Anti-Biofilm Treatments: Single and Combination Antibiotic Therapy

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The public health challenge of antibacterial resistance has escalated considerably over recent decades. Of all potentially pathogenic species of bacteria those that form biofilm, complex surface-attached communities of bacteria held together by self-produced polysaccharide extracellular matrices, show heightened resistance to antibiotics. Foremost among these is *Staphylococcus*, in particular methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). Determination of minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) facilitates improved treatment of *S. aureus* biofilm infections. Although current approaches to combination therapy, typically using an antibiotic alongside an anti-biofilm agent, can achieve successful patient outcomes, complete removal of biofilm remains extremely difficult. Ongoing research aims to develop better means to address this important clinical concern.

Biofilm Antibiotic Single therapy Combination therapy Antibacterial resistance Staphylococcus MRSA VRSA

1. Introduction

Antibiotics can be used both as prevention and therapy. In terms of current treatments, different strategies include raising dosage concentrations and combining therapy with other antimicrobial agents [1]. The maturity of the mass of a biofilm should be considered as mature and therefore as less susceptible to treatment [2][3]. This applies to a wide range of species of both facultative aerobic and facultative anaerobic bacteria that form biofilm. These include the classically non-motile Gram-positive *Staphylococcus aureus, S. epidermidis*, *Enterococcus faecium* and Gram-negative *Acinetobacter baumannii* and *Klebsiella pneumoniae*, as well as the flagellated Gram-negative *Pseudomonas aeruginosa* and *Enterobacter* spp. [4][5].

In selecting a suitable antibiotic sufficient biofilm penetration is an important consideration. Hence, tetracyclines, macrolides, rifamycins, lincosamides, quinolones, fusidic acid, oxazolidinones, sulfonamides and nitroimidazole are preferred to glycopeptides, aminoglycosides, polymyxins and β-lactamases as they have the capability to penetrate deeper [6]. In addition to biofilm age and level of resistance to a given antibiotic, broader considerations for treatment include appropriate duration of antibiotic regimen and dosage optimization [7].

2. Bacterial resistance

There are several tolerance mechanisms utilized by bacteria that enable them to show resistance and persistence in the face of antibiotic treatment. A growing concern surrounds the fact that biofilms are not only resistant to antibiotics, but frequently also to the host immune response [8]. In order to combat the thorny problem of antibiotic resistance, suggested solutions include gaining a deeper knowledge of phenotypic and genotypic characteristic features of biofilm [9]. Mounting evidence indicates that acquiring resistant genes via genetic exchange and through extracellular polymeric substances plays a pivotal role in antibacterial tolerance [10][11].

A specific feature of biofilm is 'recalcitrance', a term used to describe its capability to survive in the presence of high doses of antibiotics [12]. Bacteria within biofilm can exhibit resistance to multiple treatments, even in the presence of high concentrations of bactericidal and bacteriostatic antibiotics and toxic compounds, in stark contrast to their planktonic existence. Noteworthy among various mechanisms by which this complex phenomenon may occur are antibiotic efflux, enzyme activity and reduced permeability. MIC can be used as a quantitative measure of antibiotic resistance; the higher the MIC, the more resistant. Resistance and tolerance each has a potential role in biofilm recalcitrance. Exposure to both bacteriostatic and bactericidal antibiotics can lead to resistance, while it is only the use of bactericidal antibiotics that may result in tolerance [12][13][14].

3. Antibiotic sensitivity tests

The MIC and minimum bactericidal concentration (MBC) are the lowest levels of an antimicrobial agent, typically an antibiotic, required to prevent visible growth upon overnight incubation (i.e., to cause cell stasis) and to kill a particular bacterium, respectively [15]. Similarly, minimum biofilm inhibitory concentration (MBIC) and MBEC refer to the lowest concentrations needed to achieve inhibition and eradication, respectively [16]. MIC is much higher for those bacteria that form biofilm compared to those than do not [17]. This concurs with the observation that biofilms are resistant to antibiotics concentrations up to 1000 × greater than those required to kill free-living bacteria [1], which signifies a pressing need to use combination therapy instead of monotherapy. The emergence of *S. aureus* isolates that are resistant to multiple antibiotics is a real concern, especially as it is exaggerated among MRSA strains [18][19].

