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Biofilm prevention concentrations (BPC) of minocycline compared to polymyxin B, meropenem, and amikacin against *Acinetobacter baumannii*

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ABSTRACT

Infections caused by *Acinetobacter baumannii* are difficult to treat as they are often multidrug resistant (MDR) and frequently form biofilms. We investigated the activities of minocycline, polymyxin B, meropenem, and amikacin against diverse *Acinetobacter baumannii* strains with biofilm formation classified as weak versus moderate/strong. At clinically achievable concentrations, minocycline prevented biofilm formation for 96% of isolates versus 54% for polymyxin B, 29% for meropenem and 29% for amikacin. Minocycline and polymyxin B demonstrated highest in vitro activity against *A. baumannii* and prevented biofilm formation for a majority of isolates.

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1. Introduction

Acinetobacter baumannii is a multidrug-resistant (MDR) pathogen associated with hospital-acquired infections and nosocomial outbreaks. (Dettori et al., 2014) It is particularly difficult to eradicate as it can survive in dry conditions, and form biofilm by attaching to biotic and abiotic surfaces. (Duarte et al., 2016; Longo et al., 2014; Rodriguez-Bano et al., 2008) Consequently, it thrives in a hospital environment and causes various clinical syndromes including bloodstream infections, ventilator-associated pneumonia, urinary tract infections, wound infections, and others. (Dettori et al., 2014; Duarte et al., 2016; Longo et al., 2014; Rodriguez-Bano et al., 2008) It especially targets elderly and critically ill patients with weakened immune systems, (MacVane, 2016) and patients with implanted medical devices including intravascular devices, urinary catheters, portacaths, and those with ventilator support. (Wright et al., 2014) Due to emergence of *A. baumannii* as a MDR, biofilm-forming organism, old drugs are being explored and utilized to optimize patient outcomes. (Goff et al., 2014)

Minocycline is of particular interest from an efficacy and safety standpoint. (Goff et al., 2014; Karageorgopoulos and Falagas, 2008; Ritchie and Garavaglia-Wilson, 2014) It is a primarily bacteriostatic, semi-synthetic derivative of tetracycline that interferes with bacterial protein synthesis by reversibly binding to the 30S-ribosomal subunit. (Fishbain and Peleg, 2010) Data supports the use of minocycline for the treatment of *A. baumannii* infections due to, not only its antimicrobial activity, but also because it is a generally well-tolerated agent. (Goff et al., 2014; Karageorgopoulos and Falagas, 2008; Ritchie and Garavaglia-Wilson, 2014) Clinically, minocycline has been used alone, but mostly in combination with other active agents to treat a variety of *A. baumannii* infections including bacteremia, pneumonia, and urinary tract infections. (Goff et al., 2014) However, its utility against biofilm-forming organisms is less understood. Previously, tigecycline, a semi-synthetic derivative of minocycline, demonstrated activity against established *A. baumannii* biofilm in vitro, (Song et al., 2015) leading to our hypothesis that minocycline will also exhibit anti-biofilm activity. Therefore, we evaluated the ability of minocycline, as well as other anti-*Acinetobacter* antimicrobials including polymyxin B, meropenem, and amikacin, in preventing biofilm formation using previously described and validated in vitro biofilm methodology. (LaPlante and Mermel, 2007; Luther et al., 2014, 2015; McConeghy and LaPlante, 2010)

2. Materials and methods

2.1. Bacterial isolates

We examined 24 diverse strains of *A. baumannii* obtained clinically from patients at Rhode Island Hospital, Providence, RI (RIH), Veterans Affairs Medical Center, Providence, RI (VAMC), and from Biodefense

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and Emerging Infection Research Resources Repository, Manassas, VA (BEI). The following reagents were obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Acinetobacter baumannii*, Strain H72721, NR-9667; Isolate 1, NR-13374; Isolate 9, NR-13382; Strain 3–137 (OIFC137), NR-17777; Strain 5–032 (OIFC032), NR-17778; Strain IS-123, NR-17787; Strain Naval-18, NR-17785; Strain Naval-81, NR-17786; Strain OIFC109, NR-17780; Strain 5–143 (OIFC143), NR-17781; Strain 5–189 (OIFC189), NR-17782; Strain BC-5, NR-17783; Strain Naval-17, NR-17784; Strain WC-136, NR-19298; Strain WC-487, NR-19299.

