Minocycline Activity Tested Against *Acinetobacter baumannii*, *Burkholderia cepacia* Species Complex, *Stenotrophomonas maltophila*, and Select Enterobacteriaceae Isolates from a European Surveillance Program (2013)

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Introduction

Acinetobacter spp. may be found as causes of infections in critically ill patients with comorbidities and in soldiers returning with combat injuries from the Middle East. These organisms are frequently multidrug-resistant (MDR) and there are limited choices of antimicrobials available which would be active against this bacterium, and thus provide a potential therapeutic option. Resistance in these organisms may occur due to intrinsic mechanisms (cephalosporinase and efflux/permeability) or to acquired mechanisms. The usefulness of carbapenems against these organisms has diminished with the widespread occurrence of carbapenamase mediated resistance. Polymyxins may need to be used, although there are questions about appropriate dosing, resistance development and toxicity.

The tetracyclines, discovered in the 1940s, were an early class of broad-spectrum of antimicrobials with activity against Gram-positive and -negative bacteria. Second-generation semisynthetic compounds such as doxycycline (1967) and minocycline (1972) with improved oral bioavailability were developed. In 2005, the intravenous formulation of minocycline was voluntarily withdrawn in the United States, but was reintroduced in 2009 as it is one of the few treatments with USA-FDA approval to treat *Acinetobacter* infections.

This study evaluated the contemporary activity of minocycline tested against contemporary *A. baumannii* and other Gram-negative (GN) bacilli collected at European centres during 2013.

Materials and Methods

Organisms: Isolates of *A. baumannii* (389), *Stenotrophomonas maltophila* (154), *Burkholderia cepacia* spp. complex (3), and Enterobacteriaceae (500) were selected from medical centres in Europe and the Mediterranean region. Countries and numbers of sites: Belgium (1), Bulgaria (2), Croatia (1), Czech Republic (2), France (2), Germany (6), Greece (1), Hungary (1), Ireland (2), Israel (1), Italy (4), Poland (3), Portugal (1), Romania (1), Russia (3), Slovakia (1), Slovenia (1), Spain (3), Sweden (2), Turkey (6), United Kingdom (3) and Ukraine (1). Only one isolate per infected patient episode was included and local identifications were confirmed by the monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) using biochemical methods, the VITEK 2 System (bioMerieux, Hazelwood, Missouri, USA) and MALDI-TOF (Bruker Daltonics, Bellerica, MA, USA) as necessary. CLSI interpretive criteria were applied when EUCAST criteria were not available. MDR were classified based on published recommendations (Magiorakos et. al.) adapted by Farrell et al.

Susceptibility Testing: All isolates were susceptibility tested using the reference broth microdilution method as described by the CLSI. MDR and extensively drug-resistant (XDR) Enterobacteriaceae and *A. baumannii* were classified as such per recently recommended guidelines using the following antimicrobial class representative agents and EUCAST interpretive criteria (CLSI criteria where EUCAST criteria were not available): for Enterobacteriaceae; ceftriaxone (≥2 mg/L), meropenem (≥4 mg/L), piperacillin/tazobactam (≥16/2 mg/L), levofloxacin (≥2 mg/L), gentamicin (≥4 mg/L), tigecycline (≥2 mg/L), and colistin (≥4 mg/L); and for *A. baumannii*; ceftazidime (≥16 mg/L; CLSI criteria), meropenem (≥4 mg/L), piperacillin/tazobactam (≥32/4 mg/L; CLSI criteria), levofloxacin (≥2 mg/L), gentamicin (≥8 mg/L), colistin (≥4 mg/L), trimethoprim/sulfamethoxazole (≥4/76 mg/L) and tetracycline (≥8 mg/L; CLSI criteria). Classifications were based on the following recommended parameters:
MDR = nonsusceptible to ≥1 agent in ≥3 antimicrobial classes; XDR = nonsusceptible to ≥1 agent in all but ≤2 antimicrobial classes.

