 0.05:0.95 (g) of the ciprofloxacin–amoxicillin combination versus *Staphylococcus aureus*. T = Theoretical value. E = Experimental value. Data are means ± SEM.

TABLE 2 Theoretical and experimental MIC values and interaction index

Ciprofloxacin–amoxicillin combination	Theoretical MIC values (mg/ml)	Experimental MIC values (mg/ml)	Interaction index
Cipro0.5:0.5Amoxi	0.10 ± 0.05	0.19 ± 0.10	1.88
Cipro0.8:0.2Amoxi	0.06 ± 0.02	0.07 ± 0.03	1.12
Cipro0.2:0.8Amoxi	0.15 ± 0.08	0.20 ± 0.04	1.357
Cipro0.9:0.1Amoxi	0.04 ± 0.01	0.02 ± 0.02*	0.479
Cipro0.1:0.9Amoxi	0.16 ± 0.09	0.11 ± 0.07	0.694
Cipro0.95:0.05Amoxi	0.04 ± 0.01	0.01 ± 0.03*	0.397
Cipro0.05:0.95Amoxi	0.17 ± 0.09	0.19 ± 0.07	1.138

Note: Data are means ± SEM.
*Statistical difference according to Student's *t*-test ($P < 0.05$).

evaluate combinations of antibiotics against *S. aureus*, with a special interest in combinations of vancomycin and beta-lactam antimicrobials [28–44] or daptomycin [45–52], finding results with a trend towards antimicrobial synergy. Our results show that only the 0.9:0.1 and 0.95:0.05 ratios of the ciprofloxacin-amoxicillin combination show an antimicrobial potentiation against *S. aureus*. However, most of the evaluated proportions of the ciprofloxacin–amoxicillin combination presented an additive antibacterial effect against beta-lactamase-producing *S. aureus*.

It is possible that the production of beta-lactamases by *S. aureus* explains the antagonistic antimicrobial activity of this drug combination. However, other bacterial resistance mechanisms known cannot be ruled out. Multidrug-resistant bacterial infections are a global public health problem with high morbidity and mortality [53]. Bacteria are resistant or acquire resistance by preventing drug access to targets, changes in the structure and protection of antimicrobial drug targets, and direct modification or inactivation of these antimicrobial agents [54]. Therefore, there is a need to find new molecules with an antimicrobial effect, evaluate combinations of currently available antibiotics to detect possible synergistic effects, and use nanotechnology to improve the two previous options [55].

In conclusion, the data of this study mainly show antimicrobial additive results of the ciprofloxacin–amoxicillin combination against beta-lactamase-producing *S. aureus*. Moreover, the 0.9:0.1 and 0.95:0.05 ratios of the combination produced a synergistic antimicrobial effect according to the isobolographic analysis and interaction index versus beta-lactamase-producing *S. aureus*. It would be interesting to evaluate the antimicrobial activity of this combination of antimicrobials in other experimental conditions, in vitro and in vivo.

CONFLICT OF INTEREST

Authors do not have conflict of interest.

AUTHOR CONTRIBUTIONS

Mario Alberto Isiordia-Espinoza, Flavio Terán-Rosales, and Eduardo Gómez-Sánchez conceived and designed the study. Lorenzo Franco-de la Torre and Ronell Bologna-Molina analyzed the results, including the abstract and body of the article, figures, and tables. Adriana Hernández-Gómez and Nicolás Addiel Serafín-Higuera helped with the statistical analysis and interpretation of the results. Angel Josabad Alonso-Castro, Ronell Eduardo Bologna-Molina, and Mario Alberto Isiordia-Espinoza helped with the writing of the original draft. Mario Alberto Isiordia-Espinoza, Flavio Terán-Rosales, Eduardo Gómez-Sánchez, and Adriana Hernández-Gómez helped with the review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the authors.