Performing antibiotic sensitivity tests is necessary to select an appropriate choice and dose of treatment. Determination of MIC and MBEC of bacteria can inform tailored treatments and help to reduce the spread of resistant strains. Staphylococcal isolates from biofilm show a much higher breakpoint for MBEC than for MIC, indicating the importance of applying both biofilm susceptibility tests [20]. While vancomycin MBEC and MIC of planktonic cells are similar, for biofilm-producing isolates they are markedly different, so from a clinical perspective MBEC is the preferred measure [21]. Despite the availability of standardized methods to treat biofilm, most successful approaches were determined on planktonic cells. Although MBEC and MBIC values are proposed, this is confounded by limited evidence and complexity of correlation between innate activity towards planktonic cells and those in biofilm [22].

4. Multi-drug resistance

Resistance to a range of antibiotics has become common among MRSA strains. The formerly frontline β-lactam antibiotic methicillin targets penicillin-binding proteins (PBPs), enzymes that are essential to peptidoglycan synthesis. Yet, due to genetic mutation under the selective pressure imposed by overuse, PBP and PBP2a have become principal resistance factors [23][24]. One study from Nepal showed that the vast majority of multi-drug resistant isolates are MRSA with potential to produce biofilm [25]. Similarly, all strains of MRSA from nasal carriers possessed the capacity to form a biofilm that showed resistance to multiple antibiotics [26]. However, another study reported no difference between methicillin-sensitive *S. aureus* and MRSA strains to form biofilm [27], implying that there is no direct correlation between the ability of an isolate to form biofilm and its pattern of antibiotic resistance. In some cases, antibiotic therapy may not be completely successful due to low permeability to the biofilm matrix [28]. When this occurs, either removal of the foreign body, long-term single antibiotic treatment at high dosage and/or combination therapy is advised.

5. Combination therapy

A currently largely successful *S. aureus* anti-biofilm agent is the glycopeptide antibiotic vancomycin, which acts by interrupting cell wall synthesis [29][30]. Vancomycin is the preferred treatment for MRSA at present, although recently VRSA has been reported. The emergence of these strains, a major public health concern, could be for one or more reasons. Vancomycin is a large compound, which can lead to its weak penetration of biofilm. Additionally, as it inhibits oxygen and nutrient uptake [31]. In order to address this issue, combination therapy with antibiotics like rifampin and linezolid is proposed [32][33][34][35]. Many tested strains of *S. aureus* and most of *S. epidermidis* are susceptible to rifampin, which can penetrate biofilm [36][37]. In keeping with this, rifampin was shown to be the only good candidate for biofilm therapy in isolates with relatively high MBEC for each of vancomycin, rifampin and gentamicin [38]. The efficacy of rifampin in combination with vancomycin is due to reducing bacterial adhesion [39]. On a cautionary note, as resistance to rifampin is acquired rapidly it should not be used alone [40]. The lipopeptide antibiotic daptomycin, an alternative treatment option for MRSA and VRSA, effectively targets biofilm [41]. Both rifampin and daptomycin can disrupt MRSA biofilm at lower concentrations than that of tigecycline required to eradicate mature biofilm, especially when used in combination. Other antibiotics are able only to prevent cell attachment [42].

