

# Activity of linopristin-flopristin (NXL103) Against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*

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## INTRODUCTION

The major bacterial pathogens responsible for community-acquired respiratory tract infections comprise *Streptococcus pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis*. To this list must now be added community-acquired MRSA, especially post-influenza A.

Antibacterial resistance among these species is significantly impacting availability of preferred therapies. Pneumococcal resistance to penicillin G, other  $\beta$ -lactam, and non- $\beta$ -lactam compounds for example has increased worldwide at an alarming rate (7,11). The problem has been recently exacerbated by appearance of pan-resistant non-vaccine type 19A strains in children (otitis media) which are not amenable to treatment with any drug approved by the FDA (7,17). The predominant resistance mechanism of *Haemophilus spp.* is  $\beta$ -lactamase production but  $\beta$ -lactamase negative ampicillin resistant (BLNAR) strains are increasingly identified with accurate techniques (6, 11).

Previously most *S. aureus* infections have been nosocomial, but the past few years have witnessed a worrisome emergence of community-acquired strains many of which produce Panton-Valentine leukocidin (PVL) and cause serious and life-threatening infections including community-acquired pneumonia. Most of community MRSA are quinolone-resistant; and nosocomially-acquired MRSA strains are often multiresistant. Since the first report in 1995 of hVISA and VISA strains from Japan these strains have been described globally as well. The incidence of vancomycin non-susceptible MRSA is likely to be higher than currently reported (2, 10,12,13,16).

For empiric therapy of respiratory tract infections where either of these pathogens and resistant isolates might be causative there is a need for new effective antibiotics. Linopristin-flopristin (NXL103; XRP 2868) is an investigational oral streptogramin, being a 30:70 ratio of linopristin and flopristin (1, 8, 9). The current study test:

- 1) Activity of linopristin-flopristin, pristinamycin, quinupristin-dalfopristin, erythromycin A, azithromycin, clarithromycin and clindamycin against 261 pneumococci by agar dilution MIC;
- 2) Activity of all of the latter compounds except clindamycin against 150 *H. influenzae* and 26 *H. parainfluenzae* strains by microdilution MIC.
- 3) Activity of linopristin-flopristin compared to those of vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, azithromycin, clarithromycin, clindamycin, and quinupristin-dalfopristin against 200 strains of MRSA by agar dilution MIC.

## METHODS

### Bacteria

Pneumococci comprised 86 penicillin G susceptible (MICs  $\leq 0.06$  mg/l), 81 penicillin G intermediate (MICs 0.125-1.0 mg/l) and 94 penicillin G resistant (MIC 2.0-16.0 mg/l) strains. Of these, 120 were erythromycin A resistant (MICs  $\geq 1.0$  mg/l); 65 had the *erm(B)* gene and 32 had the *mef(A)* gene, 1 had *erm(B)* and *mef(A)*, 19 mutations in L4 and 3 mutations in 23S rRNA (A2059G). One hundred and fifty *H. influenzae* and 26 *H. parainfluenzae* strains were tested. *H. influenzae* strains comprised 146 recent nontypeable isolates and 4 type b strains.  $\beta$ -Lactamase testing was performed by the Cefinase disk method (BBL Microbiology systems, Cockeysville, Md).

The 200 recent MRSA isolates comprised 127 community and 40 hospital acquired organisms. Strains isolated from across the US, include AZ-1, CA-7, AK-7, CO-2, CT-4, FL-7, HI-5, IA-4, IN-5, KS-2, KY-8, ME-3, MI-4, MO-12, NC-1, NV-7, NJ-4, NY-4, OH-5, PA-5, TN-6, TX-7, UT-4, VT-4, VA-5, and WA-4. Of the 200 strains, 127 were obtained from JMI Labs, Liberty City, IA. We also included 3 Hershey hVISA strains, 25 VISA, and 5 VRSA organisms. Some of the latter two groups were obtained from NARSA through Eurofins Laboratories, Herndon, Va. Vancomycin non-susceptible strains were identified by overnight as well as macro Etest methodology, and hVISAs were confirmed by population analysis (2).

### Antibacterials and MIC testing.

Drug substances were obtained from Novexel Laboratories, Romainville, France. Agar dilution methodology, using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md) (supplemented with 5% sheep blood for pneumococci) was used. Calcium was added for daptomycin and fresh drug substance used for tigecycline testing (5).

