SUPPLEMENT ARTICLE

Update on *Acinetobacter* Species: Mechanisms of Antimicrobial Resistance and Contemporary In Vitro Activity of Minocycline and Other Treatment Options

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Among *Acinetobacter* species, *A. baumannii* and other closely related species are commonly implicated in nosocomial infections. These organisms are usually multidrug resistant (MDR), and therapeutic options to treat *A. baumannii* infections are very limited. Clinicians have been resorting to older antimicrobial agents to treat infections caused by MDR *A. baumannii*, and some of these agents have documented toxicity and/or are not optimized for the infection type to be treated. Recent clinical experience supported by antimicrobial susceptibility data suggests that minocycline has greater activity than other tetracyclines and glycylcyclines against various MDR pathogens that have limited therapeutic options available, including *Acinetobacter* species. An intravenous formulation of minocycline has recently become available for clinical use, and in contrast to most older tetracyclines, minocycline has high activity against *Acinetobacter* species. In this report, we summarized some of the characteristics of the tetracycline class, and quantified the minocycline activity against contemporary (2007–2011) isolates and its potential therapeutic role against a collection of 5477 *A. baumannii* and other relevant gram-negative organisms when compared directly with tetracycline, doxycycline, and other broad-spectrum antimicrobial agents. *Acinetobacter baumannii* strains were highly resistant to all agents tested, with the exception of minocycline (79.1% susceptible) and colistin (98.8% susceptible). Minocycline (minimum inhibitory concentration that inhibits 50% and 90% of the isolates [$MIC_{50/90}$]: 1/8 μg/mL) displayed greater activity than doxycycline ($MIC_{50/90}$: 2/>8 μg/mL) and tetracycline hydrochloride (HCL) (only 30.2% susceptible) against *A. baumannii* isolates, and was significantly more active than other tetracyclines against *Burkholderia cepacia*, *Escherichia coli*, *Serratia marcescens*, and *Stenotrophomonas maltophilia* isolates. In vitro susceptibility testing using tetracycline HCL as a surrogate for the susceptibility other tetracyclines fails to detect minocycline-susceptible isolates and the potential utility of minocycline for the treatment of many MDR *A. baumannii* infections and other difficult-to-treat species, where there are often limited choices of antimicrobials.

Keywords. *Acinetobacter* spp; minocycline; surrogate testing.

The tetracyclines in the 1940s became the first broad-spectrum antimicrobial class to be described [1]. These compounds were derived from *Streptomyces* species (*S. rimosus* and *S. aureofaciens*), and this class was expanded by semisynthetic processes to include tetracycline hydrochloride (HCL) and the more lipophilic agents doxycycline and minocycline. Their mode of action targeted the bacterial ribosome, resulting in the inhibition of protein synthesis [2]. Tetracycline HCL is considered short-acting, and doxycycline and minocycline are long-acting, each having extended serum half-lives [1, 3, 4]. Long-lasting tetracyclines possess more potent spectrums against some bacterial species, particularly the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), *Staphylococcus aureus*

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(including methicillin-resistant *S. aureus* [MRSA]), and nonfermentative gram-negative bacilli such as *Acinetobacter* species (including multidrug-resistant [MDR] strains) [3, 5].

The tetracycline molecule is formed by 4 linear tetracyclic rings, the hydronaphthacene nucleus, and a carboximide at the position C-2, which are essential to antibacterial activity [1, 3, 6]. In comparison with the tetracycline molecule, minocycline possesses a-dimethylamino in position C-7 and no substituent in position C-6, whereas doxycycline is formed through the removal of a hydroxyl group at C-6 and an addition of a hydroxyl in position C-7 [1, 6]. These alterations increase the molecule lipophilic properties facilitating tissue penetration and improving antibacterial activity [3]. Minocycline is the most lipophilic of all tetracyclines, and this compound has been recognized as the most potent agent in this class, followed by doxycycline [6]. Furthermore, minocycline and doxycycline have the capability to overcome many tetracycline resistance mechanisms [3].

Tetracyclines enter the bacteria through an energy-dependent process [1, 7], using outer membrane protein channels in gram-negative organisms [2]. Once in the cell, these compounds bind to the 30S unit of the ribosome blocking the entry of aminoacyl transfer RNA into the site A of the ribosome, which prevents the incorporation of amino acids into elongation peptide chain. The binding is reversible, and this most likely provides bacteriostatic activity to these compounds [7]. Additionally, interactions with the cytoplasmic membrane enhance the activity of many tetracyclines, including minocycline, providing bactericidal properties to these compounds [1].

Spectrum of Activity and Susceptibility Testing

Tetracyclines exhibit activity against most aerobic and anaerobic gram-positive and -negative organisms, atypical bacteria (including chlamydiae and mycoplasma), rickettsiae, and protozoan parasites [7]. Elevated minimum inhibitory concentration (MIC) values for these agents are observed among *Pseudomonas* species (MIC that inhibits 50% and 90% of the isolates [$MIC_{50/90}$]: 8/32 μg/mL for *P. aeruginosa*) and various aerobic gram-negative organisms, including species of *Proteus*, *Providencia*, *Salmonella*, and *Shigella* (MIC_{50}: ≥8, >8, 2, and 2 μg/mL, respectively) [1].

It was previously stated that all tetracyclines have similar spectrum of activity against all gram-negative organisms [7]; however, differences among the tetracyclines have been documented, and minocycline has been described to be more potent than tetracycline against *Acinetobacter* species [1, 3], *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* [1]. Minocycline is also active against *Nocardia* species, whereas other members of this class have limited activity against this pathogen; similarly, doxycycline can be more active than other tetracyclines against *Neisseria gonorrhoeae* [1, 7].

Guidelines for susceptibility testing of tetracyclines have dated from the earliest years of standardized methods development, with breakpoints appearing in the initial interpretive tables of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [8]). More than 3 decades ago, all tetracyclines were interpreted by a MIC breakpoint of ≤4 μg/mL for susceptibility and ≥16 μg/mL for resistance using correlate disk diffusion interpretive criteria with application to all pathogens tested [6]. Today, the published criteria vary widely by the pathogen tested and the published international guidelines utilized, but tetracycline HCL testing has long been recommended as a surrogate to predict susceptibility to other compounds from the same class.

Resistance Mechanisms

The tetracyclines have been used in human and animals, which has consequently resulted in strong selective pressure and emergence of resistant organisms. There are several genes currently known to confer resistance to tetracyclines among gram-negative organisms. The most common tetracycline resistance mechanisms are due to efflux pump– and ribosomal protection–encoding genes [1]. Other and less common resistance mechanisms include chemical molecule modification and target site modifications [3].

There are currently 29 efflux pump–encoding genes that encode resistance to tetracyclines [9]. These genes encode for proteins that belong to the major facilitator superfamily. These proteins are located in the cytoplasmic membrane and decrease the tetracycline intracellular concentration by exchanging a proton for the tetracycline-cation complex [10]. A total of 26 efflux pump–encoding genes have been detected among gram-negative organisms [9]. These encoded proteins are effective in transporting out tetracycline and doxycycline, except for *tet* (B), which also exports the synthetic derivative minocycline [10]. Newer-generation tetracycline molecules, such as tigecycline, were designed to overcome the efflux pump systems or ribosomal protection mechanisms [11]. Six efflux pump–encoding genes have been reported in *Acinetobacter* species, including *tet*(A), *tet*(B), *tet*(G), *tet*(H), *tet*(L), and *tet*(39) [9].

There are currently 12 ribosomal protection proteins described in the microbiology literature [9]. Although these genes possess a G + C content similar to that of gram-positive organisms, they have been detected among both gram-positive and -negative genera. However, *tetB*(P), *otr*(A), and *tet* have been observed among environmental isolates only [9, 12]. These genes encode for cytoplasmic proteins, which prevent tetracycline, doxycycline, and minocycline from binding to the ribosome, causing in vivo and in vitro resistance [2]. These protection proteins interact with the base of h34 protein within the ribosome, causing disruption of the primary tetracycline binding site. Consequently, the tetracycline molecule binding is reduced or released from the ribosome,

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which maintains or returns to a conformational state that allows protein synthesis [12].

