Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/107.2023

Esther Ríos[1,2]
Marta Pérez[3]
Juan Carlos Sanz[3]
Alberto Delgado-Iribarren[1,2]
Iciar Rodríguez-Avial[1,2]

Efficacy of delafloxacin alone and in combination with cefotaxime against cefotaxime non-susceptible invasive isolates of *Streptococcus pneumoniae*

[1]Departamento de Medicina, Facultad de Medicina, Universidad Complutense de Madrid.
[2]IdISSC, España; Servicio Microbiología, Hospital Clínico San Carlos, Madrid.
[3]Laboratorio Regional de Salud Pública (Madrid). Edificio Usos Múltiples Hospital Enfermera Isabel Zendal.

Article history
Received: 5 September 2023; Revision Requested: 17 October 2023; Revision Received: 20 November 2023; Accepted: 19 December 2023; Published: 16 January 2024

ABSTRACT

Objectives. We assessed the *in vitro* activity of delafloxacin and the synergy between cefotaxime and delafloxacin among cefotaxime non-susceptible invasive isolates of *Streptococcus pneumoniae* (CNSSP).

Material and methods. A total of 30 CNSSP (cefotaxime MIC > 0.5 mg/L) were studied. Serotyping was performed by the Pneumotest-Latex and Quellung reaction. Minimum inhibitory concentrations (MICs) of delafloxacin, levofloxacin, penicillin, cefotaxime, erythromycin and vancomycin were determined by gradient diffusion strips (GDS). Synergistic activity of delafloxacin plus cefotaxime against clinical *S. pneumoniae* isolates was evaluated by the GDS cross method.

Results. Delafloxacin showed a higher pneumococcal activity than its comparator levofloxacin (MIC_{50}, 0.004 versus 0.75 mg/L and MIC_{90}, 0.047 versus >32 mg/L). Resistance to delafloxacin was identified in 7/30 (23.3%) isolates, belonging to serotypes 14 and 9V. Synergy between delafloxacin and cefotaxime was detected in 2 strains (serotypes 19A and 9V). Antagonism was not observed. Addition of delafloxacin increased the activity of cefotaxime in all isolates. Delafloxacin susceptibility was restored in 5/7 (71.4%) strains.

Conclusions. CNSSP showed a susceptibility to delafloxacin of 76.7%. Synergistic interactions between delafloxacin and cefotaxime were observed *in vitro* among CNSSP by GDS cross method.

Keywords: Delafloxacin, *Streptococcus pneumoniae*, Synergy, Cefotaxime.

Correspondence:
Esther Ríos Dueñas
Departamento de Medicina, Facultad de Medicina, Universidad Complutense de Madrid.
Plaza Ramón y Cajal, s/n 28040 Madrid
Email: erios02@ucm.es

Eficacia de delafloxacino solo y en combinación con cefotaxima frente a aislados invasivos de *Streptococcus pneumoniae* no sensible a cefotaxima

RESUMEN

Objetivos. Evaluamos la actividad in vitro de delafloxacino y la sinergia entre cefotaxima y delafloxacino entre aislados invasivos de *Streptococcus pneumoniae* no sensibles a cefotaxima (SPNSC).

Material y métodos. Se estudiaron un total de 30 SPNSC (CIM de cefotaxima > 0,5 mg/L). El serotipado se realizó mediante la reacción Pneumotest-Latex y Quellung. Las concentraciones mínimas inhibitorias (CMI) de delafloxacino, levofloxacino, penicilina, cefotaxima, eritromicina y vancomicina se determinaron mediante tiras de difusión en gradiente (GDS). La actividad sinérgica de delafloxacino y cefotaxima frente aislados clínicos de *S. pneumoniae* se evaluó mediante el método cruzado GDS.

Resultados. Delafloxacino mostró una mayor actividad neumocócica que su comparador levofloxacino (CIM50, 0,004 versus 0,75 mg/L y MIC90, 0,047 versus > 32 mg/L). Se identificó resistencia a delafloxacino en 7/30 (23,3%) aislados, pertenecientes a los serotipos 14 y 9V. Se detectó sinergia entre delafloxacino y cefotaxima en 2 cepas (serotipos 19A y 9V). No se observó antagonismo. La adición de delafloxacino aumentó la actividad de cefotaxima en todos los aislados. La sensibilidad a delafloxacino se restableció en 5/7 (71,4%) cepas.