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REFERENCES

1. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*. 2021;12(1):547-569. doi:10.1080/21505594.2021.1878688
2. McCaig LF, McDonald LC, Mandal S, et al. *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. *Emerg Infect Dis*. 2006;12(11):1715-1723. doi:10.3201/eid1211.060190
3. Bouiller K, Hocquet D, Sauget M, Bertrand X, Chirouze C. Epidemiology and risk factors of *Staphylococcus aureus* CC398 bone and joint infections. *BMC Infect Dis*. 2020;20(384):1-7. doi:10.1186/s12879-020-05098-0
4. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
5. Lee AS, de Lencastre H, Garau J, et al. Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers*. 2018;4(1):18033. doi:10.1038/nrdp.2018.33
6. McGuinness WA, Malachowa N, De Leo FR. Vancomycin resistance in *Staphylococcus aureus*. *Yale J Biol Med*. 2017;90(2):269-281.
7. Ahmad-Mansour N, Loubet P, Pouget C, et al. *Staphylococcus aureus* toxins: an update on their pathogenic properties and potential treatments. *Toxins (Basel)*. 2021;13(10):677. doi:10.3390/toxins13100677
8. Suaifan GARY, Mohammed AAM, Alkhawaja BA. Fluoroquinolones' biological activities against laboratory microbes and cancer cell lines. *Molecules*. 2022;27(5):1658. doi:10.3390/molecules27051658
9. Huttner A, Bielicki J, Clements MN, et al. Oral amoxicillin and amoxicillin-clavulanic acid: properties, indications and usage. *Clin Microbiol Infect*. 2020;26(7):871-879. doi:10.1016/j.cmi.2019.11.028
10. Cerra H., Fernández M.C., Horak C., et al. Manual de Microbiología aplicada a las Industrias Farmacéutica, Cosmética y de Productos Médicos. Asociación Argentina de Microbiología, pp 343-363.
11. Aquiahualt-Ramos MA, Pérez-Chabela ML Manual de prácticas de laboratorio de microbiología general. UAM Iztapalapa 2004.
12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. *J Pharm Anal*. 2016;6(2):71-79. doi:10.1016/j.jpha.2015.11.005
13. Córdova-Guerrero I, Aragon-Martinez OH, Díaz-Rubio L, et al. Antibacterial and antifungal activity of Salvia apiana against clinically important microorganisms. *Rev Argent Microbiol*. 2016;48(3):217-221. doi:10.1016/j.ram.2016.05.007
14. Franco de la-Torre L, Alonso-Castro ÁJ, Zapata-Morales JR, et al. Antinociception and less gastric injury with the dexketoprofen-tapentadol combination in mice. *Fundam Clin Pharmacol*. 2021;35:371-378.
15. Tallarida RJ. *Drug Synergism and Dose-Effect Data Analysis*. Boca Raton, FL: Chapman & Hall/CRC Press; 2000:26-131. doi:10.1201/9781420036107.
16. Tallarida RJ. Combination analysis. *Adv Exp Med Biol*. 2010;678:133-137. doi:10.1007/978-1-4419-6306-2_17
17. Tallarida RJ. Drug combinations: tests and analysis with isoboles. *Cur Protoc Pharmacol*. 2016;72:9.19.1-9.19.19.