Strains were chosen to depict various biofilm-forming capabilities including weak and moderate/strong biofilm-formers. A known strong biofilm-forming isolate (ATCC 19606) and weak biofilm-forming isolate (ATCC 17978) (Rodriguez-Bano et al., 2008) were used as quality controls to ensure consistent and accurate categorization of biofilm-formation. Isolates were kept frozen at −80 °C.

2.2. Antimicrobial agents

Minocycline HCl powder (Sigma-Aldrich; Lot # 116M4026V), meropenem (Fresenius Kabi, LLC; Lot# 0004D51), amikacin (Teva Pharmaceuticals; Lot # 8210415), and polymyxin B (X-Gen Pharmaceuticals; Lot#AB7600) drug products were used in the experiments. Drug products were supplied by the Providence Veterans Affairs Medical Center Pharmacy Department.

2.3. Media

Cation-adjusted (calcium, 25 μg/ml and magnesium, 12.5 μg/ml) Mueller-Hinton broth (CAMHB; Difco Laboratories, Sparks, MD, USA) was used to determine minimum inhibitory concentrations. (CLSI, 2015a, 2015b) Tryptic Soy Broth (TSB; Difco Laboratories, Sparks, Maryland, USA) supplemented with 1% dextrose was used to quantify biofilm and evaluate biofilm growth in presence of antimicrobial agents, a slight modification from previously utilized media. (Song et al., 2015; Tomaras et al., 2008) This broth was previously evaluated in the investigators laboratory and demonstrated favorable biofilm formation in *A. baumannii* compared to other broths. Colony counts were determined using Tryptic Soy Agar (TSA, Difco, Becton Dickinson Co., Sparks, MD, USA) plates.

2.4. Susceptibility testing

Minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) were determined in duplicate in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria. (CLSI, 1999, 2015a, 2015b) MICs were also evaluated in using tryptic soy media to ensure there is no variation media between the assays.

2.5. Quantification of biofilm formation

Quantification of biofilm was conducted using a previously described colorimetric microtiter plate assay (Christensen et al., 1985; LaPlante and Mermel, 2007, 2009; Luther et al., 2015; McConeghy and LaPlante, 2010; Stepanovic et al., 2007) modified to allow 48 hours of growth for *A. baumannii*. (Rodriguez-Bano et al., 2008) Starting inocula for biofilm assays, as well as MICs, were 10^6 colony-forming units per milliliter (CFU/mL). At 24 hours, broth was removed and fresh broth was added. After 48-hour growth, adherent bacteria were stained with 0.1% crystal violet (CV) for 15 minutes. CV was resolubilized in 33% glacial acetic acid (GAA) for 60 minutes before the optical density (OD) of each well was determined photometrically at 570 nm using a spectrophotometer (EL800x, Bio-Tek Instruments, Inc., Winooski, VT). Previous *A. baumannii* biofilm studies defined an isolate as biofilm-forming by its ability to produce an OD_{570} reading at least twice that of a negative control. (Duarte et al., 2016; Rodriguez-Bano et al., 2008) As all our isolates were biofilm-formers in accordance with this definition, we further categorized biofilm-formation as: weak ($OD_{570} < 1$), or strong/moderate ($OD_{570} \geq 1$) based on previous definitions. (Mohamed et al., 2004; Rodriguez-Bano et al., 2008)

