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In vitro antimicrobial activity of daptomycin alone and in adjunction with either amoxicillin, cefotaxime or rifampicin against the main pathogens responsible for bacterial meningitis in adults

Thomas Maldiney[a], Dorian Bonnot[b], Nelson Anzala[b], Sandrine Albac[b], Delphine Labrousse[b], Emmanuelle Varon[c], Lucie Amoureux[d,e], Angélique Chapuis[d,e], Julien Bador[d,e], Catherine Neuwirth[d,e], Delphine Croisier[b], Pascal Chavanet[a,b,*]

[a] *Infectious Diseases Department, University Hospital of Dijon, 14 rue Paul Gaffarel, 21000, Dijon, France*
[b] *Vivexia, Résidence Richelieu, 10 Boulevard Carnot, 21000, Dijon, France*
[c] *National Centre for Pneumococci, Centre Hospitalier Intercommunal Créteil, 40 avenue de Verdun, 94000, Créteil, France*
[d] *Department of Bacteriology, University Hospital of Dijon, BP 37013, 21070, Dijon Cedex, France*
[e] *UMR/CNRS 6249 Chrono-environnement, University of Bourgogne-Franche-Comté, 2 Place Saint-Jacques, Besançon, France*

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ABSTRACT

Objectives: As daptomycin adjunction is currently under clinical evaluation in the multicentre phase II AddaMAP study to improve the prognosis of pneumococcal meningitis, the present work aimed at evaluating the *in vitro* antimicrobial activity of daptomycin-based combinations against some of the most frequent species responsible for bacterial meningitis.

Methods: Clinically relevant strains of *Streptococcus pneumoniae, Listeria monocytogenes, Haemophilus influenzae* and *Neisseria meningitidis* were obtained from National Reference Centers. The antimicrobial activity of amoxicillin, cefotaxime and rifampicin, either alone or in association with daptomycin, was explored through the determination of minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) as well as time–kill assay (TKA) using the broth microdilution method.

Results: All species taken together, the adjunction of daptomycin had no deleterious impact on the antimicrobial activity of amoxicillin, cefotaxime or rifampicin *in vitro*. Regarding Gram-positive bacteria, FICI and TKA analysis confirmed a global improvement of growth inhibition and bactericidal activity due to the adjunction of daptomycin. The synergistic effect prevailed for *L. monocytogenes* as demonstrated by FICI mainly <0.5 and a dynamic TKA-based synergy rate $>50\%$. In addition, daptomycin-based associations did not modify the activity of β-lactam antibiotics or rifampicin against Gram-negative bacteria, notably *N. meningitidis*.

Conclusion: These results bring comforting evidence towards the clinical potential of daptomycin adjunction in the treatment of bacterial meningitis, which supports the ongoing AddaMAP clinical trial.

1. Introduction

Bacterial meningitis remains one of, if not the most, severe and deadly infectious diseases [1]. Owing to intrinsic virulence factors, *Streptococcus pneumoniae* is responsible for the highest morbimortality associated with infectious meningitis worldwide as well as long-term neurological and cognitive impairment [2–4]. Moreover, the recent resurgence in Europe and North America of pneumococcal meningitis due to non-vaccine serotypes as well as an increased fraction of *S. pneumoniae* strains with reduced sensitivity to third-generation cephalosporins in France [5] highlighted the need for a novel approach regarding prevention and treatment [6]. Despite a global reliance on the use of β-lactam antibiotics [7,8], several studies suggest a significant risk of worsening cerebral lesions and vasculitis owing to the release of pro-inflammatory toxins during bacterial cell lysis [9,10]. As non-bacteriolytic antibiotics may help

* Corresponding author at: Mailing address: Infectious Diseases Department, University Hospital of Dijon, 14 rue Paul Gaffarel, 21000 Dijon, France.
E-mail address: pascal.chavanet@chu-dijon.fr (P. Chavanet).