There are only 3 genes currently associated with the enzymatic inactivation of tetracyclines. These genes are *tet*(X), *tet*(34), and *tet*(37) [9]. These genes encode for cytoplasmic proteins (oxidoreductase) that adds a hydroxyl group to the C-11a position of tetracyclines in the presence of nicotinamide adenine dinucleotide phosphate and oxygen, except for *tet*(34), which is more similar to the xanthine-guanine phosphoribosyl transferase [10, 12]. These proteins modify the first and second generation of tetracyclines, and also recognize tigecycline as a substrate [13]. *tet*(X) has been detected among several species of gram-negative isolates, including clinical isolates of *Acinetobacter* species, *Enterobacter cloacae*, *Comamonas testosteroni*, *Escherichia coli*, *K. pneumoniae*, *Delftia acidovorans*, *Enterobacter* species, and other members of Enterobacteriaceae and Pseudomonadaceae [13, 14]. The *tet*(34) gene has been observed among *Pseudomonas* species, *Serratia* species, and *Vibrio* species [10].

Other mechanisms include *tet*(U) and *otr*(C), which have been detected exclusively in anaerobes. *tet*(U) encodes for a small protein that confers low-level tetracycline resistance [10]. In addition, chromosomal mutations have been rarely associated with tetracycline resistance in *N. gonorrhoeae* [3]. However, more recently, several efflux pump systems belonging to the resistance/nodulation/division family present in Enterobacteriaceae (AcrAB) and *A. baumannii* (AdeABC, AdeIJK, AdeFGH, AbeM, and AdeDE) have been reported to extrude numerous antimicrobial agents, including older tetracyclines and tigecycline when mutations cause overexpression of these systems [14, 15].

Acinetobacter species represent a worldwide challenge for antimicrobial therapy [16], and isolates belonging to the *Acinetobacter calcoaceticus-baumannii* complex (herein named *A. baumannii*), the most clinically relevant group among this genus [3, 17], are often MDR. These organisms have become a more frequent cause of nosocomial infections [16]. This recent increase in difficult-to-treat MDR organisms, including *Acinetobacter* species and carbapenem-resistant Enterobacteriaceae, motivated clinicians to use established but older agents that are often toxic or not recommended for the indication to be treated. In this study, we assessed the contemporary activity of minocycline and other antimicrobial agents against *A. baumannii* and other non-*Pseudomonas* gram-negative pathogens. We queried the large organism resistance surveillance collection of the SENTRY Antimicrobial Surveillance Program (2007–2011) for >5000 *A. baumannii* and other organisms, including 57 493 Enterobacteriaceae, 1706 *S. maltophilia*, and 191 *B. cepacia* isolates.

Contemporary Spectrum Analyses

A total of 64 867 isolates were collected between 2007 and 2011 from medical centers located worldwide (United States, Europe, Latin America, and the Asia-Pacific) and submitted for reference identification and susceptibility testing. Local identifications were confirmed by the monitoring laboratory using biochemical algorithms and Vitek 2 under Good Laboratory Practice/Clinical Laboratory Improvement Amendments–certified conditions (JMI Laboratories, North Liberty, Iowa).

These organisms included *A. baumannii* (5478), *S. maltophilia* (1706 strains), *B. cepacia* (191 strains), and 57 493 Enterobacteriaceae. Among the latter group, the major species groups were *E. coli* (23 977), *Klebsiella* species (14 808), *Enterobacter* species (7441), *Serratia* species (3525), *Proteus mirabilis* (2662), *Citrobacter* species (2001), indole-positive Proteae (1958), and another 1121 isolates representing other species.

These selected gram-negative bacilli were tested for susceptibility to the tetracyclines by reference broth microdilution methods [18]. The validated broth microdilution panels were produced under Good Manufacturing Practices conditions at ThermoFisher Scientific (Cleveland, Ohio). Interpretations of all MIC results applied current CLSI and European Committee on Antimicrobial Susceptibility Testing breakpoints [19, 20]. Quality control (QC) was assured by using CLSI-recommended strains: *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *P. aeruginosa* ATCC 27853. All QC results were observed to be within published QC ranges [19].

Analyses were applied to determine (1) spectrums of activity (percentage susceptible) for each drug according to established CLSI breakpoint criteria, (2) cross-susceptibility accuracy using tetracycline HCL results to predict minocycline (or doxycycline) susceptibility, and (3) cross-susceptibility and -resistance for all categories for the tetracyclines.

RESULTS AND DISCUSSION

Acinetobacter baumannii isolates generally displayed elevated MIC values for most antimicrobial agents tested that are listed in the current CLSI interpretive tables (Table 1) [19]. Minocycline was the second most active (79.1% susceptible) agent, only exceeded by colistin (98.8% susceptibility using current breakpoints of ≤2 μg/mL). All other classes of agents had susceptibility rates of less than 41.9% (tobramycin; Table 1).

A direct comparison of the activity of minocycline with other tetracyclines was performed for *A. baumannii* and selected gram-negative organisms (Table 2). Minocycline potency against *A. baumannii* (MIC_{50}: 1 μg/mL) was 2- and ≥8-fold greater than doxycycline and tetracycline HCL, respectively. Against Enterobacteriaceae, minocycline displayed 2-fold greater potency than doxycycline agents against *E. coli* (MIC_{50}: 1 and 2 μg/mL, respectively; Table 2), but the MIC_{50} activity of these 2 molecules was similar for all other organisms analyzed. As expected, these 2 agents were more potent than tetracycline HCL

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Table 1. Minocycline Activity Compared With Selected Comparator Agents Tested Against 5478 *Acinetobacter baumannii* Clinical Isolates (2007–2011)

	MIC, μg/mL		% Susceptible by	
Antimicrobial Agent	50%	90%	CLSI	EUCAST[a]
Minocycline	1	8	79.1[b,c]	. . .
Doxycycline	2	>8	59.6	. . .
Tetracycline HCL	>8	>8	30.2	. . .
Piperacillin/tazobactam	>64/4	>64/4	17.7	. . .
Ampicillin/sulbactam	>16/4	>16/4	25.9	. . .
Cefepime	>16	>16	21.9	. . .
Ceftazidime	>16	>16	20.8	. . .
Ceftriaxone	>8	>8	7.2	. . .
Imipenem	>8	>8	37.4	34.3
Meropenem	>8	>8	36.4	32.8
Amikacin	>32	>32	34.4	31.7
Gentamicin	>8	>8	29.5	29.5
Tobramycin	>16	>16	41.9	41.9
Ciprofloxacin	>4	>4	20.5	20.5
Levofloxacin	>4	>4	21.8	21.0
Trimethoprim/sulfamethoxazole	>2/38	>2/38	28.5	28.5
Colistin	≤0.5	1	98.8[b]	98.8[b]

Interpretations were made using CLSI and EUCAST criteria [19, 20].

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; HCL, hydrochloride; MIC, minimum inhibitory concentration.

[a] ". . ." indicates no published breakpoint criteria.

[b] Most active agents are underlined.

[c] A statistically significant greater susceptibility rate for minocycline compared with peer tetracyclines ($P < .05$) was noted (see underline).

against Enterobacteriaceae isolates. For *B. cepacia* and *S. maltophilia*, minocycline potency (MIC_{50}: 2 and 0.5 μg/mL, respectively) was 2- and 4-fold greater than doxycycline and ≥8-fold greater than tetracycline HCL, respectively. At CLSI susceptibility breakpoints, minocycline coverage for *A. baumannii* (79.1% susceptible) was 29.5% more than doxycycline (59.6%), and 58.9% more than tetracycline HCL (Table 2). In contrast, minocycline, doxycycline, and tetracycline have similar rates of susceptibility using CLSI current breakpoints when tested against *Klebsiella* species (73.6%–75.7%), *Enterobacter* species (81.1%–81.4%), and *Citrobacter* species (81.7%–84.8%). However, a significantly wider spectrum/rate of susceptibility was observed for minocycline vs *E. coli* (78.8% vs 57.9%–61.0%), *Serratia* species (77.7% vs 8.6%–52.8%), and Enterobacteriaceae (73.7% vs 60.3%–64.2%) overall (Table 2).

Table 3 compares the differing rates of minocycline susceptibility and potencies across the 4 sampled geographic regions. Across all regions, minocycline was the most active tetracycline against *A. baumannii*, with activity highest in Latin America (MIC_{50}: 0.5 μg/mL; 91.7% susceptible) and lowest against strains isolated in Europe (MIC_{50}: 2 μg/mL; 72.5% susceptible). Minocycline was most active against Enterobacteriaceae in the United States and Europe and only slightly less active against isolates from Latin America and the Asia-Pacific regions (Table 3). Minocycline susceptibility among *S. maltophilia* (≤4 μg/mL) was similar across regions and exceeded 97.0% across all geographic regions. Additionally, for *B. cepacia*, minocycline was most active against US isolates (88.2% susceptible to ≤4 μg/mL).