2.6. Biofilm prevention assay

Biofilm prevention concentration (BPC), defined as the concentration of drug where no biofilm attachment was present (i.e. OD value of test well is equal to OD value of negative control), was determined for minocycline, polymyxin B, meropenem and amikacin, using previously described methodology with modification. (Christensen et al., 1985; LaPlante and Mermel, 2007, 2009; Luther et al., 2015; McConeghy and LaPlante, 2010; Stepanovic et al., 2007) Increasing concentrations (0.03-128 μg/mL) of each agent were combined with bacteria in a non-tissue culture 96-well plate (Costar 3370), and incubated for 48 hours in a shaking (50 rpm) incubator at 35 °C. Drug and broth were removed after 24 hours and replaced with freshly made drug and broth, and re-incubated for total of 48 hours of growth. After incubation, optical density (OD) of each well was determined photometrically using the same methods described for biofilm quantification. Wells without drug were classified as positive controls, and wells containing sterile media only (i.e. no drug or isolate) were considered negative controls for each isolate individually based on previous definitions. (LaPlante and Mermel, 2007, 2009; Rodriguez-Bano et al., 2008) Mean OD values of negative controls were subtracted from the OD values of the test wells including the positive control. (LaPlante and Mermel, 2009) Each test was carried out in quadruplicate, and average and standard deviation of ODs were taken to determine presence of biofilm at each concentration (data not shown).

3. Results

Twenty-four unique *A. baumannii* isolates were evaluated. MICs were validated and did not differ between tryptic soy and Mueller Hinton media (data not shown), allowing us to use MICs as a reference point for evaluating BPCs. All isolates were biofilm-formers in accordance with previous definitions of *A. baumannii* biofilm formation. (Duarte et al., 2016; Rodriguez-Bano et al., 2008) When using more stringent definitions, we further categorized the isolates as strong/moderate ($n = 12$) or weak ($n = 12$). The MIC_{50} and MIC_{90} for each antimicrobial agent is summarized in Table 1. Compared to median MICs, median concentrations required to prevent biofilm formation over 48 hours decreased for minocycline, remained the same for polymyxin, and increased for meropenem and amikacin. Additionally, minocycline prevented biofilm formation at or below the CLSI susceptibility breakpoint (i.e. clinically achievable concentrations) for 96% of isolates versus 54% for polymyxin B, 29% for meropenem, and 29% for amikacin. However, it is important to note that these percentages are dependent on the strains evaluated, and many isolates were amikacin-resistant. When evaluating isolate-specific changes in susceptibility, minocycline prevented biofilm formation at or below the isolate MIC for 92% of isolates versus 63% for polymyxin B, 29% for meropenem and 58% for amikacin. Isolate characteristics (MICs, MBCs, and BPCs) are further detailed in Table 2.

Incidentally, we observed the “skip wells” phenomenon (i.e. wells where no growth appears despite growth appearing at higher

Table 1
Summary of minimum inhibitory concentrations (MICs) and biofilm prevention concentrations (BPC) for all isolates.

Drug	MIC_{50}	MIC_{90}	MIC Range	BPC_{50}	BPC_{90}	BPC Range
Minocycline	1	8	0.06–16	0.5	4	0.03–8
Polymyxin B	2	4	0.125–8	2	16	0.06–64
Meropenem	1	64	0.5–128	8	64	0.5–128
Amikacin	64	>128	1- > 128	128	>128	1- >128

Table 2
Susceptibility testing and biofilm prevention concentration (μg/ml) results.

