Investigating the Interaction between Omadacycline and other Antibacterial Agents against Gram-positive and Gram-negative Bacteria

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BACKGROUND

- Omadacycline (OMC), a semi-synthetic derivative of the tetracycline class, is indicated for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia[1].
- OMC is currently undergoing evaluation in the US, in adults with uncomplicated urinary tract infections and acute pyelonephritis[2].
- Due to increasing antibiotic resistance and multidrug-resistance among pathogens, combination therapy is becoming more common.
- As a result, it is important to evaluate potential interactions between antimicrobial agents.
- In this study, the interaction between OMC and a variety of antibiotics was evaluated *in vitro* for *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium*.

METHODS

- The interaction between OMC and other agents was determined using checkerboard assays in which fractional inhibitory concentrations (FIC) and FIC indices (FICI) were calculated[3].
- Test isolates consisted of clinical isolates from the Micromyx (MMX) repository including reference isolates from the American Type Culture Collection (ATCC) and the Network on Antimicrobial Resistance in *S. aureus* (NARSA).
- A total of 6 isolates each of *E. coli* (including isolates with extended-spectrum β-lactamases [ESBL]), *S. aureus* (including prevalent PFGE types of community-acquired [CA] and hospital-acquired [HA] methicillin-resistant isolates [MRSA]), *S. pneumoniae* (including penicillin-intermediate [PISP] and penicillin-resistant isolates [PRSP]), and *Enterococcus* spp. (*E. faecalis* and *E. faecium* including vancomycin-resistant isolates [VRE]).
- Test agents included ampicillin (AMP), ceftazidime (CAZ), ceftriaxone (CRO), imipenem (IPM), piperacillin/tazobactam (P/T), gentamicin (GM), vancomycin (VAN), daptomycin (DAP), linezolid (LZD), and OMC.
- MIC and FIC values were determined by broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[4,5].
- An example of a checkerboard with growth indicating synergy is shown below:

		Agent X (µg/mL)											
		1	2	3	4	5	6	7	8	9	10	11	12
Agent Y (µg/mL)	A	64/8	32/8	16/8	8/8	4/8	2/8	4/8	0.5/8	0.25/8	0.12/8	0.06/8	0/8
	B	64/4	32/4	16/4	8/4	4/4	2/4	1/4	0.5/4	0.25/4	0.12/4	0.06/4	0/4
	C	64/2	32/2	16/2	8/2	4/2	2/2	1/2	0.5/2	0.25/2	0.12/2	0.06/2	0/2
	D	64/1	32/1	16/1	8/1	4/1	2/1	1/1	0.5/1	0.25/1	0.12/1	0.06/1	0/1
	E	64/0.5	32/0.5	16/0.5	8/0.5	4/0.5	2/0.5	1/0.5	0.5/0.5	0.25/0.5	0.12/0.5	0.06/0.5	0/0.5
	F	64/0.25	32/0.25	16/0.25	8/0.25	4/0.25	2/0.25	1/0.25	0.5/0.25	0.25/0.25	0.12/0.25	0.06/0.25	0/0.25
	G	64/0.12	32/0.12	16/0.12	8/0.12	4/0.12	2/0.12	1/0.12	0.5/0.12	0.25/0.12	0.12/0.12	0.06/0.12	0/0.12
	H	64/0	32/0	16/0	8/0	4/0	2/0	1/0	0.5/0	0.25/0	0.12/0	0.06/0	0/0

shaded cells indicate microbial growth

- FICI values were calculated by row as follows:
  $FIC_{agent\ X}$ = A/$MIC_{agent\ X}$, where A = the lowest inhibitory concentration of agent X in the row
  $FIC_{agent\ Y}$ = B/$MIC_{agent\ Y}$, where B = the lowest inhibitory concentration of agent Y in the row
  $FIC_{agent\ X}$ + $FIC_{agent\ Y}$ = FIC index (FICI)
  e.g., for row F in the above example, FICI = 0.25/2 + 0.25/2 = 0.12 + 0.12 = 0.24
- In the instance where an MIC of a test agent was off-scale, the MIC was set to the next highest 2-fold concentration for determination of the FIC.
- Mean FICI values were calculated using the FICI values across the rows of the checkerboard panel.
- FICI values were interpreted using criteria described by Odds[6] as follows: ≤0.5 = synergy, >0.5-4 = additive/indifferent, and >4 = antagonism.
- In select instances, time-kill (TK) assays were conducted to further evaluate the interaction between agents by testing them alone and in combination[3].
- For the TK assay, a target inoculum of 10^6 CFU/mL was used, OMC and the combination agent were evaluated at 0.25X and 0.5X the MIC alone and together, and viable bacteria were enumerated at 2, 4, 6, and 24 hr.
- An antagonistic/synergistic effect was defined as a 2-log increase or decrease, respectively, in log CFU/mL for the drugs tested together when compared to the log CFU/mL observed for the drugs alone[3].