As recommended in CLSI documents only until recently, tetracycline HCL breakpoints were used to predict minocycline or doxycycline susceptibilities in the 5477 *A. baumannii* isolates. When this analysis was performed in these contemporary isolates (Table 4), only 1654 isolates were susceptible to tetracycline HCL, but 2684 isolates were minocycline susceptible, with an additional 639 strains having an intermediate result (MICs: at 8 μg/mL) for minocycline (11.7%; Table 4). The number of *A. baumannii* strains resistant (MICs: >8 μg/mL) was markedly different among minocycline (500 isolates), tetracycline HCL (3135), and doxycycline (2119).

Acinetobacter species, in particular species belonging to the *A. calcoaceticus-baumannii* complex that includes *A. baumannii*, *A. calcoaceticus*, *A. nosocomialis* (previously *Acinetobacter* genospecies 13TU), and *A. pitti* (previously *Acinetobacter* genospecies 3) [17], are clinically important nosocomial pathogens. These organisms are a common cause of bloodstream infections, hospital-acquired pneumonia, and wound and other surgical site infections, and MDR *A. baumannii* has emerged as one of the most challenging organisms for appropriate antimicrobial therapy [21]. Among antimicrobials tested and considered as candidate regimens for *A. baumannii*, minocycline and colistin were the only 2 agents that had susceptibility rates (per CLSI criteria) exceeding 50% (79.1% and 98.8%, respectively) in this study [19], confirming that very few options are available for therapy. Additionally, against the *A. baumannii* isolates tested, the rank order of potency among tetracyclines was minocycline, followed by doxycycline and tetracycline HCL with the lowest activity (79.1%, 59.6%, and 30.2% susceptible at CLSI current breakpoints, respectively).

Minocycline also displayed good activity against other nonfermentative organisms tested that included *B. cepacia* ($MIC_{50/90}$: 2/8 μg/mL; 83.3% susceptible to ≤4 μg/mL) and *S. maltophilia* ($MIC_{50/90}$: 0.5/2 μg/mL; 98.9% susceptible to ≤4 μg/mL) and certain Enterobacteriaceae species. This tetracycline had markedly greater activity against *E. coli* and *S. marcescens* (ESKAPE pathogens) that might also display MDR phenotypes and challenge available therapeutic options.

More than 99.0% of isolates susceptible to tetracycline HCL were also susceptible to minocycline; however, this long-lasting tetracycline was active at ≤4 μg/mL against an additional 49.0% of *A. baumannii* isolates that were nonsusceptible to

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Table 2. Comparative Activity of Minocycline and Other Tetracyclines Tested Against *Acinetobacter baumannii* and Other Gram-Negative Strains From a Worldwide Surveillance Program (2007–2011)

Species (No. Tested) and Antimicrobial Agent	Cumulative % Inhibited at MIC, µg/mL							MIC, µg/mL	
	≤0.12	0.25	0.5	1	2	4[a]	8	50%	90%
Acinetobacter baumannii (5477)[b]									
Minocycline	19.1	29.5	45.9	59.2	64.3	79.1[b,c]	90.9	1	8
Doxycycline	17.7	24.6	38.1	48.4	56.4	59.6[c]	61.3	2	>8
Tetracycline	. . .	0.1	0.8	4.1	20.5	30.2[c]	42.8	>8	>8
Burkholderia cepacia (191)									
Minocycline	2.1	3.1	10.5	31.4	54.2	83.3[c]	92.2	2	8
Doxycycline	2.1	7.3	12.6	21.5	37.7	64.9	81.2	4	>8
Tetracycline	. . .	0.0	1.1	1.8	10.0	15.8	16.8	>8	>8
Stenotrophomonas maltophilia (1706)									
Minocycline	7.6	33.7	67.5	87.3	96.0	98.9[c]	99.9	0.5	2
Doxycycline	0.1	0.8	5.1	31.1	75.5	94.7	98.5	2	4
Tetracycline	. . .	<0.1	<0.1	0.1	0.5	3.1	22.0	>8	>8
Enterobacteriaceae (57 493)									
Minocycline	0.1	1.3	11.5	31.7	58.1	73.7[c]	83.0	2	>8
Doxycycline	0.1	0.4	5.6	30.0	54.8	64.2	73.4	2	>8
Tetracycline	. . .	. . .	. . .	. . .	55.2	60.3	63.8	≤2	>8
Escherichia coli (23 977)									
Minocycline	0.2	2.7	24.8	53.2	70.3	78.8[c]	87.4	1	>8
Doxycycline	<0.1	0.4	8.5	41.2	56.6	61.0	72.0	2	>8
Tetracycline	. . .	. . .	. . .	. . .	56.1	57.9	58.2	≤2	>8
Serratia species (3525)									
Minocycline	0.1	0.1	0.2	3.8	30.9	77.7[c]	94.4	4	>8
Doxycycline	<0.1	0.1	0.2	2.6	19.9	52.8	85.2	4	>8
Tetracycline	. . .	. . .	. . .	. . .	1.2	8.6	34.1	>8	>8
Klebsiella species (14 808)									
Minocycline	0.1	0.3	1.5	21.7	59.9	75.7	84.6	2	>8
Doxycycline	0.1	0.3	7.1	34.3	65.4	73.6	78.9	2	>8
Tetracycline	. . .	. . .	. . .	. . .	65.3	74.4	78.1	≤2	>8
Enterobacter species (7441)									
Minocycline	0.1	0.2	0.9	12.2	54.2	81.4	88.6	2	>8
Doxycycline	0.1	0.1	0.6	12.2	63.2	81.4	87.8	2	>8
Tetracycline	. . .	. . .	. . .	. . .	71.7	81.1	85.2	≤2	>8
Citrobacter species (2001)									
Minocycline	0.1	0.8	12.6	37.5	71.9	84.8	90.5	2	>8
Doxycycline	<0.1	0.1	4.4	30.8	71.4	81.7	87.1	2	>8
Tetracycline	. . .	. . .	. . .	. . .	79.6	84.2	86.8	≤2	>8

Abbreviations: *B. cepacia, Burkholderia cepacia*; HCL, hydrochloride; MIC, minimum inhibitory concentration; *S. maltophilia, Stenotrophomonas maltophilia*.

[a] Clinical and Laboratory Standards Institute breakpoints [19]; no criteria for doxycycline and tetracycline HCL when testing *B. cepacia* and *S. maltophilia*.

[b] All other agents had very low susceptibility rates at ≤41.9% (includes amikacin [34.4% susceptible], cefepime [21.9%], ceftazidime [20.8%], gentamicin [29.5%], imipenem [37.4%], levofloxacin [21.8%], meropenem [36.4%], piperacillin/tazobactam [17.7%], and tobramycin [41.9%], see Table 2; and tigecycline inhibited 80.7% of strains at ≤1 µg/mL).

[c] A statistically significant greater susceptibility rate for minocycline compared to peer tetracyclines ($P < .05$) was noted (see underline).

tetracycline HCL. Tetracycline-resistant and minocycline-susceptible isolates have been considered a common phenotype [21]; thus, minocycline susceptibility should not be determined using a surrogate class representative approach (tetracycline HCL). Minocycline should be tested directly by CLSI reference methods or validated commercial systems using the appropriate interpretive criteria to guide treatment caused by these nonfermentative species, where there are often limited therapeutic choices. This recommendation is also reflected in the current version of CLSI documents (M-100, 2014).