6. Hi-tech delivery of antibiotics

In response to a substantial increase in reports of MRSA and VRSA in recent years, a range of modern medical technologies, such as laser therapy and nanoparticles, have been investigated in attempts to enhance antibiotic efficacy. There are several benefits of harnessing nanoparticles including their high surface area to volume ratio, capacity for drug transportation and antibiotics protection against exposure to pH and enzymes, each of which enhances the efficacy of an administered antibiotic [6][43][44]. When gold nanoparticles were used alongside laser therapy to combat resistant strains of *S. aureus* and *P. aeruginosa* biofilm viability reduced and, conversely, antibiotic sensitivity increased [45]. In another study in which gold nanoparticles were conjugated to antibody specific to *S. aureus* peptidoglycan and activated by exposure to laser, bacterial cell counts were substantially

reduced [23]; potentially, such technology could be used in tandem with antibiotics to boost their efficacy. Continuing research is exploring how to effectively harness enzymes as anti-biofilm agents. Enzymatic degradation is a potentially suitable replacement to using toxic compounds to facilitate antibiotic penetration of biofilm. For example, *Mycobacterium* proteases have shown promise [46].

References

1. Suresh, M.K.; Biswas, R.; Biswas, L. An update on recent developments in the prevention and treatment of Staphylococcus aureus biofilms. Int. J. Med. Microbiol. 2019, 309, 1–12.
2. Hengzhuang, W.; Wu, H.; Ciofu, O.; Song, Z.; Høiby, N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid Pseudomonas aeruginosa biofilms. Antimicrob. Agents Chemother. 2011, 55, 4469–4474.
3. Høiby, N.; Ciofu, O.; Johansen, H.K.; Song, Z.-J.; Moser, C.; Jensen, P.Ø.; Molin, S.; Givskov, M.; Tolker-Nielsen, T.; Bjarnsholt, T. The clinical impact of bacterial biofilms. Int. J. Oral Sci. 2011, 3, 55–65.
4. Ciofu, O.; Tolker-Nielsen, T. Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—How P. aeruginosa can escape antibiotics. Front. Microbiol. 2019, 10, 913.
5. Chen, M.; Yu, Q.; Sun, H. Novel strategies for the prevention and treatment of biofilm related infections. Int. J. Mol. Sci. 2013, 14, 18488–18501.
6. Wu, H.; Moser, C.; Wang, H.-Z.; Høiby, N.; Song, Z.-J. Strategies for combating bacterial biofilm infections. Int. J. Oral Sci. 2015, 7, 1–7.
7. Høiby, N. Recent advances in the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. BMC Med. 2011, 9, 32.
8. Sun, F.; Qu, F.; Ling, Y.; Mao, P.; Xia, P.; Chen, H.; Zhou, D. Biofilm-associated infections: Antibiotic resistance and novel therapeutic strategies. Future Microbiol. 2013, 8, 877–886.
9. Ciofu, O.; Rojo-Molinero, E.; Macià, M.D.; Oliver, A. Antibiotic treatment of biofilm infections. APMIS 2017, 125, 304–319.
10. Flemming, H.C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S.A.; Kjelleberg, S. Biofilms: An emergent form of bacterial life. Nat. Rev. Microbiol. 2016, 14, 563–575.
11. Ranall, M.V.; Butler, M.S.; Blaskovich, M.A.; Cooper, M.A. Resolving biofilm infections: Current therapy and drug discovery strategies. Curr. Drug Targets 2012, 13, 1375–1385.
12. Lebeaux, D.; Ghigo, J.M.; Beloin, C. Biofilm-related infections: Bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol. Mol. Biol. Rev. 2014, 78, 510–543.