RESULTS

- OMC and comparator activity and the resulting mean FICI values for OMC in combination with the comparators are shown for *E. coli* in **Table 1**, *S. aureus* in **Table 2**, *Enterococcus* spp. in **Table 3**, and *S. pneumoniae* in **Table 4**.
- Where the mean FICI value indicated synergy or antagonism, the cell is shaded green or red, respectively. Where the FICI in an individual row indicated synergy or antagonism, the mean FICI is shown in **green** or **red** font, respectively.
- OMC had MIC values of 0.5-4 µg/mL against *E. coli* including ESBL-positive isolates, 0.25-1 µg/mL against *S. aureus* including MRSA, and 0.015-0.12 µg/mL against *S. pneumoniae* and enterococci including VRE.
- Indifferent mean FICI values were observed for OMC in combination with all agents and all isolates excluding 3 of 6 *S. aureus* and 1 of 3 *E. faecium* where antagonism with IPM and CRO, respectively, was observed by mean FICI.

Table 1. Activity of OMC and comparators alone and in combination - *E. coli*

Drug	ATCC 25922 non-ESBL		MMX 6411 non-ESBL		MMX 2232 ESBL		MMX 2267 ESBL		MMX 2269 ESBL		MMX 2499 ESBL	
	MIC (QC)	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI
AMP	8 (2-8)[a]	1.00	8	1.12	>64	1.29	>64	1.23	>64	1.29	>64	1.37
CAZ	0.5 (0.06-0.5)	0.74	0.25	1.11	4	1.04	2	1.05	16	0.75	1	1.12
CRO	0.06 (0.03-0.12)	1.12	0.03	1.87	>64	1.12	>64	1.02	>64	1.12	>64	1.12
IPM	0.12 (0.06-0.24)	1.91	0.12	0.62	0.12	1.12	0.06	1.23	0.12	1.37	0.25	0.85
P/T	4/4 (1/4-4/4)	0.62	2/4	0.75	16/4	2.37	4/4	1.62	2/4	1.12	2/4	1.12
GM	0.5 (0.25-1)	0.74	2	0.53	0.5	2.04	32	1.73	0.5	1.12	0.5	1.24
VAN	>64	1.37	>64	1.12	>64	1.29	>64	1.23	>64	1.00	>64	1.37
DAP	>64	1.37	>64	1.29	>64	1.23	>64	1.19	>64	1.29	>64	1.29
LZD	>64	1.37	>64	1.87	>64	1.29	>64	1.23	>64	1.29	>64	1.37
OMC[b]	0.5 (0.25-2)	NA	0.5-1	NA	1-2	NA	1-4	NA	0.5-1	NA	0.5-1	NA

[a] CLSI QC range shown in parentheses
[b] MIC range for all OMC MIC results (n=9/isolate) during FIC testing

