Review

Jorge Parra-Ruiz[1]
Céline Vidaillac[2,3]
Michael J. Rybak[4]

Macrolides and staphylococcal biofilms

[1]Infectious Diseases Unit. Hospital Universitario San Cecilio. Granada-Spain.
[2]UMR 7565 CNRS,
[3]Platform ABC, Nancy-University, Nancy, France.
[4]Wayne State University, Anti-Infective Research Laboratory, Detroit, MI. USA

ABSTRACT

Medical device-associated infections represent a growing problem with limited or no therapeutic options beyond implant removal. Bacterial biofilm is the major and the final determinant of the poor prognosis of these difficult-to-treat infections. Due to the high antimicrobial resistance level of bacteria organized in biofilms, combination therapy is most often recommended, and macrolides may represent antibiotics of choice. Their anti-biofilm activity has been successfully used *in-vitro* and *in-vivo* against biofilm-associated infections caused by *Pseudomonas aeruginosa* and other Gram-negative bacilli. However there is only little data regarding their clinical interest against infections involving staphylococcal biofilms. Despite controversial reports, there is growing *in-vitro* and *in-vivo* evidences of anti-staphylococcal biofilm activity of macrolides that could represent a significant advance in the battle against implant-related infections.

Macrólidos y biopelículas estafilocócicas

RESUMEN

Las infecciones asociadas a dispositivos médicos implantables han ido aumentando en los últimos años, y se han convertido en un problema de enorme relevancia dado que existen pocas opciones terapéuticas. La formación de una biopelícula es el factor característico de estas infecciones, lo que confiere una elevada resistencia al tratamiento antibacteriano, motivando el empleo de un tratamiento combinado. En este sentido los macrólidos, gracias a sus efectos anti-biopelículas demostrado en estudios in vitro e in vivo frente a biopelículas de bacterias gramnegativas se han convertido en opción terapéutica de creciente utilización. No obstante, pocos datos existen en la literatura acerca de su empleo en biopelículas de bacterias grampositivas. En esta revisión presentamos los datos existentes in vitro e in vivo del efecto de los macrólidos en biopelícula estafilocócicas, lo que podría otorgar a estos antibióticos un papel relevante, también, en el tratamiento de las infecciones asociadas a biopelículas estafilocócicas.

INTRODUCTION

Management of medical device associated infections, such as prosthetic heart valves, cardiac pacemakers, and prosthetic joint replacements, has become a conventional procedure of modern medical care. The use of foreign material however, is not free of complications with bacterial infections being the most devastating adverse event and *Staphylococci* spp. isolates being the most common pathogens involved (table 1). Biofilm producing bacteria are capable of adhesion to foreign body materials, forming organized community of adherent cells living in layers of slime. Once mature, the bacterial biofilm developed on implanted medical devices exhibits an increased tolerance to antimicrobial agents[1,2], increased withstanding of physical forces (including shear forces produced by blood or surgical lavage)[3] and an extraordinary resistance to phagocytosis[4,5]. Therapeutic options available to treat biofilm-associated infections are therefore limited, in most cases, to the removal of the infected device[6]. Despite the advances in understanding the properties of biofilms and the development of novel technologies, prevalence, mortality and morbidity rates as well as estimated cost of biofilm associated infections have increased[7,8]. Recent estimates suggest that cost of prosthetic joint infections could be up to $50,000 per patient and $250,000 million per year[9]. This highlights the urgent need for novel therapeutic strategies to treat bacterial and yeast biofilm related infections. For instance, in prosthetic joint infections (PJI), especially late-onset PJIs, combination therapy with rifampin is typically used after debridement and retention of the infected implant. Rifampin based combination has already demonstrated anti-biofilm efficacy *in-vitro* and *in-vivo*[10-12], however PJI often involved the formation of biofilm that precludes therapeutic success without surgery. Indeed,

Correspondence:
Jorge Parra-Ruiz
Service of Infectious Diseases
Hospital Universitario San Cecilio
Avda Dr. Olóriz 16. 18012
Granada – Spain
jordi@ugr.es

Table 1 **Causative agents, prevalence and economic outcomes of major indwelling medical devices infections[8,54-59].**