For *Haemophilus* strains, MICs were determined by the CLSI microdilution method (5) using commercially prepared frozen panels (TREK, Inc., Cleveland, OH) using freshly prepared *Haemophilus* test medium. Inocula were prepared from chocolate agar plates incubated a full 24 h by the direct colony suspension method as recommended by CLSI. Trays were covered and incubated overnight at 35°C in ambient air (5,6).

## RESULTS

Results of agar dilution MICs with pneumococcal strains classified by penicillin G susceptibility in Table 2. As can be seen, linopristin-flopristin, pristinamycin and quinupristin-dalfopristin yielded low MICs irrespective of the strain's penicillin G or erythromycin A susceptibility status, with MICs ranging between 0.06-1.0 mg/l (linopristin-flopristin); 0.125-1.0 mg/l (pristinamycin);  $\leq 0.06$ -1.0 mg/l (quinupristin-dalfopristin). Streptogramin MICs against macrolide resistant strains were not influenced by erythromycin A resistance mechanisms. Macrolide MICs in ribosomal mutants were raised compared to the wild type. Streptogramin MICs were uninfluenced by macrolide susceptibility status or resistance mechanisms.

Pneumococcal MICs of erythromycin A, azithromycin, clarithromycin and clindamycin increased in concordance with those of penicillin G. Lower clindamycin MICs in erythromycin A resistant strains reflected presence of *mef* genes and ribosomal protein mutations: such strains were clindamycin-susceptible. Complete cross-resistance occurred between erythromycin A, azithromycin and clarithromycin.

Of the 150 *H. influenzae* strains, 79 produced  $\beta$ -lactamase. Of the 26 *H. parainfluenzae* strains tested, 8 were  $\beta$ -lactamase positive. Twenty-one of the 71  $\beta$ -lactamase negative *H. influenzae* strains were ampicillin resistant (MICs  $\geq 1.0$  mg/l) and were classified as BLNAR. Microdilution MICs are presented in Tables 3 and 4. There were no significant differences in MICs based upon  $\beta$ -lactamase production, ampicillin resistance or serotype. MICs of all compounds had a mono-modal distribution, with MIC<sub>50</sub> and MIC<sub>90</sub> values (mg/l) as follows: linopristin/flopristin, 0.25 and 1.0 mg/l; pristinamycin, 1.0 and 2.0 mg/l; quinupristin/dalfopristin, 2.0 and 4.0; mg/l; erythromycin A, 4.0 and 8.0 mg/l; azithromycin, 1.0 and 2.0 mg/l; clarithromycin, 8.0 and 16.0 mg/l. MICs for all compounds for *H. parainfluenzae* were generally 1-2 dilutions higher than those for *H. influenzae* and mono-modal distributions were observed.

Staphylococcal MICs are listed in Table 5. Linopristin-flopristin showed good activity against all vancomycin susceptible strains tested irrespective of phenotype with MICs ranging from 0.125-0.5 mg/l, and MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.25 and 0.25-0.5 mg/l, respectively. Against vancomycin non-susceptible strains, linopristin-flopristin had an MIC range of 0.06-2 mg/l, with MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.5 and 1 mg/l. All strains were susceptible to tigecycline, quinupristin-dalfopristin, and linezolid and all except 21 strains (all VISA) had non-susceptible daptomycin MICs between 2-4 mg/l. Previous reports have confirmed higher daptomycin MICs against VISA strains and the propensity of daptomycin therapy to select for such resistance phenotypes (12). All community-acquired strains, and the majority of others, were macrolide resistant.

## CONCLUSION

The results of this study support and broaden earlier work by other authors (8, 9) that demonstrated good antibacterial activity of the linopristin-flopristin combination against *S. pneumoniae*, *H. influenzae* and *S. aureus* of a variety of resistance phenotypes. Activity against *H. influenzae* is significantly greater than that of quinupristin-dalfopristin. Other reports have confirmed activity of other drugs tested in this study (3, 4,14,15,18).

If results of toxicity, PK/PD (1) and experimental animal studies are encouraging, this compound shows potential for treatment of community-acquired respiratory tract infections.