Table 2. Activity of OMC and comparators alone and in combination - *S. aureus*

Drug	ATCC 29213 MSSA		MMX 7789 MSSA		NRS387 HA-MRSA (USA800)		NRS382 HA-MRSA (USA100)		NRS123 CA-MRSA (USA400)		MMX3982 CA-MRSA (USA300)	
	MIC (QC)	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI
AMP	1 (0.5-2)[a]	1.10	16	0.68	>64	0.70	64	0.85	64	1.04	>64	0.68
CAZ	16 (4-16)	0.94	16	1.10	>64	1.23	>64	1.19	>64	1.10	>64	1.10
CRO	4 (1-8)	0.90	2	1.60	>64	0.85	>64	1.19	>64	1.23	>64	1.04
IPM	0.015 (0.015-0.06)	1.23	0.015	1.11	0.5	7.35	16	1.69	0.5	5.73	1	7.35
P/T	1/4 (0.25/4-2/4)	0.63	1/4	0.88	8/4	3.23	128/4	1.09	64/4	1.10	32/4	1.74
GM	0.25 (0.12-1)	1.19	1	0.54	0.5	0.99	0.25	1.09	0.25	0.89	0.5	0.57
VAN	1 (0.5-2)	1.19	1	1.23	1	1.09	2	0.99	1	1.09	1	1.23
DAP	0.5 (0.12-1)	1.19	0.5	1.19	0.25	1.99	0.5	2.00	0.5	1.39	0.5	1.19
LZD	4 (1-4)	1.09	2	1.10	4	1.04	2	1.09	2	1.23	2	1.10
OMC[b]	0.25-0.5 (0.12-1)	NA	0.25-0.5	NA	0.25-0.5	NA	0.5-1	NA	0.25-0.5	NA	0.25-0.5	NA

[a] CLSI QC range shown in parentheses
[b] MIC range for all OMC MIC results (n=9/isolate) during FIC testing

Table 3. Activity of OMC and comparators alone and in combination - Enterococci

Drug	*E. faecalis* ATCC 29212 VSE		*E. faecalis* MMX 4155 VRE		*E. faecalis* MMX 4157 VRE		*E. faecium* MMX 4182 VSE		*E. faecium* MMX 752 VRE		*E. faecium* MMX 3849 VRE	
	MIC (QC)	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI
AMP	1 (0.5-2)[a]	0.99	1	0.65	1	0.88	1	1.13	64	1.13	>64	1.13
CAZ	>64	0.83	>64	1.38	>64	1.13	>64	1.29	>64	1.29	>64	1.88
CRO	>64	0.35	>64	0.91	>64	1.04	2	42.96	>64	1.29	>64	1.38
IPM	0.5 (0.5-2)	0.89	0.5	0.83	0.5	0.83	2	1.04	>64	0.80	>64	1.38
P/T	2/4 (1/4-4/4)	0.99	2/4	1.13	2/4	1.04	8/4	1.79	>256/4	1.11	>256/4	1.38
GM	16 (4-16)	0.74	4	1.13	>64	1.29	8	1.29	>64	1.29	8	1.38
VAN	2 (1-4)	1.24	>64	1.29	>64	1.24	0.5	0.79	>64	1.29	>64	1.38
DAP	2 (1-4)	0.99	0.5	1.29	1	1.13	4	2.96	2	1.96	2	1.88
LZD	2 (1-4)	1.24	1	1.29	1	1.29	2	0.88	2	0.71	1	1.38
OMC[b]	0.12 (0.06-0.5)	NA	0.03-0.06	NA	0.06-0.12	NA	0.06	NA	0.06-0.12	NA	0.03	NA

[a] CLSI QC range shown in parentheses
[b] MIC range for all OMC MIC results (n=9/isolate) during FIC testing

Table 4. Activity of OMC and comparators alone and in combination – *S. pneumoniae*

Drug	ATCC 49619 PISP		MMX 7951 PISP		MMX 7964 PISP		MMX 7839 PSSP		MMX 7812 PRSP		MMX 7830 PRSP	
	MIC (QC)	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI	MIC	mean FICI
AMP	0.12 (0.06-0.25)[a]	0.88	1	0.90	0.5	0.82	0.03	1.01	16	0.89	16	0.89
CAZ	1	0.87	2	1.05	1	3.10	0.25	1.01	32	0.87	32	0.89
CRO	0.06 (0.03-0.12)	1.13	0.12	1.11	0.25	0.94	0.015	1.01	2	0.82	1	1.02
IPM	0.03 (0.03-0.12)	1.13	0.06	0.90	0.03	0.85	0.004	0.80	1	0.89	0.5	1.30
P/T	0.5/4	1.30	1/4	0.93	0.5/4	0.81	0.015/4	1.01	4/4	1.05	4/4	1.02
GM	16	1.05	16	0.83	4	1.05	2	2.40	8	1.11	16	0.80
VAN	0.25 (0.12-0.5)	1.05	0.5	0.67	0.5	1.10	0.25	1.40	0.25	1.55	0.25	1.14
DAP	0.25 (0.06-0.5)	1.11	0.12	1.24	0.12	1.20	0.06	2.30	0.12	1.51	0.12	1.24
LZD	1 (0.25-2)	1.02	1	1.11	1	0.91	0.5	1.13	0.5	1.05	1	0.89
OMC[b]	0.03-0.06 (0.015-0.12)	NA	0.06-0.12	NA	0.12	NA	0.015-0.03	NA	0.06	NA	0.06	NA