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to contain such an excessive inflammatory burst of the host response, a number of research groups have evaluated the potential use of clindamycin, rifampicin and daptomycin in order to decrease the global level of cerebrospinal fluid (CSF) inflammation in experimental models of pneumococcal meningitis [11]. On the basis of additional promising results from this preclinical development *in vivo*, the adjunction of daptomycin to standard treatment with a third-generation cephalosporin was demonstrated as a good candidate to attenuate brain damage and to hope for an improved clinical outcome of patients with pneumococcal meningitis [12]. Following a similar approach, the adjunction of daptomycin was intended for clinical evaluation in a multicentre phase II study to improve the prognosis and survival of pneumococcal meningitis (AddaMAP Study; ClinicalTrials.gov ID: NCT03480191), with possible inclusion upon clinical suspicion only. However, its impact on the activity of standard treatment towards other species associated with bacterial meningitis remains unknown. The present study aimed at evaluating the *in vitro* antimicrobial activity of daptomycin-based associations against the most frequent species responsible for bacterial meningitis.

2. Materials and methods

2.1. Bacterial strains and culture

The main characteristics of all bacterial species and isolates are summarised in Supplementary Table S1. Four *S. pneumoniae* clinical strains were isolated from either blood culture or CSF from patients with pneumococcal meningitis and were kindly provided by the National Centre for Pneumococci (Dr Emmanuelle Varon). *Listeria monocytogenes* reference clinical and food isolates were kindly provided by the National Reference Center and WHO Collaborating Centre *Listeria* at the Institut Pasteur (Prof. Marc Lecuit). Three *Haemophilus influenzae* non-typeable clinical strains were selected from among reference isolates (ATCC 49766) and CSF cultures of patients hospitalised with bacterial meningitis in University Hospital of Dijon (Dijon, France). Four *Neisseria meningitidis* clinical strains (serotypes W, B and C) were isolated from blood cultures of patients with meningococcal meningitis and were kindly provided by the National Reference Center for Meningococci (Prof. Muhamed-Kheir Taha).

All bacterial species and isolates were stored in CryoBeads™ at –80°C. Isolates of *S. pneumoniae* (under 5% CO_2) and *L. monocytogenes* were incubated to stationary phase at 37°C on Columbia agar with 5% sheep blood (bioMérieux) for 18–24 h. Isolates of *H. influenzae* (under 5% CO_2) and *N. meningitidis* were grown on chocolate agar PolyViteX (bioMérieux). Mueller–Hinton broth (Difco) supplemented with 50 mg/L Ca^{2+}, 12.5 mg/L Mg^{2+}, 5% lysed horse blood and 20 mg/L β-NAD (MHF) was prepared according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)/Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2019 guidelines regarding susceptibility testing of fastidious micro-organisms. MHF was used for all broth microdilution minimum inhibitory concentration (MIC), fractional inhibitory concentration index (FICI) and time–kill kinetics experiments.

2.2. Minimum inhibitory concentration (MIC) determination

MICs were determined by the standard broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines as described elsewhere [13,14]. The inoculum suspension was adjusted in sterile water to a 0.5 McFarland suspension and was subsequently diluted in MHF so that each well contained ~5 × 10^5 CFU/mL. Antibiotic solutions were prepared following a proper dilution in MHF of aliquots of stock solution stored at –80°C after reconstitution of daptomycin (Cubicin 350 mg; Novartis), amoxicillin (Amoxicilline 1 g; Panpharma), cefotaxime (Céfotaxime 1 g; Mylan) and rifampicin (Rifadin 600 mg; Sanofi Aventis) powders following the manufacturer's instructions. All microdilution plates were incubated at 37°C for 16–20 h in an ambient air incubator right after adding the inoculum, adjusting the final volume to 100 μL per well and careful sealing with a plastic film to prevent drying. MICs were defined as the lowest concentration of antimicrobial agent that completely inhibited growth of the organism as detected by a microplate reader equipped with a standard absorbance filter at 595 nm (iMark™ Microplate Reader; Bio-Rad). Each MIC experiment was performed in triplicate. EUCAST clinical breakpoint tables v.11.0 (valid from 1 January 2021) were used throughout the study for breakpoint interpretation criteria.