Results

- Based on the definition of non-susceptible to ≥1 agent in ≥3 antimicrobial classes, 92.8% of *A. baumannii* and 14.6% of Enterobacteriaceae were MDR (Table 1).
- Colistin was the most active agent tested against all *A. baumannii* with a MIC_{90} value of 2 mg/L (94.1% susceptible; Table 2). Minocycline was the second most active agent and the most active "tetracycline" with a MIC_{50} value of 4 mg/L (58.6% susceptible). Susceptibility to doxycycline was lower at 35.2% and tetracycline susceptibility was further diminished at 14.9%. Susceptibility to gentamicin/amikacin/levofloxacin was 18.0/17.3/8.2%, respectively.
- Among the MDR *A. baumannii*, colistin was the most active agent exhibiting a MIC_{90} value of 2 mg/L (93.6% susceptible; Table 2). Minocycline was the second most active agent exhibiting 55.4% susceptibility. Poorer susceptibility was noted with doxycycline (30.2%) and tetracycline (8.9%).
- The majority of *A. baumanni* were XDR (80.5%; Table 1). Colistin was the most active agent (MIC_{90}, 2 mg/L; 92.6% susceptible) and minocycline was the second most active agent (MIC_{50}, 8 mg/L; 49.8% susceptible) and the most active "tetracycline" (Table 2).
- The MIC results for minocycline for the three *Burkholderia cepacia* spp. complex isolates ranged from 2-4 mg/L for minocycline, 4->8 mg/L for doxycycline and 4->32 mg/L for tetracycline (Table 3).
- The most active agent against *S. maltophilia*, (MIC_{90}, 1 mg/L; 98.7% susceptible) was minocycline (Table 3). Doxycycline was slightly less active (MIC_{90}, 4 mg/L; 94.8% susceptible) and tetracycline susceptibility was markedly lower at only 4.5% (MIC_{90}, 32 mg/L).
- Against Enterobacteriaceae, meropenem, tigecycline and gentamicin exhibited susceptibility rates of 97.2, 96.4 and 89.8%, respectively. A total of 85.6% (MIC_{90}, 8 mg/L) of Enterobacteriaceae were susceptible to minocycline, 76.6% (MIC_{90}, >8 mg/L) to doxycycline and 66.4% (MIC_{90}, >32 mg/L) to tetracycline. MIC_{50} values for minocycline for *Escherichia coli* (87.0% susceptible), *Klebsiella* spp. (83.0% susceptible), *Enterobacter* spp. (79.0% susceptible), *Serratia* spp. (88.0% susceptible), and *Citrobacter* spp. (91.0% susceptible), ranged from 1-2 mg/L (MIC_{90}, >8 mg/L except *Citrobacter* spp. [4 mg/L]) (data not shown).
- For the MDR Enterobacteriaceae, tigecycline, meropenem and colistin were the most active agents at 82.2, 80.8, and 72.2% susceptible, respectively (Table 4). Minocycline was the next most active agent exhibiting 49.3% susceptibility with doxycycline and tetracycline susceptibility at 41.1 and 32.9%, respectively.

Table 1. Summary of minocycline activity tested against selected Gram-negative bacterial isolates from European and Mediterranean region medical centers (2013).

Organism	No. of Isolates	No. of organisms (cumulative %) inhibited at minocycline MIC in mg/L of: ≤0.06	0.12	0.25	0.5	1	2	4	8	> 8	MIC_{50}	MIC_{90}
Acinetobacter baumannii	389	21(5.4)	18 (10.0)	27 (17.0)	27 (23.9)	33 (32.4)	39 (42.4)	63 (58.6)	74 (77.6)	87 (100.0)	4	> 8
MDR	361	4 (1.1)	12 (4.4)	21 (10.3)	27 (17.8)	33 (26.9)	39 (37.8)	63 (55.3)	74 (75.8)	87 (100.0)	4	> 8
XDR	313	2 (0.6)	6 (2.5)	10 (5.8)	20 (12.1)	29 (21.4)	32 (31.6)	57 (49.8)	73 (73.2)	84 (100.0)	4	> 8
Burkholderia cepacia complex	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	2 (100.0)	--	--	4	--
Stenotrophomonas maltophilia	154	1 (0.6)	22 (14.9)	53 (49.4)	45 (78.6)	21 (92.2)	8 (97.4)	2 (98.7)	2 (100.0)	--	0.5	1
Enterobacteriaceae	500	0 (0.0)	0 (0.0)	12 (2.4)	79 (18.2)	109 (40.0)	141 (68.2)	87 (85.6)	30 (91.6)	42 (100.0)	2	8
MDR	73	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (11.0)	8 (21.9)	20 (49.3)	16 (71.2)	21 (100.0)	8	> 8
XDR	13	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	3 (30.8)	0 (30.8)	9 (100.0)	> 8	> 8
Escherichia coli	100	0 (0.0)	0 (0.0)	5 (5.0)	40 (45.0)	26 (71.0)	8 (79.0)	8 (87.0)	2 (89.0)	11 (100.0)	1	> 8
Klebsiella spp.	100	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.0)	28 (31.0)	34 (65.0)	18 (83.0)	8 (91.0)	9 (100.0)	2	8
Enterobacter spp.	100	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22 (22.0)	44 (66.0)	13 (79.0)	9 (88.0)	12 (100.0)	2	> 8
Serratia spp.	100	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	12 (13.0)	37 (50.0)	38 (88.0)	8 (96.0)	4 (100.0)	2	8
Citrobacter spp.	100	0 (0.0)	0 (0.0)	7 (7.0)	35 (42.0)	21 (63.0)	18 (81.0)	10 (91.0)	3 (94.0)	6 (100.0)	1	4