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Table 3. Geographic Variations of Minocycline Activity Directed Against *Acinetobacter baumannii* and Other Gram-Negative Organisms From the SENTRY Antimicrobial Surveillance Program (2007–2011)

Organism/ Parameter	Region			
	United States	Europe	Latin America	Asia-Pacific
Acinetobacter baumannii				
(No. tested)	(760)	(1196)	(1498)	(2024)
MIC, μg/mL				
50%	1	2[a]	0.5[b]	2
90%	>8	>8	4	8
% inhibited				
≤2 μg/mL	66.1	57.3	88.2	50.2
≤4 μg/mL[c]	75.1	72.5[a]	91.7[b]	75.3
≤8 μg/mL	89.6	85.3	95.5	91.2
Enterobacteriaceae				
(No. tested)	(18 507)	(20 430)	(7075)	(11 481)
MIC, μg/mL				
50%	2[b]	2	2	4[a]
90%	>8	>8	>8	>8
% inhibited				
≤2 μg/mL	64.6	61.6	52.4	45.1
≤4 μg/mL[c]	78.2[b]	75.6	68.2	66.3[a]
≤8 μg/mL	85.8	84.3	79.0	78.6
Burkholderia cepacia				
(No. tested)	(34)	(29)	(37)	(91)
MIC, μg/mL				
50%	1[b]	2	2[a]	2
90%	8	5	>8	8
% inhibited				
≤2 μg/mL	70.6	65.5	59.5	52.8
≤4 μg/mL[c]	88.2[b]	82.8	78.4[a]	83.5
≤8 μg/mL	94.1	90.0	86.5	94.5
Stenotrophomonas maltophilia				
(No. tested)	(607)	(479)	(183)	(437)
MIC, μg/mL				
50%	0.5	0.5	0.5[b]	0.5[a]
90%	2	2	1	2
% inhibited				
≤2 μg/mL	96.4	97.1	96.7	93.8
≤4 μg/mL[c]	99.5	99.0	100.0[b]	97.7[a]
≤8 μg/mL	100.0	99.8	100.0	99.8

Abbreviation: MIC, minimum inhibitory concentration.

[a] Lowest activity for minocycline by species among the 4 monitored geographic regions.

[b] Minocycline had greatest activity for this species in this region.

[c] Susceptible breakpoint per Clinical and Laboratory Standards Institute criteria [19].

Table 4. Correlations (Accuracy) of Using Tetracycline Minimum Inhibitory Concentration Results to Predict Minocycline or Doxycycline Susceptibility When Testing *Acinetobacter baumannii* (5477 Strains)[a]

Antimicrobial Agent Predicted	MIC, μg/mL	Tetracycline MIC, μg/mL			
		≤2	4	8	>8
Minocycline	>8			3	497
	8		4	1	638
	4		5[b]	0	806[c]
	2	5[b]	6[b]	7[c]	480[c]
	1	10[b]	36[b]	128[c]	339[c]
	≤0.5	1105[b]	483[b]	549[c]	375[c]
Doxycycline	>8	1	4	1	2113
	8		0	4	90
	4		5[b]	2[c]	166[c]
	2	6[b]	3[b]	16[c]	414[c]
	1	9[b]	27[b]	231[c]	298[c]
	≤0.5	1104[b]	495[b]	433[c]	54[c]

Abbreviation: MIC, minimum inhibitory concentration.

[a] Horizontal and vertical lines show the breakpoint concentrations for each agent (≤4 μg/mL = susceptible; >8 μg/mL = resistant) by Clinical and Laboratory Standards Institute criteria [19].

[b] Number of strains having tetracycline MIC values at ≤4 μg/mL (susceptible) and also susceptible to minocycline (99.76% accuracy) or doxycycline (99.70% accuracy).

[c] False nonsusceptible strains for minocycline (2684 occurrences [49.0%]) and doxycycline (1624 occurrences [29.7%]).

In previous studies, minocycline has been reported to be active against 82.0% of *A. baumannii* isolates collected in 2009 worldwide, and in 76.0% of 3103 meropenem-resistant *A. baumannii* isolates [5]. Similar to the data from the SENTRY database, minocycline was recently shown to be highly active against a worldwide collection of >3500 clinical isolates of *Acinetobacter* species. In that study, >80% of isolates were susceptible to ≤4 μg/mL of minocycline; in the subset of 1660 isolates considered to be MDR, 67.9% were susceptible, compared with ≤22.5% for amikacin, levofloxacin, and various β-lactam antibiotics [22].

Despite the favorable in vitro activity, clinical data are limited for treatment of *A. baumannii* infections, but a few studies demonstrate favorable clinical outcomes in therapies that include minocycline. In 2 studies evaluating the use of minocycline for the treatment of ventilator-associated pneumonia caused by MDR *A. baumannii*, cures were achieved for 80.6%–86.0% of the patients receiving minocycline-based treatments [23, 24]. The number of patient cases was limited in both studies, and most isolates were tetracycline HCL susceptible [23, 24]; however, these 2 independent investigations showed that minocycline or doxycycline might be valuable choices for the treatment of this high-mortality infection when other agents are not active or are inappropriate.

Alternative therapeutic options are needed to treat MDR *Acinetobacter* infections and infections caused by other MDR organisms, with minocycline being a valuable option. In view

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of limited choices for the treatment of MDR isolates of *Acinetobacter* and other nonfermentative bacilli, an intravenous formulation of minocycline (Minocin IV) has been reintroduced into the US market. Minocycline is among the few antimicrobial agents with Food and Drug Administration approval for the treatment of *Acinetobacter* species infections. These results and other recent publications describe the clinical use of this agent as treatment for a variety of infections due to *Acinetobacter* species, as there is increasing interest in seeking alternatives to polymyxins in patients infected with MDR isolates.

Notes

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References

1. Zhanel GG, Homenuik K, Nichol K, et al. The glycylcyclines: a comparative review with the tetracyclines. Drugs **2004**; 64:63–88.
2. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev **2001**; 65:232–60.
3. Bishburg E, Bishburg K. Minocycline—an old drug for a new century: emphasis on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. Int J Antimicrob Agents **2009**; 34:395–401.
4. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother **2006**; 58:256–65.
5. Pogue JM, Marchaim D, Kaye D, Kaye KS. Revisiting "older" antimicrobials in the era of multidrug resistance. Pharmacotherapy **2011**; 31:912–21.
6. Sader HS, Ferraro MJ, Reller LB, Schreckenberger PC, Swenson JM, Jones RN. Reevaluation of Clinical and Laboratory Standards Institute disk diffusion breakpoints for tetracyclines for testing Enterobacteriaceae. J Clin Microbiol **2007**; 45:1640–3.
7. Versalovic J, Carroll KC, Funke G, Jorgensen J, Landry ML, Warnock DW. Manual of clinical microbiology. 10th ed. Washington, DC: ASM Press, **2011**.
8. National Committee for Clinical Laboratory Standards. M2-A, Performance standards for antimicrobial disc susceptibility tests. 1st ed. Villanova, PA: NCCLS, **1976**.
9. Roberts MC. Tetracycline Genes References. Available at: http://faculty.washington.edu/marilynr/tetweb1.pdf. Accessed 15 November 2013.
10. Roberts MC. Environmental macrolide-lincosamide-streptogramin and tetracycline resistant bacteria. Front Microbiol **2011**; 2:40.
11. Chopra I. New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. Drug Resist Updat **2002**; 5:119–25.
12. Roberts MC. Update on acquired tetracycline resistance genes. FEMS Microbiol Lett **2005**; 245:195–203.
13. Aminov RI. Evolution in action: dissemination of tet(X) into pathogenic microbiota. Front Microbiol **2013**; 4:192.
14. Deng M, Zhu MH, Li JJ, et al. Molecular epidemiology and mechanisms of tigecycline resistance in clinical isolates of *Acinetobacter baumannii* from a Chinese university hospital. Antimicrob Agents Chemother **2014**; 58:297–303.
15. Stein GE, Babinchak T. Tigecycline: an update. Diagn Microbiol Infect Dis **2013**; 75:331–6.
16. Giamarellou H, Poulakou G. Multidrug-resistant gram-negative infections: what are the treatment options? Drugs **2009**; 69:1879–901.
17. Nemec A, Krizova L, Maixnerova M, et al. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). Res Microbiol **2011**; 162:393–404.
18. Clinical and Laboratory Standards Institute. M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition. Wayne, PA: CLSI, **2012**.
19. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement. CLSI document M100-S23. Wayne, PA: CLSI, **2013**.
20. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.0, January 2013. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed 2 January 2013.
21. Gilad J, Carmeli Y. Treatment options for multidrug-resistant *Acinetobacter* species. Drugs **2008**; 68:165–89.
22. Hawser S, Hackel M, Morrissey I, et al. Update on the global activity of tigecyline and comparator agents tested against *Acinetobacter* spp, including agents against MDR isolates (TEST 2011–2012). In: Presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, Colorado, **2013**.
23. Wood GC, Hanes SD, Boucher BA, Croce MA, Fabian TC. Tetracyclines for treating multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. Intensive Care Med **2003**; 29:2072–6.
24. Chan JD, Graves JA, Dellit TH. Antimicrobial treatment and clinical outcomes of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. J Intensive Care Med **2010**; 25:343–8.