2.3. Fractional inhibitory concentration index (FICI) determination

The antimicrobial activity of daptomycin-based associations with either amoxicillin, cefotaxime or rifampicin was assessed using the checkerboard dilution assay [15]. First, serial two-fold increasing concentrations of the selected antibiotics spanning susceptible to resistant breakpoints were distributed in a 96-well microplate. As for MIC testing, the inoculum suspension was adjusted in sterile water to a 0.5 McFarland suspension and was subsequently diluted in MHF so that each well contained ~5 × 10^5 CFU/mL with a final volume of 150 μL per well. The microplates were finally incubated at 37°C for 16–20 h in an ambient air incubator right after sealing with a plastic film to prevent drying. FICIs were calculated after microplate absorbance reading at 595 nm on the basis of a previously described protocol [16]. Synergy was defined as FICI $\leq$ 0.5, indifference as FICI >0.5 to 4, and antagonism as FICI > 4. Each FICI experiment was performed in triplicate.

2.4. Time–kill kinetics assay

Microdilution time–kill methodology was adapted from the protocol described by Clark et al. [17]. Following bacterial growth in MHF, antimicrobial concentrations were adjusted in separate wells of a microplate to reach the final volume of 300 μL at one dilution above the MIC and one to two dilutions below the MIC for synergy testing (Table 1). Owing to lack of antimicrobial activity of daptomycin against Gram-negative bacteria, the daptomycin concentration was set at 8 mg/L for synergy testing against *N. meningitidis* and *H. influenzae*. Drug-free controls were systematically included. After proper sealing with a plastic film to prevent drying, microdilution plates were incubated at 37°C under gentle shaking for a total of 24 h in an ambient air incubator. Viability counts were performed by single plate-serial dilution spotting according to an optimised protocol described by Thomas et al. [18]. Briefly, a 15-μL aliquot was sampled at 0, 1, 3, 5 and 24 h (for all bacterial species except *S. pneumoniae*) or 0, 0.5, 1.5, 5 and 24 h (for *S. pneumoniae* owing to the rapid bactericidal activity of daptomycin) for subsequent serial dilution at 10^1, 10^2, 10^4 up to 10^6 in filter-sterilised distilled water. Starting from the last dilution, either Columbia or chocolate agar was used for the final spotting of a 20-μL microdrop from each serial dilution. Plates were then allowed to dry for 5–10 min before incubation at 37°C as required for the selected bacterial species. Only plates with <300 colonies were enumerated. Each time–kill kinetics assay was performed in triplicate and was integrated as area under the curve over a 24-h period (AUC_{0-24}). Synergy was considered starting from a minimum 2 $\log_{10}$ decrease in CFU/mL between the combination and its most active component at a definite time point between 30 min and 24 h. In addition, any decrease of the starting inoculum $\geq$3 $\log_{10}$ was considered as a killing effect.

Table 1
Antibiotic concentrations for time–kill kinetics assay

Species	Strain	Concentration (mg/L or fold MIC)						
		DPT	AMX	CTX	RMP	DPT/AMX	DPT/CTX	DPT/RMP
Streptococcus pneumoniae	SP 1 (48570)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	MIC/MIC	MIC/MIC
	SP 2 (51510)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	MIC/MIC	MIC/MIC
	SP 3 (56510)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	MIC/MIC	MIC/MIC
	SP 4 (56787)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	MIC/MIC	MIC/MIC
Listeria monocytogenes	LM 1 (EGD-e)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	MIC/MIC	MIC/MIC
	LM 2 (CLIP 2007/01481)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	MIC/MIC	0.5 × MIC/0.5 × MIC	MIC/MIC
	LM 3 (CLIP 2007/00596)	2 × MIC	2 × MIC	2 × MIC	2 × MIC	0.5 × MIC/0.5 × MIC	0.5 × MIC/0.5 × MIC	MIC/MIC
Haemophilus influenzae	HI 1 (ATCC 49766)	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
	HI 2 ("DUM")	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
	HI 3 ("ROU")	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
Neisseria meningitidis	NM 1 (29859)	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
	NM 2 (30496)	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
	NM 3 (30430)	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC
	NM 4 (30095)	8	2 × MIC	2 × MIC	2 × MIC	8/2 × MIC	8/2 × MIC	8/2 × MIC

MIC, minimum inhibitory concentration; DPT, daptomycin; AMX, amoxicillin; CTX, cefotaxime; RMP, rifampicin.