**Table 1.** Agar dilution MICs (mg/l) of 261 pneumococcal strains classified by penicillin susceptibility

Drug	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Penicillin G</b>			
Penicillin S (86)	0.016-0.06	0.03	0.03
Penicillin I (81)	0.125-1.0	0.25	1
Penicillin R (94)	2.0-16.0	2	4
<b>Linopristin-flopristin</b>			
Erythromycin A S	0.06-0.25	0.125	0.25
Erythromycin A R	0.06-1.0	0.25	0.5
<b>Pristinamycin</b>			
Penicillin S	0.125-0.5	0.25	0.25
Penicillin I	0.125-1.0	0.25	0.5
Penicillin R	0.125-1.0	0.25	0.5
<b>Quinupristin-Dalfopristin</b>			
Penicillin S	$\leq 0.06$ -1.0	0.5	0.5
Penicillin I	0.125-1.0	0.5	1
Penicillin R	0.125-1.0	0.5	1
<b>Erythromycin A</b>			
Penicillin S	0.016-64.0	0.125	32
Penicillin I	0.03-64.0	0.125	>64.0
Penicillin R	0.03-64.0	>64.0	>64.0
<b>Azithromycin</b>			
Penicillin S	0.03-64.0	0.125	16
Penicillin I	0.06-64.0	0.125	>64.0
Penicillin R	0.06-64.0	>64.0	>64.0
<b>Clarithromycin</b>			
Penicillin S	$\leq 0.008$ -64.0	0.03	8
Penicillin I	0.016-64.0	0.06	>64.0
Penicillin R	$\leq 0.008$ -64.0	32	>64.0
<b>Clindamycin</b>			
Penicillin S	$\leq 0.008$ -64.0	0.06	0.06
Penicillin I	0.016-64.0	0.06	>64.0
Penicillin R	0.016-64.0	0.06	>64.0

## REFERENCES

1. Andes, D., and W.A. Craig. 2006. Antimicrob. Agents Chemother. **50**: 243-249.
2. Appelbaum, P.C. 2007. Int. J. Antimicrob. Agents **30**: 398-408.
3. Bogdanovich, T, et al. 2005. Antimicrob. Agents Chemother. **49**:3325-3333.
4. Bryskier, A. 2000. Clin. Microbiol. Infect. **6**: 661-669.
5. Clinical and Laboratory Standards Institute 2006. M7-A7.
6. Credito, K.L., et al. 2001. Antimicrob. Agents Chemother. **45**: 67-72.
7. Critchley, I., et al. 2008. Antimicrob. Agents Chemother. **52**: 2639-2643.
8. Dupuis, M., and R. Leclercq. 2006. Antimicrob. Agents Chemother. **50**: 237-242.
9. Eliopoulos, G.M., et al. 2005. Antimicrob. Agents Chemother. **49**: 3034-3039.

**Table 2.** Agar dilution MICs (mg/l) of 261 pneumococcal strains classified by erythromycin A susceptibility

Drug	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Penicillin G</b>			
Erythromycin A S	0.016-4.0	0.125	2
Erythromycin A R	0.016-16.0	1	4
<b>Linopristin-flopristin</b>			
Erythromycin A S	0.06-0.25	0.125	0.25
Erythromycin A R	0.06-1.0	0.25	0.5
<b>Pristinamycin</b>			
Erythromycin A S	0.125-0.5	0.25	0.25
Erythromycin A R	0.125-1.0	0.25	0.5
<b>Quinupristin/Dalfopristin</b>			
Erythromycin A S	$\leq 0.06$ -1.0	0.5	0.5
Erythromycin A R	0.25-1.0	0.5	1
<b>Erythromycin A</b>			
Erythromycin S	0.016-0.25	0.06	0.125
Erythromycin R	1.0-64.0	>64.0	>64.0
<b>Azithromycin</b>			
Erythromycin A S	0.03-0.25	0.125	0.125
Erythromycin A R	1.0-64.0	>64.0	>64.0
<b>Clarithromycin</b>			
Erythromycin S	$\leq 0.008$ -0.125	0.03	0.06
Erythromycin R	0.25-64.0	>64.0	>64.0
<b>Clindamycin</b>			
Erythromycin A S	$\leq 0.008$ -0.125	0.06	0.06
Erythromycin A R	0.016-64.0	1	>64.0