Table 2. Activity of minocycline and comparator agents against *Acinetobacter baumannii*.

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
Acinetobacter baumannii (389)				
Minocycline	4	>8	58.6 / 19.0 / 22.4	58.6 / 19.0 / 22.4
Doxycycline	>8	>8	35.2 / 1.6 / 63.2	35.2 / 1.6 / 63.2
Tetracycline	>32	>32	14.9 / 7.7 / 77.4	14.9 / 7.7 / 77.4
Piperacillin/tazobactam	>64	>64	6.7 / 1.3 / 92.0	- / - / -
Ceftazidime	>32	>32	8.0 / 1.5 / 90.5	- / - / -
Meropenem	>8	>8	13.1 / 2.1 / 84.8	13.1 / 2.1 / 84.8
Amikacin	>32	>32	17.8 / 4.6 / 77.6	17.3 / 0.5 / 82.2
Gentamicin	>8	>8	18.0 / 7.4 / 74.6	18.0 / 0.0 / 82.0
Tobramycin	>16	>16	44.8 / 0.8 / 54.4	44.8 / 0.0 / 55.2
Levofloxacin	>4	>4	8.5 / 6.9 / 84.6	8.2 / 0.3 / 91.5
Trimethoprim/sulfamethoxazole	>4	>4	24.2 / 0.0 / 75.8	24.2 / 6.4 / 69.4
Colistin	1	2	94.1 / 0.0 / 5.9	94.1 / 0.0 / 5.9
MDR *A. baumannii* (361)				
Minocycline	4	>8	55.4 / 20.5 / 24.1	55.4 / 20.5 / 24.1
Doxycycline	>8	>8	30.2 / 1.7 / 68.1	30.2 / 1.7 / 68.1
Tetracycline	>32	>32	8.9 / 8.0 / 83.1	8.9 / 8.0 / 83.1
Piperacillin/tazobactam	>64	>64	0.3 / 1.1 / 98.6	- / - / -
Ceftazidime	>32	>32	1.4 / 1.4 / 97.2	- / - / -
Meropenem	>8	>8	6.4 / 2.2 / 91.4	6.4 / 2.2 / 91.4
Amikacin	>32	>32	11.7 / 5.0 / 83.3	11.4 / 0.3 / 88.3
Gentamicin	>8	>8	12.2 / 8.0 / 79.8	12.2 / 0.0 / 87.8
Tobramycin	>16	>16	40.8 / 0.9 / 58.3	40.8 / 0.0 / 59.2
Levofloxacin	>4	>4	1.4 / 7.5 / 91.1	1.1 / 0.3 / 98.6
Trimethoprim/sulfamethoxazole	>4	>4	18.6 / 0.0 / 81.4	18.6 / 6.6 / 74.8
Colistin	1	2	93.6 / 0.0 / 6.4	93.6 / 0.0 / 6.4
XDR *A. baumannii* (313)				
Minocycline	8	>8	49.8 / 23.4 / 26.8	49.8 / 23.4 / 26.8
Doxycycline	>8	>8	24.0 / 1.9 / 74.1	24.0 / 1.9 / 74.1
Tetracycline	>32	>32	3.8 / 7.7 / 88.5	3.8 / 7.7 / 88.5
Piperacillin/tazobactam	>64	>64	0.0 / 0.6 / 99.4	- / - / -
Ceftazidime	>32	>32	0.3 / 1.3 / 98.4	- / - / -
Meropenem	>8	>8	2.6 / 2.5 / 94.9	2.6 / 2.5 / 94.9
Amikacin	>32	>32	9.3 / 5.7 / 85.0	8.9 / 0.4 / 90.7
Gentamicin	>8	>8	5.1 / 8.6 / 86.3	5.1 / 0.0 / 94.9
Tobramycin	>16	>16	33.0 / 0.7 / 66.3	33.0 / 0.0 / 67.0
Levofloxacin	>4	>4	0.3 / 6.7 / 93.0	0.0 / 0.3 / 99.7
Trimethoprim/sulfamethoxazole	>4	>4	11.5 / 0.0 / 88.5	11.5 / 7.0 / 81.5
Colistin	1	2	92.6 / 0.0 / 7.4	92.6 / 0.0 / 7.4