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SUPPLEMENT ARTICLE

Minocycline: An Old Drug for a New Bug: Multidrug-Resistant *Acinetobacter baumannii*

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Keywords. *Acinetobacter baumannii*; antimicrobial resistance; minocycline; stewardship.

Acinetobacter baumannii is listed in the Centers for Disease Control and Prevention's (CDC) report "Antibiotic Resistance Threats in the United States, 2013" as 1 of 18 microorganisms whose threat level is "urgent," "serious," or "concerning" according to their current and projected health and economic impacts [1]. The *A. baumannii* threat level is ranked as "serious" and carries a warning that this organism requires prompt and sustained action by healthcare providers to ensure that this problem pathogen does not continue to become more resistant to antimicrobials and spread. The CDC estimates that nearly 7000 of 12 000 (63%) healthcare-associated *Acinetobacter* infections are multidrug resistant (MDR), defined as resistance to ≥3 different classes of antimicrobials. Hospitals around the world are witnessing the loss of antibiotics for the treatment of MDR *A. baumannii* (MDR-AB) infections [2]. The lack of clinically effective antimicrobials to treat *A. baumannii* infections has led clinicians to reevaluate other "older" agents for the treatment of MDR-AB.

Minocycline is an "old drug" that was first introduced in the 1960s. It is available both intravenously and orally with United States Food and Drug Administration approval for the treatment of infections caused by *A. baumannii* [3]. The intravenous formulation was voluntarily withdrawn from the US market in 2005 and, due in part to the continued emergence and spread of MDR-AB, was reintroduced to the US market in 2009. The reintroduction of intravenous minocycline provides an additional agent in the limited armamentarium for treating MDR-AB. Minocycline represents an option in the treatment of MDR-AB infection, as susceptibilities to *A. baumannii* remain high, conversion from intravenous to oral therapy is available, and toxicity is relatively limited. However clinical experience with intravenous minocycline for the treatment of MDR-AB infections is limited to in vitro evaluations, case reports, or small case series.

The first article in this supplement, by Mariana Castanheira and colleagues, summarizes some of the characteristics of the tetracycline class of antimicrobials and directly compares the in vitro activity of minocycline to doxycycline, tetracycline, colistin, carbapenems, and other agents against select gram-negative organisms including *A. baumannii* collected between 2007 and 2011 from medical centers located worldwide. Minocycline and colistin were the only 2 antimicrobials that exceeded 50% susceptibility rates (79.1% and 98.8%, respectively). Importantly, they note that microbiology laboratories should not use tetracycline hydrochloride susceptibility testing as a surrogate for other tetracyclines, as minocycline is sometimes active against *A. baumannii* when tetracycline is not.

David J. Ritchie and Alexandria Garavaglia-Wilson provide a thorough review of the literature that reports successful use of intravenous minocycline for the treatment of serious MDR-AB infections, particularly for nosocomial pneumonia. After reviewing the pharmacokinetics and pharmacodynamics of minocycline, the authors describe the clinical experience observed with intravenous minocycline from reported observational

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data in the form of case reports and series. Although the data are limited. the findings are generally favorable and encouraging. Minocycline's rapid and substantial penetration into lung tissues, along with its favorable safety profile and intravenous to oral step-down therapy, supports its use as an option for treatment of MDR-AB infections.

Debra A. Goff and colleagues describe the clinical experience from The Ohio State University Medical Center with intravenous minocycline for critically ill patients with MDR-AB infections. The observed decline in susceptibility of *A. baumannii* to carbapenems and ampicillin-sulbactam required the antimicrobial stewardship program to evaluate minocycline. The observed clinical and microbiologic success rate of 73% and 78%, respectively, suggests that intravenous minocycline in combination with a second active agent, primarily intravenous colistin, warrants consideration for the treatment of MDR-AB infections, as options are exceedingly limited.

Jason Pogue et al describe the processes by which the antimicrobial stewardship committee and pharmacy and therapeutics committee at Detroit Medical Center (DMC) evaluated the utility of minocycline in the management of MDR gram-negative bacilli, brought minocycline onto formulary, and integrated it into treatment algorithms. They describe the emerging role of intravenous minocycline in management of infections due to these pathogens and the experience at DMC in treating carbapenem-resistant Enterobacteriaceae and MDR-AB infections.

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References

1. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, **2013**. Available at: http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508. Accessed 14 November 2013.
2. Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. Clin Infect Dis **2013**; 56:1310–8.
3. The Medicines Company. Minocycline for injection. Available at: http://www.minociniv.com/packageinsert. Accessed 13 January 2014.

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SUPPLEMENT ARTICLE

A Review of Intravenous Minocycline for Treatment of Multidrug-Resistant *Acinetobacter* Infections

David J. Ritchie[1,2] and Alexandria Garavaglia-Wilson[2,3]

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Options for treatment of multidrug-resistant (MDR) *Acinetobacter baumannii* infections are extremely limited. Minocycline intravenous is active against many MDR strains of *Acinetobacter*, and Clinical and Laboratory Standards Institute breakpoints exist to guide interpretation of minocycline susceptibility results with *Acinetobacter*. In addition, minocycline intravenous holds a US Food and Drug Administration indication for treatment of infections caused by *Acinetobacter*. There is an accumulating amount of literature reporting successful use of minocycline intravenous for treatment of serious MDR *Acinetobacter* infections, particularly for nosocomial pneumonia. These results, coupled with the generally favorable tolerability of minocycline intravenous, support its use as a viable therapeutic option for treatment of MDR *Acinetobacter* infections.

Keywords. minocycline; intravenous; *Acinetobacter*; multidrug-resistant.

Increasing attention has been directed at *Acinetobacter baumannii*, one of the difficult-to-treat ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) as originally highlighted by the Infectious Diseases Society of America in a 2009 position paper and in a 2013 update [1, 2]. The threat posed by MDR *Acinetobacter* was recently rated by the US Centers for Diseases Control and Prevention as "serious" and likely to worsen without ongoing public health monitoring and prevention initiatives [3]. Options for treatment of MDR *Acinetobacter* infections are becoming increasingly limited. The need for new treatments for serious infections caused by MDR strains of *A. baumannii* has become critical, especially given the lack of new antibacterial development within the pharmaceutical industry. As a result, reexamination of existing antibacterial agents with the potential for unique therapeutic activity against this pathogen has become essential.

One of the classes of antibiotics being explored for treatment of MDR *Acinetobacter* infections is the tetracyclines. Introduced shortly after the advent of penicillins and sulfonamides, tetracycline antibiotics contain a core base structure composed of 4 hexagonal rings. Minocycline, or 7-dimethylamino-6-deoxytetracycline, is a semisynthetic tetracycline derivative that was originally introduced in the 1960s [4, 5]. Historically available in both oral and intravenous dosage forms, the intravenous formulation experienced a brief hiatus followed by reappearance of this formulation in 2009 [4]. Indeed, minocycline intravenous is currently approved by the US Food and Drug Administration for treatment of minocycline-susceptible *Acinetobacter* species infections [6]. In addition, Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints for minocycline and *Acinetobacter* exist and are ≤4 μg/mL for susceptibility, 8 μg/mL for intermediate, and ≥16 μg/mL for resistance [7].

This article will briefly discuss the microbiology, pharmacokinetics, pharmacodynamics, and tolerability of minocycline, and also review the available literature

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evaluating use of minocycline intravenous specifically for treatment of MDR *Acinetobacter* infections. Relevant articles for this review were obtained via a comprehensive search of PubMed and Google Scholar and limited to the English language.

MICROBIOLOGY

In common with other tetracyclines, minocycline inhibits bacterial protein synthesis through binding with the 30S subunit of the bacterial ribosome, most typically resulting in a bacteriostatic effect. However, synergistic and bactericidal activity against MDR *Acinetobacter* has been noted with minocycline in combination with colistin or carbapenems [8, 9]. Resistance to tetracyclines generally occurs through increased efflux or ribosomal protection [4]; however, minocycline is able to evade most tetracycline resistance mechanisms, including some mechanisms expressed by MDR *Acinetobacter* that confer resistance to other tetracyclines [10]. Thus, due to the possibility of obtaining discordant results among tetracycline agents, in vitro susceptibility testing of *Acinetobacter* should include minocycline. *Acinetobacter* resistance to minocycline may occur, though, and appears to be associated with the *tet*(B) efflux gene in association with the plasmid-mediated IS*CR2* mobile element [4, 11].