Table 2
Results of minimum inhibitory concentration (MIC) determination

Species	Strain	Mean MIC [range] (μg/mL)			
		DPT	AMX	CTX	RMP
Streptococcus pneumoniae	SP 1 (48570)	0.250 [0.125–0.250]	1.00 [1.00–2.00]	2.00 [2.00–4.00]	0.0312 [0.00781–0.0312]
	SP 2 (51510)	0.125 [0.125–0.125]	0.0156 [0.0156–0.0312]	0.00781 [0.00781–0.0156]	0.00781 [0.00781–0.0156]
	SP 3 (56510)	0.250 [0.125–0.250]	16.0 [16.0–16.0]	8.00 [8.00–8.00]	0.0156 [0.0156–0.0156]
	SP 4 (56787)	0.125 [0.125–0.250]	0.250 [0.250–0.250]	2.00 [2.00–2.00]	0.0156 [0.0156–0.0156]
Listeria monocytogenes	LM 1 (EGD-e)	2.00 [1.00–2.00]	0.250 [0.125–0.250]	8.00 [8.00–16.0]	0.00781 [0.00781–0.00781]
	LM 2 (CLIP 2007/01481)	4.00 [4.00–4.00]	0.250 [0.250–0.250]	32.0 [32.0–32.0]	0.0156 [0.0156–0.0312]
	LM 3 (CLIP 2007/00596)	8.00 [8.00–8.00]	0.250 [0.250–0.250]	8.00 [8.00]	0.0312 [0.0312–0.0312]
Haemophilus influenzae	HI 1 (ATCC 49766)	>512 [>512–>512]	0.250 [0.250–0.250]	0.00781 [0.00781–0.00781]	0.250 [0.250–0.250]
	HI 2 ("DUM")	>512 [>512–>512]	2.00 [2.00–2.00]	0.0312 [0.0156–0.0312]	0.250 [0.250–0.250]
	HI 3 ("ROU")	>512 [>512–>512]	0.250 [0.250–0.250]	0.00781 [0.00781–0.00781]	0.250 [0.250–0.250]
Neisseria meningitidis	NM 1 (29859)	>512 [>512–>512]	0.0312 [0.0312–0.0625]	0.000976 [<0.000976–0.000976]	0.0312 [0.0156–0.0312]
	NM 2 (30496)	>512 [>512–>512]	0.0312 [0.0156–0.0312]	0.000976 [0.000976–0.000976]	0.0312 [0.0312–0.0312]
	NM 3 (30430)	>512 [>512–>512]	0.0312 [0.0312–0.0312]	0.00195 [0.000976–0.00195]	0.125 [0.0312–0.125]
	NM 4 (30095)	>512 [512–>512]	0.500 [0.500–0.500]	0.0625 [0.0625–0.125]	0.250 [0.250–0.250]

DPT, daptomycin; AMX, amoxicillin; CTX, cefotaxime; RMP, rifampicin.

2.5. Statistical analysis

All results are presented as then mean with standard error of the mean. Unpaired *t*-test analysis was performed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) with a 95% confidence interval. For the integrated analysis of time–kill curves, statistical analysis compared the daptomycin combination with its most active component.

3. Results

3.1. Minimum inhibitory concentration (MIC) results

The results of MIC determination are shown in Table 2. For *S. pneumoniae*, SP 1 (48570) was resistant to both amoxicillin and cefotaxime. SP 4 (56787) was susceptible to amoxicillin and resistant to cefotaxime. In contrast, SP 2 (51510) was susceptible to amoxicillin and cefotaxime, and SP 3 (56510) was resistant to both antibiotics. All *S. pneumoniae* strains displayed relatively low daptomycin MICs ranging from 0.125–0.250 μg/mL and were susceptible to rifampicin with MIC mainly <0.06 μg/mL. For *L. monocytogenes*, all strains were susceptible to amoxicillin, displayed very low rifampicin MICs but rather elevated daptomycin and cefotaxime MICs, ranging from 2–8 μg/mL for daptomycin and 8–32 μg/mL for cefotaxime.

Regarding Gram-negative bacteria, all *H. influenzae* clinical strains were susceptible to amoxicillin, cefotaxime and rifampicin. Apart from NM 4 (30095) interpreted as intermediate to amoxicillin, all strains of *N. meningitidis* were susceptible to amoxicillin, cefotaxime and rifampicin. As expected, all Gram-negative bacterial strains were highly resistant to daptomycin with MICs of >512 μg/mL.