13. Stewart, P.S.; Costerton, J.W. Antibiotic resistance of bacteria in biofilms. Lancet 2001, 358, 135–138.
14. Williams, I.; Venables, W.A.; Lloyd, D.; Paul, F.; Critchley, I. The effects of adherence to silicone surfaces on antibiotic susceptibility in Staphylococcus aureus. Microbiology 1997, 143, 2407–2413.
15. Andrews, J.M. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 2001, 48 (Suppl. S1), 5–16.
16. Thieme, L.; Hartung, A.; Tramm, K.; Klinger-Strobel, M.; Jandt, K.D.; Makarewicz, O.; Pletz, M.W. MBEC versus MBIC: The lack of differentiation between biofilm reducing and inhibitory effects as a current problem in biofilm methodology. Biol. Proced. Online 2019, 21, 18.
17. Howlin, R.P.; Brayford, M.J.; Webb, J.S.; Cooper, J.J.; Aiken, S.S.; Stoodley, P. Antibiotic-loaded synthetic calcium sulfate beads for prevention of bacterial colonization and biofilm formation in periprosthetic infections. Antimicrob. Agents Chemother. 2015, 59, 111–120.
18. Okeke, I.N.; Lamikanra, A. Export of antimicrobial drugs by West African travelers. J. Travel Med. 2003, 10, 133–135.
19. Kot, B.; Wierzchowska, K.; Piechota, M.; Grużewska, A. Antimicrobial resistance patterns in methicillin-resistant Staphylococcus aureus from patients hospitalized during 2015–2017 in hospitals in Poland. Med. Princ. Pract. 2020, 29, 61–68.
20. Brady, A.J.; Laverty, G.; Gilpin, D.F.; Kearney, P.; Tunney, M. Antibiotic susceptibility of planktonic- and biofilm-grown staphylococci isolated from implant-associated infections: Should MBEC and nature of biofilm formation replace MIC? J. Med. Microbiol. 2017, 66, 461–469.
21. Antunes, A.L.; Trentin, D.S.; Bonfanti, J.W.; Pinto, C.C.; Perez, L.R.; Macedo, A.J.; Barth, A.L. Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. APMIS 2010, 118, 873–877.
22. Coenye, T.; Goeres, D.; Van Bambeke, F.; Bjarnsholt, T. Should standardized susceptibility testing for microbial biofilms be introduced in clinical practice? Clin. Microbiol. Infect. 2018, 24, 570–572.
23. Ghasemian, A.; Najar Peerayeh, S.; Bakhshi, B.; Mirzaee, M. Comparison of biofilm formation between methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus. Iran. Biomed. J. 2016, 20, 175–181.
24. Stapleton, P.D.; Taylor, P.W. Methicillin resistance in Staphylococcus aureus: Mechanisms and modulation. Sci. Prog. 2002, 85, 57–72.
25. Manandhar, S.; Singh, A.; Varma, A.; Pandey, S.; Shrivastava, N. Biofilm producing clinical Staphylococcus aureus isolates augmented prevalence of antibiotic resistant cases in tertiary care hospitals of Nepal. Front. Microbiol. 2018, 9, 2749.

26. Rezaei, M.; Moniri, R.; Mousavi, S.; Jabbari Shiadeh, S.M. Prevalence of biofilm formation among methicillin resistance Staphylococcus aureus isolated from nasal carriers. Jundishapur J. Microbiol. 2013, 6, e9601.
27. Chen, M.; Yu, Q.; Sun, H. Novel strategies for the prevention and treatment of biofilm related infections. Int. J. Mol. Sci. 2013, 14, 18488–18501.
28. Flemming, H.C.; Wingender, J. The biofilm matrix. Nat. Rev. Microbiol. 2010, 8, 623–633.
29. Watanakunakorn, C. Mode of action and in-vitro activity of vancomycin. J. Antimicrob. Chemother. 1984, 14 (Suppl. D), 7–18.
30. Reynolds, P.E. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. 1989, 8, 943–950.
31. Costerton, J.W.; Stewart, P.S.; Greenberg, E.P. Bacterial biofilms: A common cause of persistent infections. Science 1999, 284, 1318–1322.
32. Howden, B.P.; Davies, J.K.; Johnson, P.D.; Stinear, T.P.; Grayson, M.L. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: Resistance mechanisms, laboratory detection, and clinical implications. Clin. Microbiol. Rev. 2010, 23, 99–139.
33. Vergidis, P.; Rouse, M.S.; Euba, G.; Karau, M.J.; Schmidt, S.M.; Mandrekar, J.N.; Steckelberg, J.M.; Patel, R. Treatment with linezolid or vancomycin in combination with rifampin is effective in an animal model of methicillin-resistant Staphylococcus aureus foreign body osteomyelitis. Antimicrob. Agents Chemother. 2011, 55, 1182–1186.
34. Peixoto, P.B.; Massinhani, F.H.; Netto Dos Santos, K.R.; Chamon, R.C.; Silva, R.B.; Lopes Correa, F.E.; Barata Oliveira, C.; Oliveira, A.G. Methicillin-resistant Staphylococcus epidermidis isolates with reduced vancomycin susceptibility from bloodstream infections in a neonatal intensive care unit. J. Med. Microbiol. 2020, 69, 41–45.
35. Antunes, A.L.; Bonfanti, J.W.; Perez, L.R.; Pinto, C.C.; Freitas, A.L.; Macedo, A.J.; Barth, A.L. High vancomycin resistance among biofilms produced by Staphylococcus species isolated from central venous catheters. Mem. Inst. Oswaldo Cruz 2011, 106, 51–55.
36. Burmølle, M.; Thomsen, T.R.; Fazli, M.; Dige, I.; Christensen, L.; Homøe, P.; Tvede, M.; Nyvad, B.; Tolker-Nielsen, T.; Givskov, M.; et al. Biofilms in chronic infections—A matter of opportunity—Monospecies biofilms in multispecies infections. FEMS Immunol. Med. Microbiol. 2010, 59, 324–336.
37. Hamad, T.; Hellmark, B.; Nilsdotter-Augustinsson, Å.; Söderquist, B. Antibiotic susceptibility among Staphylococcus epidermidis isolated from prosthetic joint infections, with focus on doxycycline. APMIS 2015, 123, 1055–1060.

