Tigecycline activity against macrolide resistance in *Streptococcus pneumoniae* mediated by *mefE* and *ermB* determinants: A Global Analysis 2008

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Revised Abstract

Background: Macrolide resistance in *S. pneumoniae* (MRSP) has increased steadily in the last decade. Two important determinants in MRSP are the *mefE* (clindamycin-susceptible, macrolide-resistant) and *ermB* (clindamycin-resistant, macrolide-resistant) genes. This report evaluates tigecycline and comparator drug activity from a global population of MRSP in clinical isolates collected in the T.E.S.T. program during the years 2004 to 2008. **Methods**: 4,937 *S. pneumoniae* clinical isolates were collected from 1,016 investigative sites in 52 countries and tested for erythromycin resistance. Clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by a reference laboratory using broth microdilution panels and interpreted according to CLSI guidelines. **Results**: Summary data for the 1,216/4,937 (24.6%) MRSP strains categorized by *ermB* and *mefE* determinants are as follows:

	ermB (n=699)				***mefE*** (n=517)			
Drug	**MIC_{50}**	**MIC_{90}**	**%Sus**	**%Res**	**MIC_{50}**	**MIC_{90}**	**%Sus**	**%Res**
Tigecycline	0.03	0.12	na	na	0.03	0.06	na	na
AmoxClav	0.5	8	71.8	10.7	0.25	2	94.4	1.5
Ceftriaxone	0.5	1	80.5	5.2	0.25	1	89.2	2.5
Clindamycin	>64	>64	0	100	0.06	0.12	100	0
Erythromycin	>64	>64	0	100	8	16	0	100
Levofloxacin	0.5	1	98.4	1.1	0.5	1	98.6	0.8
Linezolid	≤0.5	1	100	0	≤0.5	1	100	0
Penicillin	1	4	20.6	45.5	0.25	2	30.8	22.8

na = breakpoints not defined

Conclusions: Tigecycline demonstrated the lowest MIC_{50} and MIC_{90} *in vitro* values of all study drugs against macrolide-resistant *S. pneumoniae* with *ermB* determinants. Tigecycline *in vitro* activity suggests that tigecycline may be active against this important clinical pathogen and resistant phenotype.

Introduction

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in humans and is responsible for both respiratory tract infections, such as community acquired pneumonia (CAP), and invasive disease. Increasing prevalence of antimicrobial resistance among *S. pneumoniae* is a serious problem, and resistance to penicillin and macrolide antibiotics has been increasingly reported for *S. pneumoniae* in many countries. There are two major mechanisms of macrolide resistance in *S. pneumoniae*: efflux mediated by the *mefE* gene product and target site modification as a result of the *ermB* gene product. The *mefE* protein extrudes macrolides from the cytoplasm of *S. pneumoniae*. Efflux-positive *S. pneumoniae* usually have erythromycin MICs in the range of 1 to 32 mg/L but remain susceptible to clindamycin with MICs <0.5 mg/L (M-phenotype). The *ermB* gene product catalyses methylation of the adenine residue at the 2058 position of the 23S rRNA in the domain V of the 50S ribosomal subunit, the primary binding site of macrolides, thereby renders the binding site inactive. *ermB* –positive isolates display high-level resistance to macrolides (MICs >64 mg/L) and also to lincosamides such as clindamycin (MICs >16 mg/L) and streptogramin B (MLS_B phenotype) [1-6].

The tigecycline evaluation surveillance trial (T.E.S.T.) determines the *in vitro* activity of tigecycline and broad spectrum antimicrobial comparators against gram-negative and gram-positive species collected from over 320 hospitals globally 2004-2008.

As part of this program, this report evaluates the activity of tigecycline and comparators against a global collection of *S. pneumoniae* exhibiting macrolide resistance.