**Table 3.** MICs (mg/l) of compounds against *Haemophilus influenzae*

Drug	$\beta$ -lactamase negative (50)			$\beta$ -lactamase positive (79)			BLNAR (21)			All strains (150)		
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Linopristin-flopristin	$\leq 0.06$ -1.0	0.25	0.5	$\leq 0.06$ -1.0	0.25	1	$\leq 0.06$ -1.0	0.25	0.5	$\leq 0.06$ -1.0	0.25	1
Pristinamycin	0.5-2.0	1	2	0.12-4.0	1	2	0.25-4.0	1	1	0.12-4.0	1	2
Quinupristin-Dalfopristin	1.0-8.0	2	4	0.25-8.0	4	4	1.0-8.0	2	4	0.25-8.0	2	4
Erythromycin A	2.0-16	4	8	0.5-16	4	8	1.0-16	4	8	0.5-16	4	8
Azithromycin	0.5-2.0	1	2	0.12-2.0	1	2	0.5-8.0	1	2	$\leq 0.12$ -8.0	1	2
Clarithromycin	4.0-32	8	16	1.0-64	8	8	4.0-16	4	16	1.0-64	8	16

**Table 4.** MICs (mg/l) of compounds against *Haemophilus parainfluenzae*

Drug	$\beta$ -lactamase negative (18)			$\beta$ -lactamase positive (8)			All strains (26)		
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Linopristin-flopristin	0.12-2.0	1	2	0.12-4.0	1	1	0.12-4.0	1	2
Pristinamycin	0.5-8.0	4	4	1.0-8.0	4	4	0.5-8.0	4	4
Quinupristin-Dalfopristin	1.0-16	8	16	2.0-32	8	16	1.0-32	8	16
Erythromycin A	1.0-8.0	2	4	1.0-8.0	4	4	1.0-8.0	2	4
Azithromycin	0.25-2.0	0.5	1	0.25-1.0	0.5	1	0.25-2.0	0.5	1
Clarithromycin	2.0-16	4	8	2.0-16	8	16	2.0-20	4	16

**Table 5.** MIC results (mg/l) for MRSA strains

Drug	Community-acquired			Hospital-acquired			hVISA+VISA+VRSA		
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Linopristin-flopristin	0.125-0.5	0.25	0.25	0.125-0.5	0.25	0.5	0.06-2	0.5	1
Vancomycin	0.5-1	1	1	0.5-2	1	1	1-128	4	32
Teicoplanin	0.25-2	1	1	0.25-1	0.5	1	1-32	8	16
Linezolid	4-Feb	4	4	4-Jan	4	4	4-Jan	2	4
Daptomycin	0.5-1	0.5	1	0.5-1	1	1	4-Jan	2	4
Tigecycline	0.25-0.25	0.25	0.25	0.125-0.5	0.25	0.5	0.06-1	0.25	0.5
Azithromycin	>8-8	>8	>8	1-8	>8	>8	1-8	>8	>8
Clarithromycin	>8-8	>8	>8	0.25-8	>8	>8	0.25-8	>8	>8
Clindamycin	0.125-0.25	0.25	0.25	0.125-0.16	0.25	>16	0.06-0.16	>16	>16
Quinu-Dalfo	0.25-1	0.5	0.5	0.25-1	0.25	1	0.125-2	0.5	1

10. Fergie, J.E., and K. Purcell. 2001. Pediatr. Infect. Dis. **30**: 860-863.
11. Jacobs, M.R., et al. 2003. J. Antimicrob. Chemother. **52**: 229-246.
12. Julian, K., et al. 2007. Antimicrob. Agents Chemother. **51**: 3445-3448.
13. Kazakova, S.V., et al. 2005. N. Engl. J. Med. **352**: 468-475.
14. Leclercq, R. 2001. J. Antimicrob. Chemother. **48** (Suppl. T1): 9-23.
15. Marchese, A., and G.C. Schito. 1999. Clin. Microbiol. Infect. **5**: 488-495.
16. Naimi, T.S., et al. 2003 JAMA **290**: 2976-2984.
17. Pichichero, M., and J.R. Casey. 2007. J. Am. Med. Assoc. **298**: 1772-1778.
18. Spangler, S.K., et al. Antimicrob. Agents Chemother. **36**: 856-859.