Strain	Minocycline		Polymyxin B		Meropenem		Amikacin	
	MIC* (MBC)	BPC	MIC* (MBC)	BPC	MIC* (MBC)	BPC	MIC* (MBC)	BPC
ATCC19606^{++}	0.125 (1)	0.06	0.125 (4)	0.5	0.5 (4)	2	16 (32)	32
NR-17785^{++}	0.5 (>2)	0.25	1 (8–16)	32	64 (128)	64	128 (>128)	128
NR-19298^{++}	1 (>2)	0.06	2 (2–4)	8	0.5–1 (2–4)	1	8 (16)	8
L1054^{++}	8-16 (32-64)	1	2–4 (4–8)	0.25	32 (128)	64	>128 (>128)	>128
L1056^{++}	8 (16)	4	4 (8)	0.5	8 (64)	32	128 (>128)	128
L1085^{++}	0.5-1 (2)	1	1–2 (4)	64	0.5 (>4)	4	16 (16–32)	32
NR-13374^{++}	1 (1)	0.5	4 (16)	4	8 (64)	128	8 (8)	8
NR-17777^{++}	2 (>8)	0.5	2 (8)	4	0.5–1 (4)	8	64 (128)	>128
NR-17787^{++}	1 (2)	1	4 (8)	4	2 (2)	8	64 (>128)	128
NR-17781^{++}	0.5 (>8)	0.06	0.5–1 (8)	8	0.5 (2)	1	4 (16)	16
NR-17784^{++}	1 (>16)	8	8 (8)	16	0.5 (4)	4	4 (8)	8
NR-19299^{++}	1 (2)	0.125	1–2 (2)	8	0.5 (2)	8	16 (16)	8
L1055^{+}	8 (32)	4	4 (32)	8	16 (16)	32	>128 (>128)	>128
NR-9667^{+}	0.5–1 (>8)	0.25	2–4 (4)	0.25	16 (32)	32	16 (16)	128
NR-13382^{+}	2 (4)	4	2 (>16)	0.5	128 (>128)	128	128 (128)	128
NR-17778^{+}	0.25 (>8)	0.03	1–2 (16)	2	0.5 (2)	1	4 (8)	16
NR-17786^{+}	0.5 (1)	0.25	0.5 (1)	0.06	0.5 (1)	4	128 (>128)	128
NR-17782^{+}	4 (64)	2	2–4 (4)	4	0.5 (1)	2	64 (128)	128
NR-17783^{+}	0.5 (2)	0.5	0.5 (>4)	0.25	32 (32)	0.5	16 (32–64)	128
ATCC 17978^{+}	0.06 (0.25–0.5)	0.03	0.5 (0.5)	0.06	1 (4)	1	1 (2)	1
NR-17780^{+}	2 >8)	0.5	1–2 (2–4)	0.25	0.5–1 (4)	4	64–128 (>128)	64
L1051^{+}	16 (32)	1	2 (2)	0.06	64 (64)	64	128 (>128)	128
L1052^{+}	8 (16–32)	2	2 (2)	2	64 (128- > 128)	64	>128 (>128)	>128
L1053^{+}	8 (16)	0.5	0.5–1 (4)	0.5	16–32 (128)	64	>128 (>128)	>128

$^{++}$ Moderate/strong biofilm former; $^{+}$Weak biofilm former.
* CLSI breakpoints: minocycline ≤4 μg/mL, polymyxin B ≤ 2 μg/mL, meropenem ≤2 μg/mL, amikacin ≤16 μg/mL.

concentrations) for some polymyxin B-containing wells. This has been described in the literature for polymyxins. (Hawley et al., 2007; Landman et al., 2013) Although the definitive mechanism for skipped wells is uncertain, the cause is attributed to the cationic properties of polymyxin B, and the presence of heteroresistance in Gram-negative organisms including *A. baumannii*. (Hawley et al., 2007; Landman et al., 2013) Guidance on interpretation of "skip wells" is unclear; therefore, we excluded experiments with skipped wells from interpretation.

4. Discussion

A. baumannii is often MDR and challenging to treat particularly due to its ability to form biofilm. Several studies have demonstrated a significant association between biofilm production and multidrug resistance in *A. baumannii*. (Badave and Kulkarni, 2015; Gurung et al., 2013; Rao et al., 2008) Our study revealed that minocycline has ability to prevent biofilm formation in vitro at clinically relevant serum concentrations for a larger number of *A. baumannii* isolates than polymyxin B, meropenem and amikacin. Furthermore, as biofilm mass increases, minocycline was the only agent tested that was able to prevent biofilm formation at concentrations below the CLSI susceptibility breakpoint of ≤4 μg/mL. Our findings are supported by previous data indicating that carbapenem or colistin monotherapy lacked sufficient activity against *A. baumannii* biofilm, while tigecycline, a semi-synthetic form of minocycline, inhibited biofilm formation even at subinhibitory concentrations at one-fourth of the isolate MIC. (Song et al., 2015) Additionally, amikacin demonstrated poor activity and ability to prevent biofilm formation in the current study. Previous studies have shown that biofilm formers are associated with higher resistance rates to aminoglycosides, including amikacin (Badave and Kulkarni, 2015; Duarte et al., 2016), which may explain our findings as all isolates tested were biofilm-formers. Interestingly, minocycline prevented biofilm formation for a larger number of isolates than polymyxin B, amikacin, and meropenem

when BPC was compared to isolate-specific MIC. Although not clinically relevant for resistant isolates, these data suggest that planktonic and biofilm-forming isolates exhibit different modes of growth that may result in discrepant antimicrobial activity observations.