a. Criteria as published by the CLSI [2015] and EUCAST [2015].

Table 3. Activity of minocycline and comparator agents tested against *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia*.

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
Burkholderia cepacia species complex (3)[a]				
Minocycline	4	-	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Doxycycline	8	-	33.3 / 33.4 / 33.3	33.3 / 33.4 / 33.3
Tetracycline	>32	-	33.3 / 0.0 / 66.7	33.3 / 0.0 / 66.7
Ceftazidime	1	-	66.7 / 33.3 / 0.0	- / - / -
Levofloxacin	2	-	100.0 / 0.0 / 0.0	- / - / -
Trimethoprim/ sulfamethoxazole	≤0.5	-	100.0 / 0.0 / 0.0	- / - / -
Stenotrophomonas maltophilia (154)				
Minocycline	0.5	1	98.7 / 1.3 / 0.0	98.7 / 1.3 / 0.0
Doxycycline	2	4	94.8 / 3.9 / 1.3	94.8 / 3.9 / 1.3
Tetracycline	16	32	4.5 / 26.7 / 68.8	4.5 / 26.7 / 68.8
Ceftazidime	32	>32	33.6 / 10.5 / 55.9	- / - / -
Levofloxacin	1	>4	78.7 / 9.3 / 12.0	- / - / -
Trimethoprim/ sulfamethoxazole	≤0.5	>4	87.7 / 0.0 / 12.3	89.0 / 0.0 / 11.0

a. Criteria as published by the CLSI [2015] and EUCAST [2015].

Table 4. Activity of minocycline and comparator agents tested against Enterobacteriaceae.