Conclusiones. SPNSC mostraron una susceptibilidad a delafloxacino del 76,7%. Se observaron interacciones sinérgicas in vitro entre delafloxacino y cefotaxima entre SPNSC mediante el método cruzado GDS.

Palabras clave: Delafloxacino, *Streptococcus pneumoniae*, Sinergia, Cefotaxima.

INTRODUCTION

Streptococcus pneumoniae, a microorganism causing invasive diseases such as meningitis, sepsis, and pneumonia [1], is worldwide the fourth leading pathogen in terms of deaths associated with or attributable to antimicrobial resistance. *S. pneumoniae* along with five other pathogens were responsible for 929 000 of 1·27 million deaths attributable to antimicrobial resistance and 3·57 million of 4·95 million deaths associated with antimicrobial resistance globally [2].

Over the years, *S. pneumoniae* has developed different mechanisms of resistance to the main drugs such as beta-lactams, macrolides and fluoroquinolones, being a major problem in treating pneumococcal infections. New therapeutic modalities are necessary.

Delafloxacin (BAXDELA® in the USA; Quofenix® in the EU) is an anionic fluoroquinolone antibacterial that is approved for the treatment of community-acquired pneumonia (CAP) and acute bacterial skin and skin structure infections in adults. Delafloxacin has demonstrated *in vitro* activity against Gram-positive and Gram-negative pathogens, including drug-resistant isolates [3]. Unlike other quinolones, which usually have a binding affinity for either DNA gyrase or topoisomerase IV, delafloxacin is equally potent against both enzymes [4]. Moreover, fluoroquinolones plus beta-lactams are now recommended as an alternative option in the treatment of severe pneumonia by the latest international guidelines [5]. Thus, it would be interesting to evaluate the antipneumococcal activity of delafloxacin in combination with beta-lactams.

The present study aims to assess the *in vitro* activity of delafloxacin and the synergy between cefotaxime and delafloxacin among cefotaxime non-susceptible invasive isolates of *S. pneumoniae* (CNSSP).

MATERIAL AND METHODS

Bacterial isolates.nA total of 30 cefotaxime non-susceptible (MIC > 0.5 mg/L) invasive isolates of *S. pneumoniae* (CNSSP) from clinical samples were collected between 2012 and 2018 at the Madrid Regional Public Health Laboratory (Spain). These strains were identified by standard procedures, including Gram staining, catalase test, optochin susceptibility, and bile solubility tests. Capsular serotypes were determined by Pneumotest-Latex (Statens Serum Institut, Copenhagen, Denmark) and by Quellung reaction using commercial factor antisera (Statens Serum Institut, Copenhagen, Denmark).

Antibiotic susceptibility testing. Minimum inhibitory concentrations (MICs) of delafloxacin [DX], levofloxacin [LX], penicillin [PG], cefotaxime [CTX], erythromycin [EM] and vancomycin [VAN] were determined by gradient diffusion strips (GDS) (Liofilchem, Italy [DX]); Etest® bioMerieux, France [others]) on 5% horse blood-enriched Mueller-Hinton agar. *S. pneumoniae* ATCC49619 was used as internal quality control strains. Interpretation of MIC results was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoints [6]. Strains showing a MIC to penicillin >0.06 mg/L were categorized as non-susceptible *S. pneumoniae* isolates.

Evaluation of synergy. Synergistic activity of CTX plus DX was assessed against the 30 clinical *S. pneumoniae* isolates by the GDS cross method in the Laboratory of Microbiology, Hospital Clínico San Carlos (Madrid). Bacterial suspension prepared to a concentration of 1 to 5×10^8 (CFU)/ml was inoculated on 5% horse blood-enriched Mueller-Hinton agar and GDS were placed at 90° angles at the intersection of the MIC of each drug. Combination MIC´s were then compared with MICs for each of the antibiotics when used alone. Fractional Inhibitory Concentration (FIC) index values were determined to

Table 1 The *in vitro* activity of delafloxacin and comparators against the 30 CNSSP.