PHARMACOKINETICS AND PHARMACODYNAMICS

Pharmacokinetic and pharmacodynamic characteristics of minocycline intravenous, along with dosing recommendations, are contained in Table 1. Peak serum concentrations following a 200-mg intravenous dose of minocycline range from 2.52 to 6.63 μg/mL and average 4.18 μg/mL. Twelve-hour trough concentrations achieved at steady state with dosing of 100-mg intravenous every 12 hours, as per the US prescribing label, range from 1.4 to 1.8 μg/mL [6]. These achievable peak and trough serum concentrations with standard human doses of minocycline intravenous exceed the mutant prevention concentration of 1 μg/mL, which has been reported with *Acinetobacter* [12]. The drug is 76% protein bound and has a volume of distribution of 1.3 L/kg [13]. Minocycline has enhanced lipophilicity over earlier tetracyclines, which enhances tissue penetration [14]. The 15- to 23-hour half-life of minocycline is longer than that of the earliest tetracyclines, making it a long-acting agent within the class [6, 15]. Renal impairment appears to have little effect on the half-life or area under the concentration–time curve (AUC) of minocycline [15, 16]. Hepatic cirrhosis also appears not to affect the half-life of minocycline, but cautious use is suggested in this population [6, 13]. From a pharmacodynamics standpoint, the free drug AUC/minimum inhibitory concentration appears to be the parameter most closely associated with the antibacterial effect of minocycline [17].

Table 1. Pharmacokinetics, Pharmacodynamics, and Dosing of Minocycline Intravenous

Characteristic	Value
Pharmacokinetics [4, 6, 13, 15, 30]	
Peak concentration following 200-mg load	Mean, 4.18 μg/mL (range, 2.52–6.63 μg/mL)
Trough concentration with 100-mg dosing every 12 h	1.4–1.8 μg/mL
AUC	67–85 mg · h/L (with 200-mg intravenous dose)
Volume of distribution	1.3 L/kg
Plasma protein binding	76%
Metabolism	Up to 6 hepatic metabolites; some active
Urinary excretion	11%
Fecal elimination	20%–35%
Half-life	15–23 h
Pharmacodynamics [8, 9, 12, 17]	
Microbiologic activity	Primarily bacteriostatic, but bactericidal in combination with carbapenems or colistin against *Acinetobacter baumannii*; time-dependent
Primary pharmacodynamic index	AUC/MIC
MPC	1 μg/mL
Dosing [6, 15, 30]	
Usual dose	200-mg intravenous load, followed by 100 mg intravenous every 12 h (not to exceed 400 mg in 24 h)
Renal dosing	Not required

Abbreviations: AUC, area under the concentration–time curve; MIC, minimum inhibitory concentration; MPC, mutant prevention concentration.

CLINICAL EXPERIENCE

The favorable pharmacokinetic profile of minocycline intravenous, along with its stability to many tetracycline resistance mechanisms, suggests a potential role for minocycline intravenous for treatment of some serious MDR *Acinetobacter* infections. Data concerning antibiotic treatment of infections caused by MDR bacteria in general are limited and typically descriptive. Published data evaluating minocycline intravenous for treatment of MDR *Acinetobacter* infections generally consist of observational data in the form of case reports and series.

Minocycline Intravenous for *Acinetobacter* Pneumonia

In a retrospective case series conducted at Presley Regional Medical Center in Memphis, Tennessee, Wood et al described their experience in treating 7 critically ill trauma patients with late-onset ventilator-associated pneumonia (VAP) caused by *A. baumannii*, 4 of whom were treated with minocycline 100 mg intravenous every 12 hours for 10–20 days [18]. The

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A. baumannii strains in these 4 patients were all resistant to amikacin and sulbactam. Three of the 4 strains were also resistant to imipenem, with the fourth strain showing intermediate susceptibility to imipenem. All 4 MDR strains were susceptible to tetracycline, with appropriately inferred susceptibility to minocycline [7]. To qualify as having VAP, all patients were required to have *A. baumannii* growth of $>10^5$ colony-forming units (CFU)/mL from bronchoalveolar lavage (BAL), in addition to fever, leukocytosis or leukopenia, macroscopically purulent sputum, and new or changing infiltrate on chest radiography.

All 4 patients with *A. baumannii* VAP treated with minocycline intravenous were deemed successes, defined as clinical improvement and absence of *A. baumannii* from follow-up BAL culture. One of these 4 patients did not undergo a follow-up BAL, but was judged a success based on predefined criteria of clinical improvement and survival to hospital discharge. Two of the 4 patients deemed successes received minocycline intravenous monotherapy, whereas the other 2 patients received combination therapy with minocycline intravenous and imipenem in 1 case and trovafloxacin and amikacin in the other. The *A. baumannii* strains in these combination therapy cases were not susceptible to the agents accompanying the minocycline intravenous. The patient receiving the combination of minocycline intravenous, trovafloxacin, and trimethoprim-sulfamethoxazole was coinfected with *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. This patient ultimately died later in their hospital course due to septic shock from *P. aeruginosa* VAP, but was not deemed a minocycline failure as *A. baumannii* was not found in the follow-up BAL performed after a 14-day course of intravenous minocycline. This first report of successful use of minocycline intravenous for treatment of clinically and microbiologically confirmed VAP caused by MDR *A. baumannii* provided the basis for continued study of minocycline intravenous for serious *Acinetobacter* infections.

Chan et al subsequently conducted a retrospective analysis of 55 patients with carbapenem-resistant *A. baumannii* VAP at Harborview Medical Center in Seattle, Washington [19]. Ventilator-associated pneumonia was defined by a quantitative BAL culture of $\geq 10^4$ CFU/mL or brush specimen $\geq 10^3$ CFU/mL of carbapenem-resistant *Acinetobacter* performed by bronchoscopy during hospitalization. Clinical response was defined as resolution or improvement in signs and symptoms of VAP or microbiologic eradication of carbapenem-resistant *Acinetobacter* from subsequent BALs or sputum cultures at completion of therapy. All patients with polymicrobial VAP received combination therapy with appropriate agents directed at other causative pathogens in addition to *Acinetobacter*. Of the total population of patients, 19 received minocycline intravenous therapy at a dose of 200 mg once, followed by 100 mg intravenous every 12 hours. A clinical response was noted in 15 of 19 (78.9%) patients receiving minocycline intravenous. Patients receiving minocycline 200 mg oral or per tube every 12 hours had an 82.4% (14/17) successful response rate. Although approximately two-thirds of patients treated with minocycline received combination therapy with at least 1 other agent, clinical response rates did not differ between the minocycline monotherapy group (81.8%) vs the minocycline combination therapy group (80%). The overall minocycline clinical success rate of 80.6% also compared favorably to the 60%, 66.7%, 77.8%, and 90% response rates noted for sulbactam-, polymyxin-, aminoglycoside-, and tigecycline-based comparative therapies, respectively. Although this retrospective analysis provided only limited details of the specific clinical responses of minocycline intravenous-treated patients, it did evaluate a large number of *Acinetobacter* VAP infections that were confirmed based on a predefined strict definition. The effectiveness of step-down therapy from minocycline intravenous to oral was also suggested by this report.

In another retrospective analysis, Jankowski et al reported the results of minocycline intravenous treatment of 3 intensive care unit patients with MDR *A. baumannii* pneumonia at Ohio State University Medical Center [20]. These cases of pneumonia were included as a component of this broader report of that institution's use of minocycline intravenous for various MDR *Acinetobacter* infections. Specific definitions for infections assessed were not provided. The 3 patients received minocycline 100 mg intravenous every 12 hours for 10–13 days in combination with another active agent. Of note, minocycline susceptibility was not reliably predicted by susceptibilities to tigecycline, with some isolates exhibiting lower minimum inhibitory concentrations with minocycline than tigecycline. All 3 patients had documented or presumed eradication of *Acinetobacter*. Two of the 3 patients had a clinical response to therapy with minocycline and survived to discharge. The single patient who died experienced eradication of *Acinetobacter* from BAL and urine, but ultimately had withdrawal of care. Surviving patients were longitudinally followed and were not readmitted to the authors' hospital within 90 days after discharge.