[a] CLSI QC range shown in parentheses
[b] MIC range for all OMC MIC results (n=9/isolate) during FIC testing

RESULTS

- Where antagonism was observed by mean FICI, the interaction was further evaluated by TK as shown in **Figure 1-4** for OMC combined with IPM against MRSA and in **Figure 5** for OMC combined with CRO against *E. faecium*.
- For OMC combined with IPM, the overall interaction by TK was indifferent with the exception of NRS382 (**Figure 2**) where synergy was observed at 24 hr.
- Antagonism was only observed at 6 hr for OMC and IPM against NRS387 at 0.5X the MIC **(Figure 3),** but was not observed at any other timepoint.
- No antagonism was apparent for OMC combined with CRO against *E. faecium* (**Figure 5**).
- Similar results were observed when testing IPM at 0.25X the MIC combined with OMC at either 0.25X the MIC or 0.5X the MIC (**data not shown**).

Figure 1. Activity of OMC and IPM alone and in combination – *S. aureus* NRS123

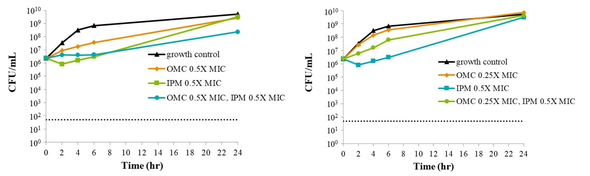


Figure 2. Activity of OMC and IPM alone and in combination – *S. aureus* NRS382

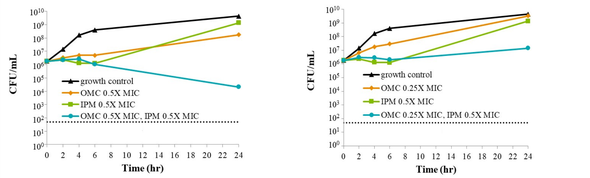


Figure 3. Activity of OMC and IPM alone and in combination – *S. aureus* NRS387

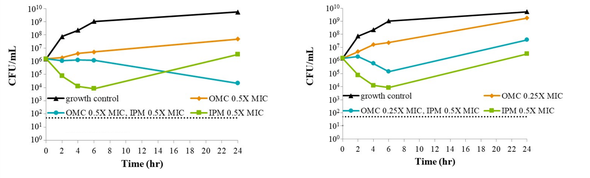


Figure 4. Activity of OMC and IPM alone and in combination – *S. aureus* MMX 3982

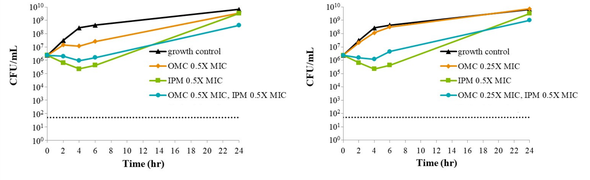
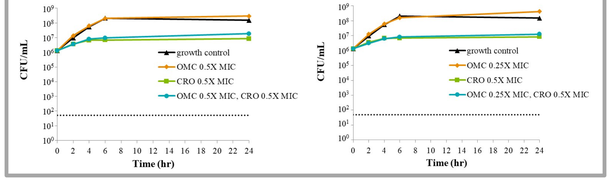


Figure 5. Activity of OMC and CRO alone and in combination – *E. faecium* MMX 4182



CONCLUSIONS

- OMC demonstrated potent activity against *E. coli*, *S. aureus*, *S. pneumoniae,* and enterococci including isolates with important resistance phenotypes (e.g. ESBL, MRSA, PRSP, and VRE).
- This activity was largely not affected when OMC was tested in combination with other agents; typically during FIC testing, FICI values indicated additive or indifferent interactions.
- In the rare instances where mean FICI values indicated antagonism, the antagonism was not confirmed by subsequent TK analysis.
- There is no apparent *in vitro* signal for synergy or antagonism between OMC and the other evaluated agents.

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