Antimicrobial agent	MIC (mg/L)			Susceptible	Non-susceptible
	Range	MIC50	MIC90	No. (%)	No. (%)
CTX	1 - 8	2	3	0	30 (100%)
PG	1 - 8	3	6	0	30 (100%)
EM	0.094 - >256	0.38	>256	15 (50%)	-
LX	0.38- >32	0.75	>32	23 (76.7%)	-
DX	0.002 - 0.38	0.004	0.047	23 (76.7%)	-
VAN	0.5 - 1	0.75	1	30 (100%)	-

CNSSP: cefotaxime non-susceptible invasive isolates of *Streptococcus pneumoniae*, CTX: cefotaxime, PG: penicillin, EM: erythromycin, LX: levofloxacin, DX: delafloxacin, VAN; vancomycin.
CTX ≤ 0.5 mg/L susceptible, and >0.5 mg/L non-susceptible; PG ≤ 0.06 mg/L susceptible, and >0.06 mg/L non-susceptible; EM ≤ 0.25 mg/L susceptible, and >0.5 mg/L resistant; LX≤ 0.001 mg/L susceptible, and >2 mg/L resistant; DX ≤ 0.03 mg/L susceptible and >0.03 mg/L resistant; VAN ≤ 2 mg/L susceptible, and >2 mg/L resistant.

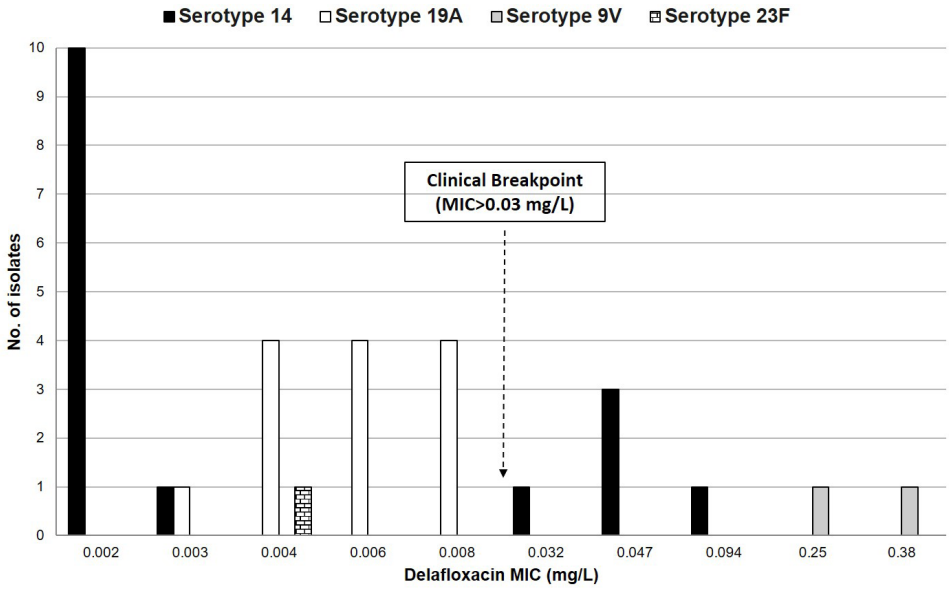

Figure 1 Distribution of delafloxacin MIC according to pneumococcal serotypes.

Table 2 Mean MIC´s to antimicrobial agents alone and in combination according to Fractional Inhibitory Concentration (FIC) index values.

FIC index ranges	No. of isolates (%)	Mean MIC (mg/L)				Interpretation
		CTX alone	CTX in combination	DX alone	DX in combination	
≤0.5	2 (6.7)	3	0.75	0.2	<0.03	Synergy
> 0.5 - ≤1	15 (50)	2	1	0.036	0.010	No interaction
>1- ≤4	13 (43.3)	2	1	0.004	0.003	No interaction
> 4	0	-	-	-	-	Antagonism

describe antibiotic interactions and results were interpreted as follows: ≤0.5 as synergy; >0.5 to ≤4 as no interaction; and >4 as antagonism [7].

