



## Original

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

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## 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<sub>50</sub>, 0.004 versus 0.75 mg/L and MIC<sub>90</sub>, 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.

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## Eficacia de delafloxacin 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 delafloxacin y la sinergia entre cefotaxima y delafloxacin 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 delafloxacin, levofloxacin, penicilina, cefotaxima, eritromicina y vancomicina se determinaron mediante tiras de difusión en gradiente (GDS). La actividad sinérgica de delafloxacin y cefotaxima frente a aislados clínicos de *S. pneumoniae* se evaluó mediante el método cruzado GDS.

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

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

**Palabras clave:** Delafloxacin, *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.** A 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.

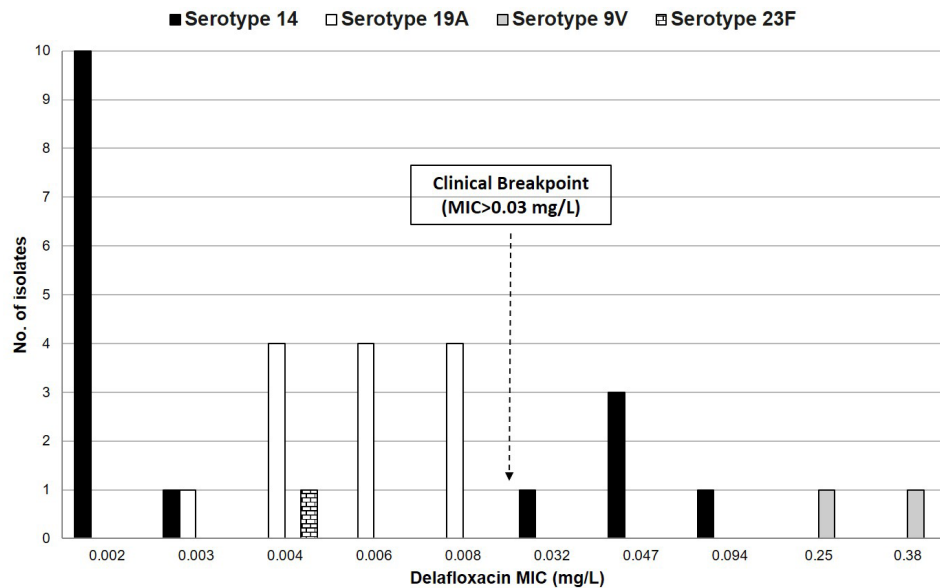
**Evaluation of synergy.** Synergistic activity of CTX plus DX was assessed against the 30 clinical *S. pneumoniae* isolates by the GDS cross method in the Laboratory of Microbiology, Hospital Clínico San Carlos (Madrid). Bacterial suspension prepared to a concentration of  $1$  to  $5 \times 10^8$  (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.

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<sub>50</sub>/MIC<sub>90</sub> were 0.004 and 0.047 mg/L, respectively. The MIC ranges and MIC<sub>50</sub> and MIC<sub>90</sub> results for the remaining antimicrobial agents against the pneumococcal isolates are shown in Table 1. Delafloxacin was at least 128-fold (MIC<sub>50</sub>) and 512-fold (MIC<sub>90</sub>) 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<sub>50</sub> and MIC<sub>90</sub>, 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 and 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  $> 0.5 - \leq 1$  (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<sub>50</sub> and MIC<sub>90</sub> 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  $\leq 0.25$  mg/L of delafloxacin [10].

Against our CNSSP, delafloxacin demonstrated higher activity than its comparator (levofloxacin), being at least 128-fold (MIC<sub>50</sub>) and 512-fold (MIC<sub>90</sub>) 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 checkerboard 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 checkerboard [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  $> 0.5 - \leq 1$ . 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.

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None to declare

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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