38. Kotulová, D.; Slobodníková, L. Susceptibility of Staphylococcus aureus biofilms to vancomycin, gemtamicin and rifampin. J. Epidemiol. Mikrobiol. Imunol. 2010, 59, 80–87.
39. Saginur, R.; Stdenis, M.; Ferris, W.; Aaron, S.D.; Chan, F.; Lee, C.; Ramotar, K. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob. Agents Chemother. 2006, 50, 55–61.
40. Raad, I.; Hanna, H.; Jiang, Y.; Dvorak, T.; Reitzel, R.; Chaiban, G.; Sherertz, R.; Hachem, R. Comparative activities of daptomycin, linezolid, and tigecycline against catheter-related methicillin-resistant Staphylococcus bacteremic isolates embedded in biofilm. Antimicrob. Agents Chemother. 2007, 51, 1656–1660.
41. Smith, K.; Perez, A.; Ramage, G.; Gemmell, C.G.; Lang, S. Comparison of biofilm-associated cell survival following in vitro exposure of meticillin-resistant Staphylococcus aureus biofilms to the antibiotics clindamycin, daptomycin, linezolid, tigecycline and vancomycin. Int. J. Antimicrob. Agents 2009, 33, 374–378.
42. Cafiso, V.; Bertuccio, T.; Spina, D.; Purrello, S.; Stefani, S. Tigecycline inhibition of a mature biofilm in clinical isolates of Staphylococcus aureus: Comparison with other drugs. FEMS Immunol. Med. Microbiol. 2010, 59, 466–469.
43. Wang, L.-S.; Gupta, A.; Rotello, V.M. Nanomaterials for the treatment of bacterial biofilms. ACS Infect. Dis. 2016, 2, 3–4.
44. Gupta, A.; Landis, R.F.; Rotello, V.M. Nanoparticle-based antimicrobials: Surface functionality is critical. F1000Research 2016, 5, 364.
45. Kirui, D.K.; Weber, G.; Talackine, J.; Millenbaugh, N.J. Targeted laser therapy synergistically enhances efficacy of antibiotics against multi-drug resistant Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Nanomedicine 2019, 20, 102018.
46. Saggu, S.K.; Jha, G.; Mishra, P.C. Enzymatic degradation of biofilm by metalloprotease from Microbacterium sp. SKS10. Front. Bioeng. Biotechnol. 2019, 7, 192.