RESULTS

Among the 30 CNSSP, the delafloxacin MIC_{50} /MIC_{90} were 0.004 and 0.047 mg/L, respectively. The MIC ranges and MIC_{50} and MIC_{90} results for the remaining antimicrobial agents against the pneumococcal isolates are shown in Table 1. Delafloxacin was at least 128-fold (MIC_{50}) and 512-fold (MIC_{90}) more active than levofloxacin. For all strains, 100% were non-susceptible to penicillin (MIC > 0.06 mg/L) and cefotaxime (MIC > 0.5 mg/L). Fifteen (50%) isolates were erythromycin resistant (MIC > 0.5 mg/L) and 7 (23.3%) showed resistance to both delafloxacin and levofloxacin. All strains were vancomycin susceptible.

Against the levofloxacin-resistant *S. pneumoniae*, delafloxacin MICs were increased 16-to 8-fold (MIC_{50} and MIC_{90}, 0.047 and 0.38 mg/L) relative to the general population. The highest MICs for delafloxacin (0.38 and 0.25 mg/L) were detected in two quinolones-resistant isolates belonging to serotype 9V.

Distribution of delafloxacin MICs among the different serotypes is shown in Figure 1. Among the 7 delafloxacin resistant isolates (MIC >0.03 mg/L), 5 belonged to serotype 14 y 2 were identified as serotype 9V. Nevertheless, most of the serotype 14 isolates (62.5%, 10/16) had delafloxacin MIC value of 0.002 mg/L. Serotype 19A isolates showed delafloxacin MICs between 0.003 and 0.008 mg/L.

Out of 30 CNSSP, 2 isolates (serotypes 19A and 9V) demonstrated a synergistic effect when delafloxacin was combined with cefotaxime. One of these two strains was resistant

to delafloxacin (serotype 9V). Antagonism was not detected. Restoration of susceptibility to the delafloxacin in the presence of cefotaxime was observed in 5 of the 7 delafloxacin-resistant isolates (71.4%).

The Mean MIC´s to antimicrobial agents alone and in combination according to Fractional Inhibitory Concentration (FIC) index values are presented in Table 2. In case of isolates displaying synergistic interaction, the mean MIC of each agent in the combination was reduced in the presence of the other (4-fold and 8-fold MIC reduction for cefotaxime and delafloxacin, respectively), even returning the susceptibility category to delafloxacin. Although the MIC-lowering effect was also observed in those isolates with categorization of 'no interaction', susceptibility to delafloxacin was only restored in those strains exhibiting a FIC index value > 0.5 - ≤1 (Table 2).

DISCUSSION

Beta-lactam and macrolide antibiotics are frequently utilized to treat pneumococcal disease [5]. In our study, all *S. pneumoniae* isolates included in our study were cefotaxime non-susceptible, although non-susceptibility to penicillin and erythromycin were also observed (66.7% and 50%, respectively). Respiratory fluoroquinolones (i.e., levofloxacin) may be considered a treatment option as monotherapy for outpatients with Community-acquired Pneumonia [5]. Among the drugs tested, vancomycin exhibited complete activity (100% susceptibility) and fluoroquinolones (both levofloxacin and delafloxacin) were the second most active antimicrobial against all strains, both of them, with 23.3% resistance rate.

The MIC_{50} and MIC_{90} values for delafloxacin against the CNSSP were 0.004 and 0.047 mg/L, respectively. These values are similar to those obtained in previous studies carried out in the USA and Canada [8, 9]. Delafloxacin have demonstrated potent activity against *S. pneumoniae* and its activity extends to strains displaying penicillin-resistant or ceftriaxone non-susceptible phenotypes [8, 10].

In our collection, serotype 9V exhibited the highest delafloxacin MICs (0.38 and 0.25 mg/L) showing resistance to levofloxacin. Our findings are consistent with a previous survey that reported the serotype 9V with the highest MIC value among highly levofloxacin-resistant pneumococcal invasive isolates [11]. In a 2014 surveillance study, the *in vitro* activity of delafloxacin against *S. pneumoniae* strains collected from medical centers in Europe and the United States were tested. All levofloxacin-resistant isolates were inhibited by ≤0.25 mg/L of delafloxacin [10].