3.2. Fractional inhibitory concentration index (FICI) results

Results of FICI tests are shown in Table 3. For *S. pneumoniae*, the rate of synergistic effect due to the adjunction of daptomycin was estimated at 50% with amoxicillin (SP 2 and SP 3) and 25% with cefotaxime (SP 3). The combination of daptomycin and rifampicin showed FICIs from 1.00–2.00, i.e. in favour of an indifferent effect against *S. pneumoniae*. The association of daptomycin and amoxicillin displayed a relative low rate of synergistic effect against *L. monocytogenes*, with only one FICI of ≤0.5 (LM 3) among the three selected strains. The rate increased up to 67% with cefotaxime for

Table 3
Results of fractional inhibitory concentration index (FICI) determination

Species	Strain	Mean FICI [range]		
		DPT + AMX	DPT + CTX	DPT + RMP
Streptococcus pneumoniae	SP 1 (48570)	0.625 [0.625–0.625]	1.25 [0.625–1.25]	1.00 [1.00–1.00]
	SP 2 (51510)	0.500 [0.500–1.06]	0.750 [0.750–1.50]	1.00 [1.00–2.00]
	SP 3 (56510)	0.500 [0.500–0.750]	0.500 [0.500–0.500]	1.00 [1.00–1.50]
	SP 4 (56787)	1.00 [1.00–1.50]	0.750 [0.750–1.12]	2.00 [2.00–2.00]
Listeria monocytogenes	LM 1 (EGD–e)	0.750 [0.500–1.03]	0.625 [0.250–1.06]	0.750 [0.500–0.750]
	LM 2 (CLIP 2007/01481)	0.531 [0.312–0.625]	0.281 [0.187–0.516]	0.750 [0.625–1.25]
	LM 3 (CLIP 2007/00596)	0.500 [0.375–1.01]	0.312 [0.156–0.531]	0.625 [0.500–1.06]
Haemophilus influenzae	HI 1 (ATCC 49766)	n/a	n/a	n/a
	HI 2 ("DUM")	n/a	n/a	n/a
	HI 3 ("ROU")	n/a	n/a	n/a
Neisseria meningitidis	NM 1 (29859)	n/a	n/a	n/a
	NM 2 (30496)	n/a	n/a	n/a
	NM 3 (30430)	n/a	n/a	n/a
	NM 4 (30095)	n/a	n/a	n/a

DPT, daptomycin; AMX, amoxicillin; CTX, cefotaxime; RMP, rifampicin; n/a, not available.
Synergy was defined as FICI ≤ 0.5, indifference as FICI >0.5 to 4, and antagonism as FICI > 4.