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
Enterobacteriaceae (500)[b]				
Minocycline	2	8	85.6 / 6.0 / 8.4	85.6 / 6.0 / 8.4
Doxycycline	2	>8	76.6 / 8.8 / 14.6	76.6 / 8.8 / 14.6
Tetracycline	2	>32	66.4 / 7.2 / 26.4	66.4 / 7.2 / 26.4
Ceftriaxone	0.12	>8	76.8 / 0.6 / 22.6	76.8 / 0.6 / 22.6
Ceftazidime	0.25	32	81.2 / 2.6 / 16.2	79.4 / 1.8 / 18.8
Meropenem	≤0.06	≤0.06	97.2 / 0.0 / 2.8	97.2 / 0.8 / 2.0
Piperacillin/tazobactam	4	64	85.2 / 7.6 / 7.2	80.8 / 4.4 / 14.8
Levofloxacin	≤0.12	>4	85.4 / 2.0 / 12.6	83.6 / 1.8 / 14.6
Gentamicin	≤1	4	90.4 / 0.4 / 9.2	89.8 / 0.6 / 9.6
Tigecycline	0.25	1	99.0 / 1.0 / 0.0	96.4 / 2.6 / 1.0
Colistin	0.5	>8	- / - / -	78.5 / 0.0 / 21.5
MDR Enterobacteriaceae (73)[c]				
Minocycline	8	>8	49.3 / 21.9 / 28.8	49.3 / 21.9 / 28.8
Doxycycline	8	>8	41.1 / 11.0 / 47.9	41.1 / 11.0 / 47.9
Tetracycline	32	>32	32.9 / 10.9 / 56.2	32.9 / 10.9 / 56.2
Ceftriaxone	>8	>8	8.2 / 2.8 / 89.0	8.2 / 2.8 / 89.0
Ceftazidime	32	>32	26.0 / 8.2 / 65.8	19.2 / 6.8 / 74.0
Meropenem	≤0.06	>8	80.8 / 0.0 / 19.2	80.8 / 5.5 / 13.7
Piperacillin/tazobactam	64	>64	27.8 / 33.3 / 38.9	12.5 / 15.3 / 72.2
Levofloxacin	>4	>4	34.2 / 8.3 / 57.5	24.7 / 9.5 / 65.8
Gentamicin	8	>8	49.3 / 1.4 / 49.3	45.2 / 4.1 / 50.7
Tigecycline	0.5	2	94.5 / 5.5 / 0.0	82.2 / 12.3 / 5.5
Colistin	0.5	>8	- / - / -	72.2 / 0.0 / 27.8
XDR Enterobacteriaceae (13)[d]				
Minocycline	>8	>8	30.8 / 0.0 / 69.2	30.8 / 0.0 / 69.2
Doxycycline	>8	>8	23.1 / 0.0 / 76.9	23.1 / 0.0 / 76.9
Tetracycline	>32	>32	23.1 / 0.0 / 76.9	23.1 / 0.0 / 76.9
Ceftriaxone	>8	>8	0.0 / 7.7 / 92.3	0.0 / 7.7 / 92.3
Ceftazidime	>32	>32	15.4 / 7.7 / 76.9	15.4 / 0.0 / 84.6
Meropenem	8	>8	30.8 / 0.0 / 69.2	30.8 / 30.7 / 38.5
Piperacillin/tazobactam	>64	>64	7.7 / 15.4 / 76.9	0.0 / 7.7 / 92.3
Levofloxacin	>4	>4	7.7 / 0.0 / 92.3	0.0 / 7.7 / 92.3
Gentamicin	>8	>8	30.8 / 7.7 / 61.5	15.4 / 15.4 / 69.2
Tigecyclinec	1	4	84.6 / 15.4 / 0.0	61.5 / 23.1 / 15.4
Colistin	2	>8	- / - / -	53.8/ 0.0 / 46.2

a. Criteria as published by the CLSI [2015] and EUCAST [2015].

b. Includes: *Citrobacter braakii* (six strains), *C. freundii* (28 strains), *C. freundii* species complex (10 strains), *C. koseri* (55 strains), *C. youngae* (one strain), *Enterobacter aerogenes* (22 strains), *E. asburiae* (one strain), *E. cloacae* (60 strains), *E. cloacae* species complex (17 strains), *Escherichia coli* (100 strains), *Klebsiella oxytoca* (20 strains), *K. pneumoniae* (80 strains), *Serratia liquefaciens* (8 strains), *S. marcescens* (90 strains), *S. odorifera* (one strain), and unspeciated Serratia (one strain).

c. MDR includes: *Citrobacter freundii* (two strains), *C. freundii* species complex (four strains), *Enterobacter aerogenes* (four strains), *E. cloacae* (six strains), *E. cloacae* species complex (seven strains), *Escherichia coli* (11 strains), *Klebsiella oxytoca* (one strain), *K. pneumoniae* (25 strains), and *Serratia marcescens* (13 strains).

d. XDR includes: *Citrobacter freundii* species complex (two strains), *Enterobacter cloacae* species complex (two strains), *Klebsiella pneumoniae* (six strains), and *Serratia marcescens* (three strains).

Conclusions

- Tetracyclines differ greatly in their activity against Gram-negative bacteria (Tables 2-4).
- Minocycline was the most active "tetracycline" tested against *A. baumannii*, *S. maltophila*, and the Enterobacteriaceae isolates collected from patients throughout Europe and the Mediterranean region during 2013.
- Minocycline, particularly the intravenous formulation, has activity against several ESKAPE pathogens and merits consideration in seriously ill patients where treatment options may be limited due to the presence of MDR bacteria.

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