Bishburg et al recently published their experience using minocycline intravenous in treating resistant gram-negative and methicillin-resistant *Staphylococcus aureus* (MRSA) infections at Newark Beth Israel Medical Center [21]. This report included 2 patients with pneumonia caused solely by *A. baumannii*. A specific definition for pneumonia was not provided. Both cases of *Acinetobacter* pneumonia clinically improved and were deemed successfully treated with minocycline 100 mg intravenous every 12 hours for durations between 7 and 14 days. Whether additional antibiotics were added to minocycline intravenous in these cases of *Acinetobacter* pneumonia was not specifically addressed. No adverse effects were noted with minocycline in either of the cases, and both patients were discharged from the hospital.

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Minocycline for Other *Acinetobacter* Infections

In addition to their aforementioned pneumonia cases, Jankowski et al also described their use of minocycline intravenous for a case of bacteremia and a case of skin and soft tissue infection caused by MDR *Acinetobacter* [20]. The patient with bacteremia received a minocycline loading dose of 200 mg intravenous, followed by 100 mg intravenous every 12 hours for 20 days, in addition to colistin for 19 of the 20 days. Bacterial eradication and clinical cure were achieved. The patient was discharged, was not continued on any antibiotics following discharge, and was not readmitted to the authors' hospital within 90 days following discharge. However, at a 90-day postdischarge evaluation, the patient was noted to have died. The other case was a wound infection caused by MDR *Acinetobacter*. This patient was treated with minocycline 100 mg intravenous every 12 hours for 10 days and colistin for 17 days. Presumed bacterial eradication was achieved, and the patient was discharged from the hospital alive. At 90-day postdischarge follow-up, the patient was still alive.

Bishburg et al recently published their experience using minocycline intravenous in treating resistant gram-negative and MRSA infections at Newark Beth Israel Medical Center [21]. This report included 3 patients with polymicrobial infections involving *A. baumannii*: 2 with skin and soft tissue infection (including a postoperative wound infection) and 1 with osteomyelitis. Specific definitions for infections assessed and details of any adjunctive therapies were not provided. Each patient with polymicrobial infection involving MDR *Acinetobacter* clinically improved and was deemed successfully treated with minocycline 100 mg intravenous every 12 hours for durations of therapy up to 7 days, followed by oral minocycline for continued therapy. No adverse effects were noted with minocycline in any of the cases, and all 3 patients were discharged from the hospital.

The availability of minocycline in both intravenous and oral dosage forms is convenient and can help promote continuity of treatment. Griffith et al reported a case series of 8 patients with specifically defined traumatic wound infections with presumptive osteomyelitis caused by MDR *Acinetobacter baumannii* from Brooke Army Medical Center in Fort Sam Houston, Texas [22]. Initial therapy with minocycline intravenous was not provided in any case. However, this study occurred during the period of time that minocycline intravenous was not available on the US market. All patients were treated with minocycline 100 mg orally twice daily for 4–7 weeks, and all isolates of *A. baumannii* were susceptible to minocycline. Three patients received prior therapy with colistin (2 cases) and imipenem (1 case). Seven of the 8 patients' infections involved copathogens, all of which were treated with other concomitant antibiotics in addition to minocycline. However, in the majority of cases, the additional antibiotic was inactive against the *Acinetobacter* strain identified. Patients were followed for an average of 6 weeks. All patients also underwent serial surgical debridement of nonviable or overtly infected tissue, and 4 patients had retained, surgically placed hardware. Treatment was deemed successful in 7 of the 8 patients treated with minocycline. The other patient was clinically responding to minocycline, but developed eosinophilia and neutropenia, which resolved upon discontinuation of minocycline. No other adverse effects of minocycline were noted in any other patient.

ADVERSE EVENTS

Minocycline is, in general, well tolerated. In an early clinical evaluation of minocycline intravenous for treatment of 24 severe infections (non-*Acinetobacter*), no toxicities or adverse effects of the agent were noted [23]. Very limited details regarding the tolerability of minocycline intravenous for treatment of MDR *Acinetobacter* infections in the previously discussed clinical reports are available. One patient receiving minocycline intravenous for treatment of a traumatic wound infection caused by MDR *Acinetobacter* experienced reversible eosinophilia and neutropenia while clinically improving on minocycline [22]. No adverse effects of minocycline intravenous were noted in any of the 8 patients receiving the drug for treatment of infections caused by MDR organisms, including 5 with MDR *Acinetobacter* infections [21]. Of interest, additional safety data are available for nonantimicrobial use of minocycline intravenous. Minocycline intravenous at doses of up to 10 mg/kg/day for 72 hours was reported to be safe and well tolerated in 60 patients receiving the drug in the setting of acute ischemic stroke, with only a single patient experiencing dose-limiting hepatic enzyme elevation [24].

As with other tetracyclines, minocycline should be avoided in pregnancy and in children aged <8 years due to its ability to cause permanent tooth discoloration [6]. Minocycline may cause photosensitivity, lightheadedness, dizziness, vertigo, gastrointestinal disturbances, and local injection site reactions [6, 25, 26]. Drug rash with eosinophilia and systemic symptoms, hepatotoxicity, *Clostridium difficile*–associated diarrhea, pseudotumor cerebri, serum sickness–like reaction, hematologic abnormalities, drug-induced lupus, and antianabolic effects may rarely occur [6, 27]. A thorough study of the possible effects of minocycline intravenous on the QTc interval has not been conducted. Overall, minocycline intravenous appears to have a favorable risk–benefit profile when considered for treatment of serious MDR *Acinetobacter* infections.

DISCUSSION

A total of 23 cases of MDR *Acinetobacter* pneumonia successfully treated with minocycline intravenous have been reported by 4 different groups of authors (Table 2). Although the reports are descriptive, often lack specific details, frequently involve use of minocycline intravenous in combination with other antibiotics,

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Table 2. Summary of Clinical Experience With Minocycline for Treatment of Infections Caused by *Acinetobacter*

Study	Description	Outcomes Evaluated	Results
Pneumonia			
Wood et al [18]	Retrospective case series VAP Critically ill trauma patients MDR *Acinetobacter baumannii* n = 4 All sensitive to tetracycline Monotherapy (n = 2) and combination therapy (n = 2) Minocycline 100 mg intravenous every 12 h Treatment duration ranged from 10 to 20 d	Success was defined as negative follow-up BAL and clinical improvement. If follow-up BAL was unavailable, then success was defined as clinical improvement and survival until hospital discharge. Failure was defined as death due to VAP complications or persistent positive BAL culture without clinical improvement.	All 4 patients achieved success. Three patients had a negative follow-up BAL. One patient did not have a follow-up BAL.
Chan et al [19]	Retrospective study VAP Trauma center Carbapenem-resistant *Acinetobacter* n = 19 Minocycline 200 mg, then 100 mg intravenous every 12 h (or 200 mg orally or per tube every 12 h) Overall average treatment duration = 13.3 d	Clinical response, defined as improvement and resolution of signs and symptoms of VAP, or microbiologic eradication from follow-up BAL or sputum culture	Clinical response to minocycline intravenous: 15/19 (78.9%) Clinical response to minocycline oral: 14/17 (82.5%) Overall clinical response to minocycline-based regimens: 29/36 (80.6%) Overall clinical response regardless of specific antibiotic therapy: 42/55 (76.4%)
Jankowski et al [20]	Retrospective case series Intensive care unit patients MDR *Acinetobacter baumannii* n = 3 Minocycline 100 mg intravenous every 12 h Treatment duration ranged from 10 to 13 d	Successful clinical outcome was defined as the absence of or partial resolution of clinical and laboratory parameters of infection. Successful microbiologic outcome was defined as documented or presumed eradication.	Successful clinical outcome: n = 2/3 Successful microbiologic outcome: n = 3/3
Bishburg et al [21]	Retrospective study Hospitalized patients *Acinetobacter baumannii* n = 2 Minocycline 100 mg intravenous every 12 h (allowed transition to minocycline oral therapy to complete the course) Treatment duration ranged from 5 to 18 d	Clinical improvement Hospital discharge	Both patients demonstrated clinical improvement and were discharged from the hospital.
Skin and soft tissue infections with or without osteomyelitis			
Jankowski et al [20]	See tabular description above n = 1 Minocycline 100 mg intravenous every 12 h Duration of 10 d	See tabular description above	Successful clinical and microbiologic outcome
Bishburg et al [21]	See tabular description above Osteomyelitis: n = 1 Skin and soft tissue infection: n = 2 (included a postoperative wound infection) Minocycline 100 mg intravenous every 12 h	See tabular description above	All 3 patients demonstrated clinical improvement and were discharged from the hospital.
Griffith et al [22]	Retrospective study Traumatic wound infection with presumptive osteomyelitis MDR *Acinetobacter calcoaceticus* complex n = 8 Sensitive to minocycline: n = 3 Susceptibility to minocycline not available: n = 5 Minocycline 100 mg orally twice daily Treatment duration ranged from 4 to 7 wk	Success was defined as no further evidence of infection as determined by symptoms, physical exam, and laboratory evaluation (leukocyte count, erythrocyte sedimentation rate, and C-reactive protein).	Successful outcome: n = 7/8
Bacteremia			
Jankowski et al [20]	See tabular description above n = 1 Minocycline 200 mg, then 100 mg intravenous every 12 h Treatment duration of 20 d	See tabular description above	Successful clinical and microbiologic outcome

Abbreviations: BAL, bronchoalveolar lavage; MDR, multidrug-resistant; VAP, ventilator-associated pneumonia.