Minocycline anti-biofilm activity may be due to its mechanism of inhibiting early protein synthesis as other agents with a similar mechanism, including tigecycline and rifampin, also exhibit anti-biofilm activity. (Croes et al., 2010; Song et al., 2015) From a pharmacodynamic standpoint, minocycline efficacy is associated most closely with a free drug area under the curve/ MIC (*f*AUC/MIC) ratio of 20–25, (Agwuh and MacGowan, 2006; Ritchie and Garavaglia-Wilson, 2014) and MIC values ≤4 μg/mL are reasonable to achieve the target parameter. (Alfouzan et al., 2017) However, unless used at higher than standard doses (e.g. 700 mg/day) or in combination with other active agents, rapid emergence of resistance is a concern. (Alfouzan et al., 2017)

Ninety-six percent of all tested isolates had a BPC below ≤4 μg/mL indicating that minocycline can potentially prevent biofilm formation by achieving clinically relevant *f*AUC/MIC concentrations over 48 hours. Consequently, prompt administration to prevent biofilm attachment, particularly in patients with implanted medical devices and/or catheters, is warranted. Due to toxicity concerns with polymyxin B and variability of resistance patterns between agents, minocycline is a viable treatment option. However, due to rapid emergence of resistance (Alfouzan et al., 2017) use should be largely in combination with other active agents, particularly as hospitals worldwide are losing therapeutic options against *A. baumannii* infections. (Goff et al., 2014) There is a scarcity of in vitro and clinical data describing the optimal treatment of biofilm-forming *A. baumannii* isolates. More studies exploring the role of antimicrobial activity in preventing and promoting biofilm formation, including optimal doses that prevent resistance, are warranted.

Disclaimer

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Conflict of interest

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References

Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother 2006;58(2):256–65.

Alfouzan WA, Noel AR, Bowker KE, Attwood MLG, Tomaselli SG, MacGowan AP. Pharmacodynamics of minocycline against Acinetobacter baumannii studied in a pharmacokinetic model of infection. Int J Antimicrob Agents 2017;50(6):715–7.

Badave GK, Kulkarni D. Biofilm producing multidrug resistant Acinetobacter baumannii: an emerging challenge. J Clin Diagn Res 2015;9(1):DC08–10.

Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 1985;22(6):996–1006.

Clinical and Laboratory Standards Institute (CLSI). M26-A. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline. CLSI document. Wayne PA: Clinical and Laboratory Standards Institute (CLSI); 1999.

Clinical and Laboratory Standards Institute (CLSI). M100-S25. Performance standards for antimicrobial susceptibility testing: 25th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2015a.

Clinical and Laboratory Standards Institute (CLSI). M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2015b.

Croes S, Beisser PS, Neef C, Bruggeman CA, Stobberingh EE. Unpredictable effects of rifampin as an adjunctive agent in elimination of rifampin-susceptible and -resistant Staphylococcus aureus strains grown in biofilms. Antimicrob Agents Chemother 2010;54(9):3907–12.

Dettori M, Piana A, Deriu MG, Lo Curto P, Cossu A, Musumeci R, et al. Outbreak of multidrug-resistant Acinetobacter baumannii in an intensive care unit. New Microbiol 2014;37(2):185–91.

Duarte A, Ferreira S, Almeida S, Domingues FC. Clinical isolates of Acinetobacter baumannii from a Portuguese hospital: PFGE characterization, antibiotic susceptibility and biofilm-forming ability. Comp Immunol Microbiol Infect Dis 2016;45:29–33.

Fishbain J, Peleg AY. Treatment of Acinetobacter infections. Clin Infect Dis 2010;51(1):79–84.