Against our CNSSP, delafloxacin demonstrated higher activity than its comparator (levofloxacin), being at least 128-fold (MIC_{50}) and 512-fold (MIC_{90}) more potent than levofloxacin. However, an increase in MIC values of delafloxacin was observed when it was tested against levofloxacin-resistant strains. These data have been previously described by other authors [10, 11, 12]. The high affinity of delafloxacin for the DNA gyrase could contribute to its lower MICs in comparison to levofloxacin [12].

Combination therapy with a beta-lactam (cefotaxime, ceftriaxone or ampicillin/sulbactam) plus a fluoroquinolone is superior to monotherapy, particularly for patients with severe for community-acquired pneumonia or bacteremic pneumococcal [5]. One of the purposes of this study was to evaluate the combination of delafloxacin with cefotaxime. We performed synergy tests using antibiotic GDS on the 30 CNSSP. Even though time–kill curves and chequerboard methods are considered as the 'gold standard', both of them are not easily adaptable to the clinical laboratory for testing of multiple isolates. Nevertheless, frequent agreement among time-kill and GDS techniques has been described, thus, GDS method can be quick and useful for synergy assessments [13].

Two (6.7%) isolates belonging to serotype 19A and 9V, respectively, displayed synergistic interactions *in vitro* between delafloxacin and cefotaxime. The *in vitro* and *in vivo* synergism of third-generation cephalosporins and fluoroquinolones have already been described in pneumococci [14-17]. A high rate of synergy (54%) was found with the combination of levofloxacin with ceftriaxone in the experiments carried out by Drago *et al.* [15] using time-kill and checkerboard methods. The low level of synergy detected in our study could be due to the very low MIC of the first antibiotic, which could mask the detection of interaction between both agents. Moreover, it is well known that the E-Test technique detects a much lower percentage of synergisms compared to bacterial kill curves and even lower than chequerboard [13].

In order to evaluate the clinical use of the combination for invasive pneumococcal disease treatment, other factors should be taken into account. Even though the GDS synergy method is not able to detect all synergistic interactions by time–kill analyses, it could identify an MIC-lowering effect for one or both agents in combination [18]. These findings were observed in our study. All isolates displayed the cefotaxime MIC-lowering effect in combination (at least 2-fold MIC reduction), while for delafloxacin this reduction occurred in most strains. Moreover, it is also important to mention that the decreased in MIC of delafloxacin in combination produced the restoration of the susceptibility category not only in isolates showing synergism but also in those with FIC index > 0.5 - ≤1. Therefore, the GDS cross method could be of interest for clinicians to identify these important MIC-lowering effects, especially if susceptibility reports indicate non-susceptibility or resistance by a small margin [18].

CONCLUSIONS

In summary, CNSSP showed a susceptibility to delafloxacin of 76.7%. To the best of our knowledge, this is the first study to investigate the synergistic activity of delafloxacin-based combinations *in S. pneumoniae*. The addition of delafloxacin improves the activity of cefotaxime against CNSSP. Synergistic interactions between delafloxacin and cefotaxime were observed *in vitro* among CNSSP by GDS cross method.

ACKNOWLEDGEMENTS

This work was presented in part in abstract form and poster at XXIV SEIMC (Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica) Congress 2023.

FUNDING

None to declare

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1 Subramanian K, Henriques-Normark B, Normark S. Emerging concepts in the pathogenesis of the *Streptococcus pneumoniae*: From nasopharyngeal colonizer to intracellular pathogen. Cell Microbiol. 2019; 21(11):e13077. doi: 10.1111/cmi.13077.

2 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022; 399:629–55. doi: 10.1016/S0140-6736(21)02724-0.

3 European Medicines Agency. Quofenix, *delafloxacin* [cited 26 May 2023]. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/quofenix.

4 Bassetti M, Melchio M, Giacobbe DR. Delafloxacin for the treatment of adult patients with community-acquired bacterial pneumonia. Expert Rev Anti Infect Ther. 2022 May;20(5):649-656. doi: 10.1080/14787210.2021.2020098.

5 Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K *et. al.* Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019 Oct 1;200(7):e45-e67. doi: 10.1164/rccm.201908-1581ST.