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and often involve copathogens, the published experiences with the use of minocycline intravenous for treatment of MDR *Acinetobacter* pneumonia are in general favorable and consistent. Moreover, minocycline penetrates rapidly and substantially into lung tissues, as well as sputum [28–30]. Whether nonantibiotic, anti-inflammatory properties characteristic of tetracyclines are involved in the overall therapeutic activity of intravenous minocycline for MDR *Acinetobacter* infections is unclear [14].

Experience with minocycline intravenous for treatment of skin and soft issue infection and bacteremias caused by MDR *Acinetobacter* is much more limited. In addition, consideration of intravenous to oral step-down minocycline therapy is supported by Chan et al's previously discussed successful use of this strategy in the setting of VAP, as well as by the aforementioned favorable results achieved by Griffith et al with minocycline oral for treatment of several traumatic wound infections with bone involvement [19, 22].

Given the lack of options available for treating MDR *Acinetobacter* infections, it is reasonable to consider use of any agent (s) testing active against a particularly resistant strain of this organism, including minocycline intravenous. Due to the possibility of obtaining discordant results among tetracycline agents, in vitro susceptibility testing with *Acinetobacter* should include minocycline. The availability of CLSI susceptibility breakpoints with *Acinetobacter* and minocycline allows accurate reporting of minocycline susceptibility results in the clinical setting.

Increasing clinical experience is accumulating with minocycline intravenous monotherapy and as a component of combination therapy for MDR *Acinetobacter* infections, especially pneumonia. The role of minocycline intravenous for treatment of MDR *Acinetobacter* infections is likely to continue to evolve with the availability of additional clinical and microbiologic data. The existing minocycline intravenous indication for treatment of *Acinetobacter* infections, the encouraging clinical results discussed herein, and the generally favorable safety profile of minocycline intravenous warrant its serious consideration for treatment of serious MDR *Acinetobacter* infections.

Notes

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References

1. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **2009**; 48:1–12.
2. Boucher HW, Talbot GH, Benjamin DK Jr, et al. 10 x '20 Progress-development of new drugs active against gram-negative bacilli: an update from the Infectious Diseases Society of America. Clin Infect Dis **2013**; 56:1685–94.
3. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, **2013**. Available at: http://www.cdc.gov/drugresistance/threat-report-2013/. Accessed 1 August 2014.
4. Bishburg E, Bishburg K. Minocycline—an old drug for a new century: emphasis on methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. Int J Antimicrob Agents **2009**; 34:395–401.
5. Redin GS. Antibacterial activity in mice of minocycline, a new tetracycline. Antimicrob Agents Chemother **1966**; 6:371–6.
6. Minocin [package insert]. San Diego, CA: Rempex Pharmaceuticals, 2013.
7. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 24th informational supplement. CLSI document M100-S24. Wayne, PA: CLSI, **2014**.
8. Tan TY, Ng LSY, Tan E, Huang G. In vitro effects of minocycline and colistin combinations on imipenem-resistant *Acinetobacter baumannii* clinical isolates. J Antimicrob Chemother **2007**; 60:421–3.
9. Liang W, Liu X-F, Huang J, Zhu D-M, Li J, Zhang J. Activities of colistin- and minocycline-based combinations against extensive drug resistant *Acinetobacter baumannii* isolates from intensive care unit patients. BMC Infect Dis **2011**; 11:109.
10. Akers KS, Mende K, Yun HC, et al. Tetracycline susceptibility testing and resistance genes in isolates of *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex from a U.S. military hospital. Antimicrob Agents Chemother **2009**; 53:2693–5.
11. Vilacoba E, Aluzara M, Gulone L, et al. Emergence and spread of plasmid-borne *tet*(B)::IS*CR2* in minocycline-resistant *Acinetobacter baumannii* isolates. Antimicrob Agents Chemother **2013**; 57:651–4.
12. Lomovskaya O, Sun D, King P, Dudley MN. Tigecycline but not minocycline selects for clinically relevant efflux-mediated resistance in *Acinetobacter* spp. (abstract C1-1087). In: Program and Abstracts of the 53rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 10–13 September 2013.
13. Thummel KE, Shen DD. Dosage and optimization of dosage regimens: pharmacokinetic data. In: Hardman JG, Limbird LE, eds. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill, **2001**:1983.
14. Bahrami F, Morris DL, Pourgholami MH. Tetracyclines: drugs with huge therapeutic potential. Mini Rev Med Chem **2012**; 12:44–52.
15. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother **2006**; 58:256–65.
16. Welling PG, Shaw WR, Uman SJ, Tse FLS, Craig WA. Pharmacokinetics of minocycline in renal failure. Antimicrob Agents Chemother **1975**; 8:532–7.
17. Bowker KE, Noel AR, MacGowan A. Pharmacodynamics of minocycline against *Staphylococcus aureus* in an in vitro pharmacokinetic model. Antimicrob Agents Chemother **2008**; 52:4370–3.
18. Wood GC, Hanes SD, Boucher BA, Croce MA, Fabian TC. Tetracyclines for treating multi-drug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. Intensive Care Med **2003**; 29:2072–6.
19. Chan JD, Graves JA, Dellit TH. Antimicrobial treatment and clinical outcomes of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. J Intensive Care Med **2010**; 25:343–8.
20. Jankowski CA, Balada-Llasat J-M, Raczkowski M, Pancholi P, Goff DA. A stewardship approach to combating multidrug-resistant *Acinetobacter baumannii* infections with minocycline. Infect Dis Clin Pract **2012**; 20:184–7.
21. Bishburg E, Shah M, Chan T. Use of intravenous minocycline for the treatment of methicillin-resistant *Staphylococcus* (MRSA) and resistant gram-negative organisms. Infect Dis Clin Pract **2014**; 22:26–31.
22. Griffith ME, Yun HC, Horvath LL, Murray CK. Minocycline therapy for traumatic wound infections caused by the multidrug-resistant *Acinetobacter baumannii–Acinetobacter calcoaceticus* complex. Infect Dis Clin Pract **2008**; 16:16–9.

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23. Rogers JH, Barnwell PA, Waterman NG, Austin FD, Raff MJ. Clinical evaluation of intravenous minocycline. Int J Clin Pharmacol **1979**; 15:194–8.
24. Fagan SC, Waller JL, Nichols FT, et al. Minocycline to improve neurologic outcomes in stroke (MINOS): a dose-finding study. Stroke **2010**; 41:2283–7.
25. Jacobson JA, Daniel B. Vestibular reactions associated with minocycline. Antimicrob Agents Chemother **1975**; 8:453–6.
26. Smith K, Leyden JJ. Safety of doxycycline and minocycline: a systematic review. Clin Ther **2005**; 27:1329–42.
27. Shapiro LE, Knowles SR, Shear NH. Comparative safety of tetracycline, minocycline, and doxycycline. Arch Dermatol **1997**; 133:1224–30.
28. Naline E, Sanceaume M, Toty L, Bakdach H, Pays M, Advenier C. Penetration of minocycline into lung tissues. Br J Clin Pharmacol **1991**; 32:402–4.
29. Watanabe A, Anzai Y, Niitsuma K, Saito M, Yanase K, Nakamura M. Penetration of minocycline hydrochloride into lung tissue and sputum. Chemotherapy **2001**; 47:1–9.
30. Saivin S, Houin G. Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet **1988**; 15:355–66.

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