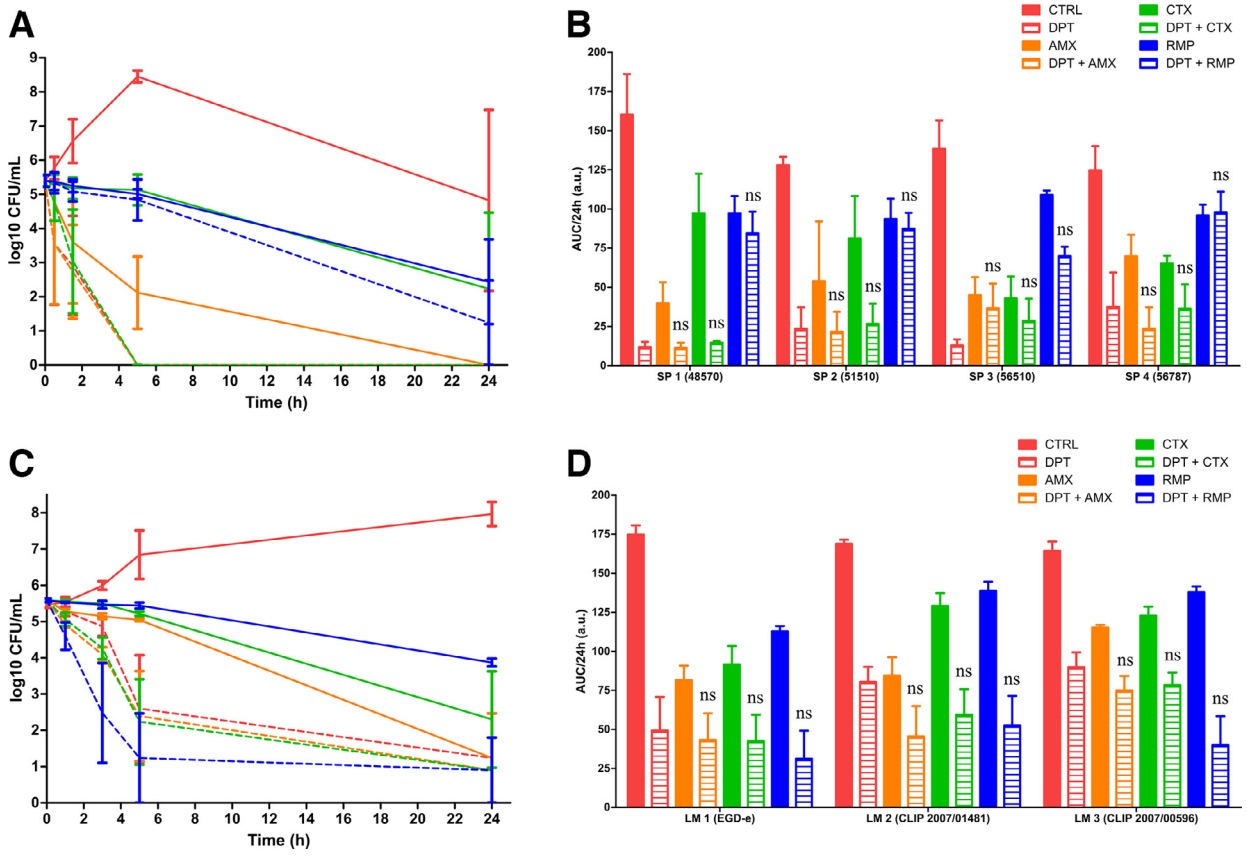

Fig. 1. Time–kill kinetics assay for Gram-positive bacteria. (A) Time–kill curves for *Streptococcus pneumoniae* SP 1 (48570). (B) Integrated analysis of time–kill curves for *S. pneumoniae* (AUC/24 h). (C) Time–kill curves for *Listeria monocytogenes* LM 1 (EGD-e). (D) Integrated analysis of time–kill curves for *L. monocytogenes* (AUC/24 h). AUC, area under the curve; CTRL, control; DPT, daptomycin; AMX, amoxicillin; CTX, cefotaxime; RMP, rifampicin; ns, not significant ($P > 0.05$).

LM 2 (CLIP 2007/01481) and LM 3 (CLIP 2007/00596). Apart from these situations, all FICIs calculated for daptomycin-based associations against *L. monocytogenes* were mainly <1, that is to say globally in concordance with a potential effect of the lipopeptide. As MICs for both *H. influenzae* and *N. meningitidis* did not display any dependence towards daptomycin concentration, we were unable to calculate FICIs for Gram-negative bacteria.

3.3. Time–kill kinetics assay

Results from the time–kill analysis for Gram-positive and Gram-negative bacteria are displayed in Fig. 1 and Fig. 2, respectively. They gather a representative time–kill curve for each bacterial species and an integrated comparison of all time–kill studies, presented as $AUC_{0–24}$. Individual synergy data for Gram-positive and