Materials & Methods

- *S. pneumoniae* T.E.S.T. program isolates were derived from blood and respiratory tract. Only one isolate per patient was accepted.
- Clinical isolates (n=4,937) were collected from 2004 to 2008 from 1,016 investigative sites globally.
- Custom broth microdilution panels were supplied by MicroScan (Dade Behring, West Sacramento, CA, USA) with the following antimicrobial agents and concentrations (expressed in mg/L): tigecycline (0.008-16); amoxicillin-clavulanic acid (0.12-32); azithromycin (0.03-64); clindamycin (0.015-64); levofloxacin (0.008-8); ceftriaxone (0.06-64); clarithromycin (0.015-64); erythromycin (0.015-64); imipenem (0.06-16); linezolid (0.5-8); and penicillin (0.06-8).
- MIC interpretive criteria followed published guidelines established by the CLSI where applicable. MIC interpretive criteria for tigecycline followed published guidelines established by the FDA where applicable.
- Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- *mefE* and *ermB* genotypes were determined phenotypically utilizing observed MICs to erythromycin and clindamycin. Macrolide-resistant/clindamycin-susceptible isolates were considered to be *mefE* positive and *ermB* negative. Macrolide-resistant/clindamycin-resistant isolates were considered to be *ermB* positive.
- Quality control of broth microdilution panels followed manufacturer's and CLSI guidelines using the following ATCC strains where applicable: *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922; *Escherichia coli* ATCC 35218; *Klebsiella pneumonia* ATCC 700603 (as positive ESBL control); *Haemophilus influenzae* ATCC 49247; *Haemophilus influenzae* ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *Pseudomonas aeruginosa* ATCC 27853.
- The collection and transportation of organisms, confirmation of identification, and construction and management of a centralized database were conducted and coordinated by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

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Results

Table 1. Comparative activity of antimicrobials against 1,413 macrolide-resistant[a] *S. pneumoniae* by source. % Resistant/MIC_{90} (mg/L).

Drug	**ALL (1,413)**	**Respiratory (1,035)**	**Blood (378)**
Tigecycline	NA (0.12)	NA (0.06)	NA (0.12)
Amox/Clav	6.2 (4)	7.2 (4)	4.5 (4)
Azithromycin	98.9 (>64)	99.1 (>64)	98.7 (>64)
Ceftriaxone	4.5 (1)	5.1 (1)	2.6 (1)
Clarithromycin	97.6 (>64)	97.6 (>64)	98.1 (>64)
Clindamycin	52.3 (16)	53.9 (>64)	49.5 (>64)
Erythromycin	100 (>64)	100 (>64)	100 (>64)
Imipenem	7.2 (0.5)	8.5 (1)	5.3 (0.5)
Levofloxacin	1.2 (1)	1.8 (1)	0 (1)
Linezolid	0 (1)	0 (1)	0 (1)
Penicillin	33 (4)	34.7 (4)	29.4 (4)

[a]Erythromycin MIC ≥1 mg/L
[b]Susceptibility breakpoints are defined by CLSI document M100-S18, 2008 where available. NA = not available. Tigecycline breakpoints for *S. pneumoniae* are undefined.

Table 2. Activity of tigecycline and comparators against *mefE* positive[a] *S. pneumoniae* (517). % S[b], I, R/$MIC_{50/90}$/mg/L.

Drug	**%S**	**%I**	**%R**	**MIC_{50}**	**MIC_{90}**
Tigecycline	NA	NA	NA	0.03	0.06
Amox/Clav	94.4	4.1	1.5	0.25	2
Azithromycin	1.2	2.3	96.5	8	64
Ceftriaxone	89.2	8.3	2.5	0.25	1
Clarithromycin	2.1	4.1	93.8	2	8
Clindamycin	100	0	0	0.06	0.12
Erythromycin	0	0	100	8	16
Imipenem	55.2	39.4	5.5	≤0.12	0.5
Levofloxacin	98.6	0.6	0.8	0.5	1
Linezolid	100	0	0	1	1
Penicillin	30.8	46.4	22.8	0.25	2

[a]Erythromycin resistant (MIC ≥1 mg/L) and clindamycin sensitive (MIC ≤0.25 mg/L).
[b]Susceptibility breakpoints are defined by CLSI document M100-S18, 2008 where available. NA = not available. Tigecycline breakpoints for *S. pneumoniae* are undefined.