18. Tallarida RJ. The interaction index: a measure of drug synergism. *Pain*. 2002;98(1):163-168. doi:10.1016/S0304-3959(02)00041-6
19. Athanassiadis B, Abbott PV, George N, et al. An in vitro study of the antimicrobial activity of some endodontic medicaments and their bases using an agar well diffusion assay. *Aust Dent J*. 2009;54(2):141-146. doi:10.1111/j.1834-7819.2009.01107.x
20. Klink MJ, Laloo N, Leudjo TA, et al. Synthesis, characterization and antimicrobial activity of zinc oxide nanoparticles against selected waterborne bacterial and yeast pathogens. *Molecules*. 2022;27(11):3532. doi:10.3390/molecules27113532
21. Abass AA, Al-Magsoosi MJN, Kadhim WA, et al. Antimicrobial effect of red roselle (*Hibiscus sabdariffa*) against different types of oral bacteria. *J Med Life*. 2022;15(1):89-97. doi:10.25122/jml-2021-0184
22. Mehdipour A, Ehsani A, Samadi N, Ehsani M, Sharifinejad N. The antimicrobial and antibiofilm effects of three herbal extracts on *Streptococcus mutans* compared with chlorhexidine 0.2% (*in vitro* study). *J Med Life*. 2022;15(4):526-532. doi:10.25122/jml-2021-0189
23. van Vuuren SF, Suliman S, Viljoen AM. The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Lett Appl Microbiol*. 2009;48(4):440-446. doi:10.1111/j.1472-765X.2008.02548.x
24. Cho H, Uehara T, Bernhardt TG. Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. *Cell*. 2014;159(6):1300-1311. doi:10.1016/j.cell.2014.11.017
25. Zang W, Li D, Gao L, Gao S, Hao P, Bian H. The antibacterial potential of ciprofloxacin hybrids against *Staphylococcus aureus*. *Curr Top Med Chem*. 2022;22(12):1020-1034. doi:10.2174/1568026622666220317162132
26. Flores-Ramos JM, Ochoa-Zaragoza MG, López-Rodriguez LL, et al. Drug interactions related to the administration of beta-lactam antibiotics. *Rev ADM*. 2016;73:227-234.
27. Davis JS, Van Hal S, Tong SY. Combination antibiotic treatment of serious methicillin-resistant *Staphylococcus aureus* infections. *Semin Respir Crit Care Med*. 2015;36(1):3-16. doi:10.1055/s-0034-1396906
28. Lozniewski A, Lion C, Mory F, Weber M. In vitro synergy between cefepime and vancomycin against methicillin-susceptible and -resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J Antimicrob Chemother*. 2001;47(1):83-86. doi:10.1093/jac/47.1.83
29. Domaracki BE, Evans AM, Venezia RA. Vancomycin and oxacillin synergy for methicillin-resistant staphylococci. *Antimicrob Agents Chemother*. 2000;44(5):1394-1396. doi:10.1128/AAC.44.5.1394-1396.2000
30. Drago L, De Vecchi E, Nicola L, et al. In vitro evaluation of antibiotics' combinations for empirical therapy of suspected methicillin resistant *Staphylococcus aureus* severe respiratory infections. *BMC Infect Dis*. 2007;7(1):111. doi:10.1186/1471-2334-7-111
31. Kobayashi Y. Study of the synergism between carbapenems and vancomycin or teicoplanin against MRSA, focusing on S-4661, a carbapenem newly developed in Japan. *J Infect Chemother*. 2005;11(5):259-261. doi:10.1007/s10156-005-0402-2
32. Ribes S, Pachón-Ibáñez ME, Domínguez MA, et al. In vitro and in vivo activities of linezolid alone and combined with vancomycin and imipenem against *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *Eur J Clin Microbiol Infect Dis*. 2010;29(11):1361-1367. doi:10.1007/s10096-010-1007-y
33. Silva LV, Araújo MT, Santos KR, et al. Evaluation of the synergistic potential of vancomycin combined with other antimicrobial agents against methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp strains. *Mem Inst Oswaldo Cruz*. 2011;106(1):44-50. doi:10.1590/S0074-02762011000100007
34. Hagihara M, Wiskirchen DE, Kuti JL, Nicolau DP. In vitro pharmacodynamics of vancomycin and cefazolin alone and in combination against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2012;56(1):202-207. doi:10.1128/AAC.05473-11
35. Leonard SN. Synergy between vancomycin and nafcillin against *Staphylococcus aureus* in an in vitro pharmacokinetic/pharmacodynamic model. *PLoS One*. 2012;7(7):e42103. doi:10.1371/journal.pone.0042103
36. Dilworth TJ, Sliwinski J, Ryan K, Dodd M, Mercier RC. Evaluation of vancomycin in combination with piperacillin-tazobactam or oxacillin against clinical methicillin-resistant *Staphylococcus aureus* isolates and vancomycin-intermediate *S. aureus* isolates in vitro. *Antimicrob Agents Chemother*. 2014;58(2):1028-1033. doi:10.1128/AAC.01888-13
37. Werth BJ, Steed ME, Kaatz GW, Rybak MJ. Evaluation of ceftaroline activity against heteroresistant vancomycin-intermediate *Staphylococcus aureus* and vancomycin-intermediate methicillin-resistant *S. aureus* strains in an in vitro pharmacokinetic/pharmacodynamic model: exploring the "see-saw effect". *Antimicrob Agents Chemother*. 2013;57(6):2664-2668. doi:10.1128/AAC.02308-12
38. Mohammadi-Berenjestanaki H, Khori V, Shirzad-Aski H, Ghaemi EA. *In vitro* synergistic effect of vancomycin and some antibacterial agents against clinical methicillin-resistant and sensitive *Staphylococcus aureus* isolates. *Microb Drug Resist*. 2020;26(3):218-226. doi:10.1089/mdr.2019.0003
39. Liao CH, Chen SY, Huang YT, Tsai HY, Hsueh PR. Comparison of in vitro synergy of various β-lactams with vancomycin against methicillin-resistant *Staphylococcus aureus*. *J Infect*. 2017;74(3):324-325. doi:10.1016/j.jinf.2016.12.013
40. García AB, Candel FJ, López L, Chiarella F, Viñuela-Prieto JM. In vitro ceftaroline combinations against methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol*. 2016;65(10):1119-1122. doi:10.1099/jmm.0.000341
41. Bakthavatchalam YD, Ralph R, Veeraraghavan B, Babu P, Munusamy E. Evidence from an in vitro study: is oxacillin plus vancomycin a better choice for heteroresistant vancomycin-intermediate *Staphylococcus aureus*? *Infect Dis Ther*. 2019;8(1):51-62. doi:10.1007/s40121-018-0224-z
42. Tran KN, Rybak MJ. β-Lactam combinations with vancomycin show synergistic activity against vancomycin-susceptible *Staphylococcus aureus*, vancomycin-intermediate *S. aureus* (VISA), and heterogeneous VISA. *Antimicrob Agents Chemother*. 2018;62:e00157-e00118.
43. Gil Romero Y, Gómez-Garcés JL. In vitro activity of ceftaroline in combination with other antimicrobials active against *Staphylococcus* spp. *Enferm Infecc Microbiol Clin (Engl Ed)*. 2020;38(1):25-27. doi:10.1016/j.eimc.2019.03.007
44. Obiang-Obounou BW, Kang OH, Choi JG, et al. In vitro potentiation of ampicillin, oxacillin, norfloxacin, ciprofloxacin, and vancomycin by sanguinarine against methicillin-resistant *Staphylococcus aureus*. *Foodborne Pathog Dis*. 2011;8(8):869-874. doi:10.1089/fpd.2010.0759
45. Snydman DR, McDermott LA, Jacobus NV. Evaluation of in vitro interaction of daptomycin with gentamicin or beta-lactam antibiotics against *Staphylococcus aureus* and enterococci by FIC index and timed-kill curves. *J Chemother*. 2005;17(6):614-621. doi:10.1179/joc.2005.17.6.614
46. Rand KH, Houck HJ. Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2004;48(8):2871-2875. doi:10.1128/AAC.48.8.2871-2875.2004
47. Mehta S, Singh C, Plata KB, et al. β-Lactams increase the antibacterial activity of daptomycin against clinical methicillin-resistant *Staphylococcus aureus* strains and prevent selection of daptomycin-resistant derivatives. *Antimicrob Agents Chemother*. 2012;56(12):6192-6200. doi:10.1128/AAC.01525-12