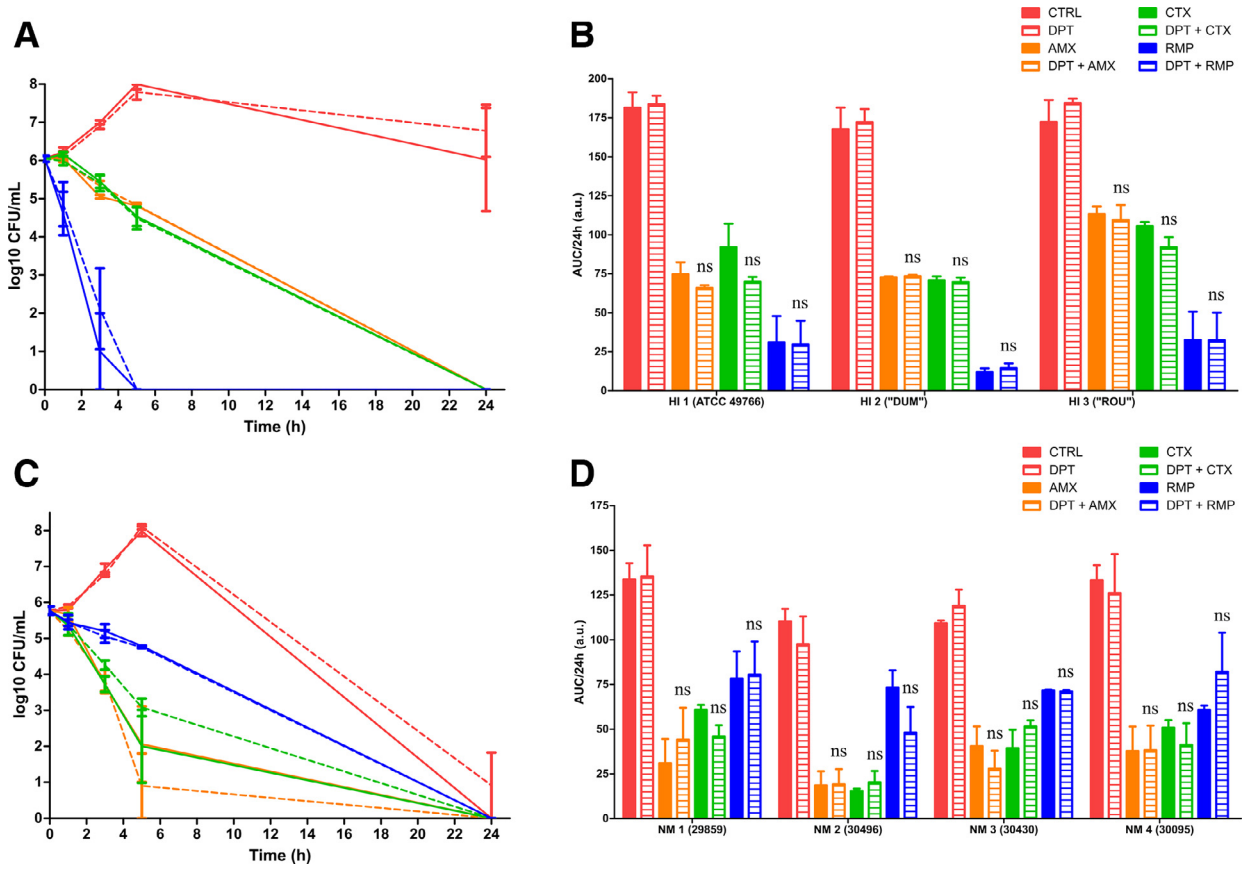

Fig. 2. Time–kill kinetics assay for Gram-negative bacteria. (A) Time–kill curves for *Haemophilus influenzae* HI 2 ("DUM"). (B) Integrated analysis of time–kill curves for *H. influenzae* (AUC/24 h). (C) Time–kill curves for *Neisseria meningitidis* NM 3 (30430). (D). Integrated analysis of time–kill curves for *N. meningitidis* (AUC/24 h). AUC, area under the curve; CTRL, control; DPT, daptomycin; AMX, amoxicillin; CTX, cefotaxime; RMP, rifampicin; ns, not significant ($P > 0.05$).

Gram-negative bacteria are available in Supplementary Fig. S1 and Fig. S2, respectively. For *S. pneumoniae*, analysis of time–kill curves demonstrates a rather quick bactericidal activity of daptomycin alone within a few hours only (Fig. 1A; Supplementary Fig. S1). Unfortunately, the selected experimental conditions and concentration were unable to reveal any synergistic effect due to the adjunction of daptomycin with either amoxicillin, cefotaxime or rifampicin against *S. pneumoniae*. Although not significant, the results from integrated time–kill curves on pneumococcus showed a general trend associated with an improved bactericidal activity of β-lactam antibiotics owing to the adjunction of daptomycin (Fig. 1B). Time–kill analysis of daptomycin-based associations towards *L. monocytogenes* confirmed a synergistic effect with rifampicin (Fig. 1C). Moreover, the data shown in Supplementary Fig. S1 also reveal a synergistic effect due to the association of daptomycin with either amoxicillin or cefotaxime against *L. monocytogenes* LM 2 (CLIP 2007/01481). The integration of time–kill curves illustrates once more a clearly improved bactericidal activity of daptomycin-based associations, no matter which antibiotic or *L. monocytogenes* genoserotype (Fig. 1D). The results from time–kill analysis for *H. influenzae* and *N. meningitidis* (Fig. 2) appear quite different from those obtained with Gram-positive bacteria. First, the time–kill curve from either Fig. 2A or Supplementary Fig. S2 demonstrates a strict indifference of *H. influenzae* regarding the adjunction of daptomycin, regardless of the antibiotic or the isolate. Similar conclusions can be drawn from the integrated data displayed in Fig. 2B. Interestingly, data from Fig. 2C,D confirm a comparable trend with daptomycin-based associations against *N. meningitidis*. No matter which meningococcus strain, the adjunction of daptomycin showed no antagonistic effect (Supplementary Fig. S2).