Table 3. Activity of tigecycline and comparators against *ermB* positive[a] *S. pneumoniae* (699). % S[b], I, R/$MIC_{50/90}$/mg/L.

Drug	**%S**	**%I**	**%R**	**MIC_{50}**	**MIC_{90}**
Tigecycline	NA	NA	NA	0.03	0.12
Amox/Clav	71.8	17.5	10.7	0.5	8
Azithromycin	0	0	100	>64	>64
Ceftriaxone	80.5	14.3	5.2	0.5	1
Clarithromycin	0	0.1	99.9	>64	>64
Clindamycin	0	0	100	>64	>64
Erythromycin	0	0	100	>64	>64
Imipenem	50.8	39.8	10.2	0.25	1
Levofloxacin	98.4	0.4	1.1	1	1
Linezolid	100	0	0	≤0.5	1
Penicillin	20.6	33.9	45.5	1	4

[a]Erythromycin resistant (MIC ≥1 mg/L) and clindamycin resistant (MIC ≥1 mg/L).
[b]Susceptibility breakpoints are defined by CLSI document M100-S18, 2008 where available. NA = not available. Tigecycline breakpoints for *S. pneumoniae* are undefined.

Table 4. Activity of tigecycline and comparators vs. *S. pneumoniae* at erythromycin MICs (n=4,937).

	% Susceptible[a]							
Drug	≤0.25	0.5	1	2	4	8	16	≥32
Tigecycline	NA	NA	NA	NA	NA	NA	NA	NA
Amox/Clav	99.2	95.8	100	100	96.4	94.4	91.3	77.8
Azithromycin	99.9	54.2	20	3.2	0.9	0.4	0	0
Ceftriaxone	98.2	95.8	100	100	82.7	89.6	90.7	81.8
Clarithromycin	99.9	91.7	46.7	9.7	0.9	0.4	0	0
Clindamycin	99.9	95.8	100	93.5	97.3	95.2	97.3	21.1
Erythromycin	100	0	0	0	0	0	0	0
Imipenem	85.8	70.6	28.6	60.9	49.3	57	55.2	54.3
Levofloxacin	99.5	95.8	100	96.8	97.3	98.7	1	1
Linezolid	100	100	100	100	100	100	100	100
Penicillin	77.1	54.2	66.7	38.7	21.8	27.3	36.7	25.4

[a]Susceptibility breakpoints are defined by CLSI document M100-S18, 2008 where available. NA = not available. Tigecycline breakpoints for *S. pneumoniae* are undefined.

Conclusions

- Tigecycline demonstrated the lowest MIC_{90} of all drugs tested against macrolide resistant *S. pneumoniae* regardless of source (respiratory tract or blood). Lowest levels of resistance were for both levofloxacin and linezolid (no resistant breakpoints defined for tigecycline).
- Tigecycline MIC_{50} and MIC_{90} of 0.03 and 0.06 mg/L, respectively, was the lowest amongst drugs tested against *mefE* positive *S. pneumoniae*. Clindamycin $MIC_{50/90}$ of 0.06 and 0.12 mg/L demonstrate *in vitro* activity against *mefE* positive isolates.
- Tigecycline $MIC_{50/90}$ of 0.03 and 0.12 mg/L, respectively, was the lowest of all drugs tested against *ermB* positive *S. pneumoniae* that have clindamycin MIC ≥1 mg/L with a MIC_{90} of >64 mg/L.
- The *in vitro* activity of drugs such as amoxicillin/clavulanic acid, ceftriaxone, imipenem and penicillin as well as all macrolides, diminish due to *ermB* phenotypes.
- In this global study of susceptibilities in *S. pneumoniae*, 24.6% (1,216/4,937) of isolates were macrolide resistant. 40.5% of macrolide resistant isolates demonstrated the *mefE* phenotype, while 49.5% (699/1,413) of macrolide resistant isolates demonstrated the *ermB* phenotype. Remaining macrolide resistant isolates yielded indeterminate MICs.