48. Barber KE, Werth BJ, Ireland CE, et al. Potent synergy of ceftobiprole plus daptomycin against multiple strains of *Staphylococcus aureus* with various resistance phenotypes. *J Antimicrob Chemother*. 2014;69(11):3006-3010. doi:10.1093/jac/dku236
49. Leonard SN, Rolek KM. Evaluation of the combination of daptomycin and nafcillin against vancomycin-intermediate *Staphylococcus aureus*. *J Antimicrob Chemother*. 2013;68(3):644-647. doi:10.1093/jac/dks453
50. Werth BJ, Barber KE, Ireland CE, Rybak MJ. Evaluation of ceftaroline, vancomycin, daptomycin, or ceftaroline plus daptomycin against daptomycin-nonsusceptible methicillin-resistant *Staphylococcus aureus* in an in vitro pharmacokinetic/pharmacodynamic model of simulated endocardial vegetations. *Antimicrob Agents Chemother*. 2014;58(6):3177-3181. doi:10.1128/AAC.00088-14
51. Mishra NN, Lew C, Abdelhady W, et al. Synergy mechanisms of daptomycin-fosfomycin combinations in daptomycin-susceptible and -resistant methicillin-resistant *Staphylococcus aureus*: *In vitro*, *ex vivo*, and *in vivo* Metrics. *Antimicrob Agents Chemother*. 2022;66(1):e0164921. doi:10.1128/AAC.01649-21
52. Tsai CE, Yang CJ, Chuang YC, et al. Evaluation of the synergistic effect of ceftaroline against methicillin-resistant *Staphylococcus aureus*. *Int J Infect Dis*. 2022;122:230-236. doi:10.1016/j.ijid.2022.05.057
53. Frieri M, Kumar K, Boutin A. Antibiotic resistance. *J Infect Public Health*. 2017;10:369-378.
54. Blair JM, Webber MA, Baylay AJ, et al. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*. 2015;13(1):42-51. doi:10.1038/nrmicro3380
55. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. *Microb Pathog*. 2016;95:32-42. doi:10.1016/j.micpath.2016.02.009

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