4. Discussion

This study brings additional arguments regarding the antimicrobial activity of daptomycin, alone or in association with either amoxicillin, cefotaxime or rifampicin, against selected bacterial clinical strains associated with invasive infection, mainly meningitis. As a whole, the adjunction of daptomycin was associated with no antagonistic effect on meningococcus, either indifference or mild synergy with *S. pneumoniae*, and synergy against *L. monocytogenes*.

First, the results from MIC tests regarding the activity of both β-lactam antibiotics and rifampicin towards *H. influenzae* were highly consistent with those described in the literature, CLSI's Subcommittee on Antimicrobial Susceptibility Testing as well as recent EUCAST 2021 guidelines [19]. Apart from one or a maximum of two serial dilutions, the reported MICs of β-lactam antibiotics and rifampicin towards *S. pneumoniae, L. monocytogenes* and *N. meningitidis* were comparable with those established by the National Reference Centers (data not shown). Regarding Gram-positive bacteria, the MICs of daptomycin were highly similar to those described from other groups, ranging from 1–8 μg/mL for *L. monocytogenes* [20] and ~0.25 μg/mL for *S. pneumoniae* [21,22]. For Gram-negative bacteria, *N. meningitidis* NM 4 (30095) was interpreted as intermediate to amoxicillin but considered resistant by the National Reference Center for Meningococci. Such slight difference might be explained by the possible discrepancies encountered with *N. menin-*

gitidis when switching from Etest to standard broth microdilution method, sometimes greater than 25% depending on the tested antibiotic [23].

Comparison of the results from FICI tests and time–kill analysis for Gram-positive bacteria highlighted a lack of strict correlation between the two methods. As an example, associations containing daptomycin showed a synergistic effect against *S. pneumoniae* with FICIs <0.5 with amoxicillin and cefotaxime but no significant synergy from time–kill kinetics assay. As reported previously and contrary to the dynamic view of time–kill analysis, the interpretation of FICI tests provides a static vision of antimicrobial associations [24]. Thereby, in the present case, both techniques should be considered complementary for a better understanding of daptomycin-based associations. All Gram-positive bacteria taken together, the results both from FICI and time–kill analysis seem to reassure a global improvement of β-lactam antimicrobial activity due to the adjunction of daptomycin. Unfortunately and probably due to the rapid bactericidal activity of daptomycin alone against *S. pneumoniae*, the dynamic synergistic effect of daptomycin associations was not fully demonstrated from the present time–kill results on pneumococcus. Interestingly, the adjunction of daptomycin to either amoxicillin, cefotaxime or rifampicin was not associated with any antagonistic effect against *H. influenzae* and *N. meningitidis*. Although encouraging, these preliminary results *in vitro* would require further confirmation in experimental models to assess the global safety of daptomycin-based associations and to confirm the exact mechanism of antimicrobial activity, notably their ability to decrease bacterial cell lysis.

Altogether, these results constitute reassuring *in vitro* confirmation paving the way to a potential use of daptomycin adjunction in the treatment of Gram-positive bacterial meningitis, without deleterious effect regarding the antimicrobial activity of β-lactam antibiotics towards *N. meningitidis*. Such additional *in vitro* data unveil critical supplementary information supporting the ongoing AddaMAP clinical trial, which seeks to show a possible benefit from the addition of daptomycin to the recommended treatment of pneumococcal meningitis.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.03.007.

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