6 The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Versión 12.0, 2022. (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf)

7 Canut Blasco A, Collazos Blanco A, Díez Aguilar M, Rodríguez Gascón A, Seral García C. Métodos microbiológicos para la determinación *in vitro* de la actividad de combinaciones de antimicrobianos 70. Procedimientos en Microbiología Clínica. Morosini Reilly MI (Coordinadora). Cercenado Mansilla E, Cantón Moreno R (Editores). Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). 2000. https://seimc.org/contenidos/documentoscientificos/procedimientosmicrobiologia/seimc-procedimiento70.pdf

8 Flamm RK, Rhomberg PR, Huband MD, Farrell DJ. In Vitro Activity of Delafloxacin Tested against Isolates of *Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*. Antimicrob Agents Chemother. 2016 Sep 23;60(10):6381-5. doi: 10.1128/AAC.00941-16.

9 Zhanel GG, Palatnick L, Nichol KA, Bellyou T, Low DE, Hoban DJ. 2003. Antimicrobial resistance in respiratory tract S*treptococcus pneumoniae* isolates: results of the Canadian Respiratory Organism Susceptibility Study, 1997 to 2002. Antimicrob Agents Chemother 47:1867–1874. doi: 10.1128/AAC.47.6.1867-1874.2003.

10 Pfaller MA, Sader HS, Rhomberg PR, Flamm RK. In vitro activity of delafloxacin against contemporary bacterial pathogens from the United States and Europe, 2014. Antimicrob Agents Chemother. 2017 Mar 24;61(4):e02609-16. doi: 10.1128/AAC.02609-16.

11 Cercenado E, Loras C, Cobos A, Sanz JC. *In vitro* activity of delafloxacin against highly levofloxacin-resistant invasive isolates of *Streptococcus pneumoniae*. Enferm Infecc Microbiol Clin (Engl Ed). 2022 Mar;40(3):131-133. doi: 10.1016/j.eimce.2020.09.009.

12 Jorgensen SCJ, Mercuro NJ, Davis SL, Rybak MJ. Delafloxacin: place in therapy and review of microbiologic, clinical and pharmacologic properties. Infect Dis Ther 2018 Jun;7(2):197-217. doi: 10.1007/s40121-018-0198-x.

13 White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Antimicrob Agents Chemother. 1996 Aug;40(8):1914-8. doi: 10.1128/AAC.40.8.1914.

14 Flatz L, Cottagnoud M, Kühn F, Entenza J, Stucki A, Cottagnoud P. Ceftriaxone acts synergistically with levofloxacin in experimental meningitis and reduces levofloxacin-induced resistance in penicillin-resistant pneumococci. J Antimicrob Chemother 2004 Feb;53(2):305-10. doi: 10.1093/jac/dkh082.

15 Drago L, Nicola L, Rodighiero V, Larosa M, Mattina R, De Vecchi E. Comparative evaluation of synergy of combinations of beta-lactams with fluoroquinolones or a macrolide in *Streptococcus pneumoniae*. J Antimicrob Chemother. 2011 Apr;66(4):845-9. doi: 10.1093/jac/dkr016.

16 Tsai HY, Liao CH, Liu CY, Huang YT, Hsueh PR. In vitro synergy of penicillin, ceftriaxone and levofloxacin against serotype 19A *Streptococcus pneumoniae*. *I*nt J Antimicrob Agents. 2015 Dec;46(6):722-5. doi: 10.1016/j.ijantimicag.2015.07.018.

17 Cottagnoud P, Cottagnoud M, Acosta F, Flatz L, Kühn F, Stucki A et al. Meropenem prevents levofloxacin-induced resistance in penicillin-resistant pneumococci and acts synergistically with levofloxacin in experimental meningitis. Eur J Clin Microbiol Infect Dis. 2003 Nov;22(11):656-62. doi: 10.1007/s10096-003-1016-1.

18 Avery LM, Nicolau DP. Feasibility of routine synergy testing using antibiotic gradient diffusion strips in the clinical laboratory. J Antimicrob Chemother 2018 Aug 1;73(8):2264-2265. doi: 10.1093/jac/dky165.