Device	Prevalent causative pathogen		Average rate of infection (%)	Estimated average cost
	Principal	Secondary		
Central venous catheters	*Staphylococcus* spp.	GNB, *Candida* spp., *Enterococcus* spp.	5-8.5/1000 d*	USD 56,000
Mechanical heart valves	*Staphylococcus* spp.	*Streptococcus* spp., GNB, *Enterococus* spp.	4	USD 50,000
Ventricular assist devices	*Staphylococcus* spp.	*Candida* spp., *Pseudomonas* spp.	40	USD 50,000
Coronary stents	*Staphylococcus* spp.	*Pseudomonas* spp., *Candida* spp.	4	USD 35,000
Neurosurgical ventricular shunts	*Staphylococcus* spp.	*Streptococcus* spp., *Corynebacterium* GNB	2-12	USD 40,000
Orthopedic prostheses	*Staphylococcus* spp.	*Streptococcus* spp., GNB, *Enterococcus* spp.	2	USD 30,000
Fracture-fixation devices	*Staphylococcus* spp.	*Propionibacterium* spp., *Corynebacterium* spp.	5	USD 15,000
Endovascular grafts	Enteric GNB	*Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp.	0.6-3	USD 85,000
Peritoneal dialysis catheters	*Staphylococcus* spp.	*Pseudomonas* spp., GNB, *Candida* spp.	3	USD 35,000
Urethral catheters	*E. coli*	*Candida* spp., *Staphylococcus* spp., *Enterococcus* spp.		
Inflatable penile implants	*Staphylococcus* spp.	Enteric GNB, *Pseudomonas* spp., fungi.		
Breast implants	*Staphylococcus* spp.	*E. coli*, *Propionibacterium* spp., *Streptococci* spp.	2	USD 20,000
Cochlear implants	*Staphylococcus* spp.	*Pseudomonas* spp., *Streptococcus* spp.	<1	UNKNOWN

*** Average infection rate expressed as episodes per 1000 central-line days**

success rates reported for implant removal for rifampin based combination therapy range between 7-80%[13,14]. There is an urgent need for new strategies to manage biofilm related infection; therefore, the usefulness of other anti-biofilm antimicrobials like macrolides should be investigated. Some agents of this class (erythromycin, clarithromycin and azithromycin) have already demonstrated a potent *in-vitro* and *in-vivo* anti-biofilm activity against numerous Gram-negative bacteria, by inhibiting the production of alginate, a key component of the matrix of biofilm in *P. aeruginosa* for example[15-19]. *In-vitro* and *in-vivo* data available on anti Gram-positive biofilm properties of macrolides are limited (table 2)[20-23] but very encouraging for future investigations.

As a value to practicing physicians and infectious diseases pharmacists, we briefly review the general architecture of biofilm, to further discuss on the impact and the potential clinical interest of macrolides for the treatment of Gram-positive biofilm related infections.

Bacterial biofilm: general architecture and strategies to eradicate biofilm

Biofilms are complex and structured communities of organisms embedded in a self-produced matrix of water and extracellular polymeric substances that include exopolysaccharides, enzymes, proteins, lipopolysaccharides, biosurfactants and extracellular DNA (eDNA) (figure 1). Each of these components are essential for the formation and maintenance of the matrix architecture, and is responsible for specific properties of the bacterial biofilm[24]. For instances, adhesion of the matrix to an inert surface is facilitated by polysaccharides, proteins, eDNA and amphiphilic molecules, whereas cohesion of cells within the matrix is ensured by neutral and charged polysaccharides and proteins (such as amyloids and lectins)[24,25]. Molecules of eDNA enclosed within the matrix are a major structural component of *P. aeruginosa* and *S. aureus* biofilms[24,26]. These molecules seem to be involved in the cohesion of embedded cells, play a role in inhibition or promotion of biofilm, and are responsible for antimicrobial properties of biofilms[24]. They usually come from lysis of embedded cells, but excretion of DNA cannot be excluded[24]. Exopolysaccharides are also essential components that intervene on the formation and maturation of bacterial biofilms[27]. These molecules differ from species to species and also differ from strain to strain within the same species. For examples, *P. aeruginosa* biofilms contain at least three major polysaccharides (alginates, Pel, Pst)[28] whereas *S. epidermidis* and S. *thermophilus* produce polysaccharide intercellular adhesion (PIA), and hetero-polysaccharides of different monomer compositions, ratios and molecular weight, respectively[24]. In addition, a large variety of extracellular enzymes capable of cleavage of proteins, lipids and polysaccharides are present within the matrix, and play key roles in the process of formation / maturation of the matrix as well as in the virulence properties of biofilms[24, 29].

In general, the formation of a scaffold of biofilm matrix molecules involves five distinct steps that include initial attachment, cell-to-cell adhesion and proliferation, maturation, and finally, detachment[30]. Bacterial adhesion to a surface is crucial in the process of biofilm formation and involves several molecules, such as polysaccharides, proteins, eDNA[31-33]. Once successfully attached, the bacterial community