Goff DA, Bauer KA, Mangino JE. Bad bugs need old drugs: a stewardship program's evaluation of minocycline for multidrug-resistant Acinetobacter baumannii infections. Clin Infect Dis 2014;59(Suppl. 6):S381–7.

Gurung J, Khyriem AB, Banik A, Lyngdoh WV, Choudhury B, Bhattacharyya P. Association of biofilm production with multidrug resistance among clinical isolates of Acinetobacter baumannii and Pseudomonas aeruginosa from intensive care unit. Indian J Crit Care Med 2013;17(4):214–8.

Hawley JS, Murray CK, Griffith ME, McElmeel ML, Fulcher LC, Hospenthal DR, et al. Susceptibility of acinetobacter strains isolated from deployed U.S. military personnel. Antimicrob Agents Chemother 2007;51(1):376–8.

Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant Acinetobacter baumannii infections. Lancet Infect Dis 2008;8(12):751–62.

Landman D, Salamera J, Quale J. Irreproducible and uninterpretable Polymyxin B MICs for Enterobacter cloacae and Enterobacter aerogenes. J Clin Microbiol 2013;51(12):4106–11.

LaPlante KL, Mermel LA. In vitro activity of daptomycin and vancomycin lock solutions on staphylococcal biofilms in a central venous catheter model. Nephrol Dial Transplant 2007;22(8):2239–46.

LaPlante KL, Mermel LA. In vitro activities of telavancin and vancomycin against biofilm-producing Staphylococcus aureus, S. Epidermidis, and enterococcus faecalis strains. Antimicrob Agents Chemother 2009;53(7):3166–9.

Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. New Microbiol 2014;37(2):119–27.

Luther MK, Mermel LA, LaPlante KL. Comparison of ML8-X10 (a prototype oil-in-water micro-emulsion based on a novel free fatty acid), taurolidine/citrate/heparin and vancomycin/heparin antimicrobial lock solutions in the eradication of biofilm-producing staphylococci from central venous catheters. J Antimicrob Chemother 2014;69(12):3263–7.

Luther MK, Bilida S, Mermel LA, LaPlante KL. Ethanol and isopropyl alcohol exposure increases biofilm formation in Staphylococcus aureus and Staphylococcus epidermidis. Infect Dis Ther 2015;4(2):219–26.

MacVane SH. Antimicrobial resistance in the intensive care unit: a focus on gram-negative bacterial infections. J Intensive Care Med 2017;32(1):25–37.

McConeghy KW, LaPlante KL. In vitro activity of tigecycline in combination with gentamicin against biofilm-forming Staphylococcus aureus. Diagn Microbiol Infect Dis 2010;68(1):1–6.

Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by enterococcus faecalis. Infect Immun 2004;72(6):3658–63.

Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of Acinetobacter baumannii. Indian J Med Microbiol 2008;26(4):333–7.

Ritchie DJ, Garavaglia-Wilson A. A review of intravenous minocycline for treatment of multidrug-resistant Acinetobacter infections. Clin Infect Dis 2014;59(Suppl. 6):S374–80.

Rodriguez-Bano J, Marti S, Soto S, Fernandez-Cuenca F, Cisneros JM, Pachon J, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implications. Clin Microbiol Infect 2008;14(3):276–8.

Song JY, Cheong HJ, Noh JY, Kim WJ. In vitro comparison of anti-biofilm effects against Carbapenem-resistant Acinetobacter baumannii: imipenem, Colistin, Tigecycline, Rifampicin and Combinations. Infect Chemother 2015;47(1):27–32.

Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007;115(8):891–9.

Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA, Actis LA. Characterization of a two-component regulatory system from Acinetobacter baumannii that controls biofilm formation and cellular morphology. Microbiology 2008;154(Pt 11):3398–409.

Wright MS, Perez F, Brinkac L, Jacobs MR, Kaye K, Cober E, et al. Population structure of KPC-producing Klebsiella pneumoniae isolates from midwestern U.S. hospitals. Antimicrob Agents Chemother 2014;58(8):4961–5.