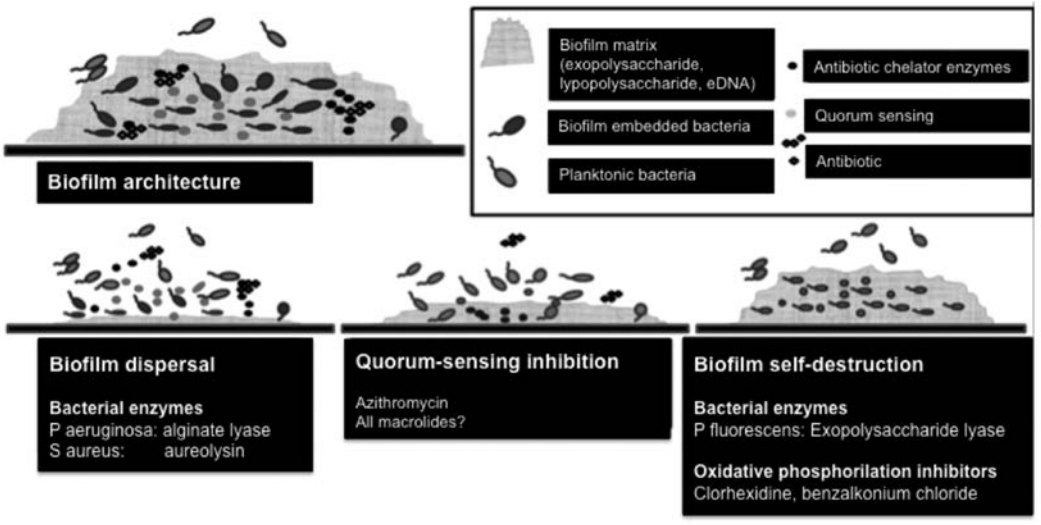

Figure 1 | **Potential strategies to eradicate bacterial biofilm.**

develops as a multilayered matrix of embedded cells. Cell to cell cohesion becomes then the determining factor, and is ensured by production of the intercellular adhesin (PIA) and accumulation of associated protein (Aap)[34,35]. Ultimately, the aggregation of new layers and the maturation of the matrix will lead to the detachment of small clusters of cells that will disseminate in the environment. Finally, biofilm dispersal and self-destruction are naturally occurring mechanisms involving bacterial enzymes (figure 1). Although these processes have not been fully elucidated, it has been reported that accumulation of extracellular substances, like the auto-inducer peptide, may trigger quorum sensing regulatory systems such as the *agr* system of *S. aureus*, leading thus to the expression of toxins and proteases[36] (figure 1) and, the reduced expression of surface adhesion[37,38]. Macrolide antibiotics, and especially azithromycin, have also been shown to interfere with bacterial quorum sensing, reducing this polysaccharide synthesis and leading to instability of the biofilm architecture[39,40]. Of note and of particular interest, other inhibitors of protein synthesis like gentamycin did not demonstrate similar properties against biofilms[41].

Effect of macrolides on biofilm

Macrolide antibiotics (such as erythromycin, clarithromycin, and azithromycin) are well-tolerated older antibiotics with a broad spectrum of activity and anti-biofilm properties. Because of these characteristics and their immunomodulatory and anti-inflammatory activities, macrolides are among the most frequently used antibiotics.

The anti-biofilm activity of macrolides was first described *in-vitro* in 1992 with sessile cells of *P. aeruginosa* exposed to clarithromycin and erythromycin[42]. A year later, Yasuda *et al.* confirmed these preliminary results against biofilm embedded cells of a clinical and non mucoid strain of *P. aeruginosa*[43]. In presence of clarithromycin (concentration up to 20 mg/L), the authors observed a net reduction of the amount of alginate and hexose, both in the colonies and the environment, suggesting destruction of the polysaccharide glycocalyx and/or the inhibition of polysaccharide synthesis by clarithromycin.

Despite these evidences about the anti-biofilm activity against gram-negative organisms, there is contradictory information regarding the effect of macrolides in biofilm formation of gram-positive organism. Rachid et al. reported an increased expression of the intercellular adhesion gene cluster (*ica)* which comprises 4 genes (*icaADBC*), in presence of erythromycin[44]. Of note, a similar activity was observed for others antibiotics, such as tetracycline or the streptogramin antibiotic, quinupristin-dalfopristin, although oxacillin, penicillin G, clindamycin or gentamycin did not seem to impact biofilm formation. More recently, Wan et al. evaluated the role of macrolides in biofilm formation and its relation to the ica status of *S. epidermidis*[45]. Exposure of erythromycin resistant strains of *S. epidermidis* to macrolides (at ¼ x MIC) resulted in an increase production of biofilm for 24 (20%) organisms, independently of the *ica* status, suggesting that other regulatory systems are involved in the mechanism of macrolide-enhanced biofilm formation.

Other authors have reported that macrolides also inhibit bilofilm formation of gram-positive organisms, being clarithromycin the most extensively studied macrolide against mature biofilm of *Staphylococcus* spp. One of the first experiments on this topic was performed by Yasuda et al. in 1994[20]. The authors incubated clarithromycin-resistant strains

Table 2 ***In vitro* and *in vivo* reports on macrolide combinations against Gram positive biofilm.**

Macrolide	2nd antimicrobial	Device/model	Species	Outcome	Reference
Clarithromycin	Vancomycin	*In vitro* bladder and kidney model	MRSA	Biofilm and planktonic cells eradication	46
Roxithromycin	Imipenem	*In vivo* biofilm skin lesion on mice	MRSA	Higher penetration of neutrophiles into the biofilm	60
Clarithromycin	Imipenem	Pieloplasty/lithotomy in 38 yo patient	*S. epidermidis* (& *P. aeruginosa*)	Patient cured	61
Erythromycin (E)	Vancomycin (V) Rifampin (R) Gentamycin (G)	*In vitro* model with polyurethane sheets	*S. epidermidis*	V+R (S) > V + E (S) > V + G > R > V > E > G	62
Clarithromycin	Vancomycin	*In vitro* and *in vivo* (mouse) model with titanium washers	*S. aureus*	Eradication of the biofilm on the washers	22 21
Clarithromycin (C)	Daptomycin (D) Moxifloxacin (M)	In vitro polycarbonate coupons	MSSA	D+ C sterilized the coupons D+C (S, B) > M+ C (S, B)	23

of *S. epidermidis* in a glass chamber with a cellulose membrane filter with different concentrations of clarithromycin and ofloxacin or cefotiam. They observed a clarithromycin dose-dependent (1 to 20 mg/L) increase in the rate of penetration of ofloxacin and cefotiam through the filters, suggesting the eradication of the glycocalyx matrix of biofilms by clarithromycin. Few years later, Sano et al explored the efficacy of clarithromycin and vancomycin against *S. aureus* isolates in an in vitro model of urinary tract infection[46]. Vancomycin and clarithromycin MIC values were 0.5 (susceptible) and > 128 mg/L (resistant), respectively. Simulated dosing regimens mimicked human pharmacokinetics of each agent. After 48 h of therapy, vancomycin eradicated methicillin-resistant *S. aureus* (MRSA) biofilm formed on the bladder model. However, withdrawal of therapy and replacement of media resulted in bacterial re-growth. As expected, clarithromycin alone had no antibacterial activity on the MRSA count. However, combination therapy resulted in a complete eradication of the MRSA within 46 h. No re-growth was observed after changing the media, suggesting eradication of persistent cells embedded in the matrix of biofilm grown on the bladder. Scanning electron microscopy confirmed persistence of biofilm with vancomycin alone, persistence of planktonic cells without biofilm with clarithromycin alone and complete eradication of biofilm and planktonic cells with combination therapy[46]. Similarly, Fujimura et al. reported the efficacy of clarithromycin on combination with cefazolin or vancomycin in an in vitro model of foreign-body infection[22] and in an *in vivo* model of implant-related infection[21]. Mature biofilm of *S. aureus* biofilm were grown at the surface of titanium washers prior exposure to clarithromycin plus cefazolin or vancomycin for 72 h. Elimination of the biofilm and complete sterilization of the media secondary to combination therapies with clarithromycin was confirmed using scanning electron microscopy, whereas monotherapy did not eradicate bacterial biofilms and did not prevent re-growth at 72 h[22]. More recently, we developed an *in vitro* pharmacokinetic/pharmacodynamic model of bacterial biofilm-related infections. We evaluated the activity of daptomycin and moxifloxacin alone and in combination with clarithromycin against a methicillin-susceptible, clarithromycin-resistant *S. aureus* strain[23]. Neither daptomycin nor moxifloxacin were able to eradicate mature biofilm, but combination of any of these antimicrobial agents with clarithromycin significantly improved the antibacterial activity, with a substantial decrease of viable bacteria. Daptomycin plus clarithromycin was the most effective combination, decreasing viable bacteria below the limit of detection (10 cells/mL). Scanning electron microscopy confirmed these results, illustrating the biofilm destruction and/or eradication in presence of the combo daptomycin plus clarithromycin (figure 2). Similarly to what has been described by Sano et al. for Staphylococci biofilms and by Yanagihara et al. or Schultz et al. for *Pseudomonas* biofilms[17,19], clarithromycin displayed anti staphylococcal biofilm activity at sub-MIC concentrations.

Azithromycin has also been evaluated against mature staphylococci biofilms. For example, Presterl et al. evaluated the activity of azithromycin combined to daptomycin, vancomycin, tigecycline, fosfomycin (5 and 20 x MIC) or ceftriaxone (100 x MIC) on mature biofilms of 12 non-related strains of *S. epidermidis* under static conditions of exposure[47]. None of the combinations were able to reduce the viable count of bacteria, although a slight decrease was observed with vancomycin and fosfomycin. Although there is limited data on azithromycin, the absence of anti-biofilm activity of this macrolide against Staphylococci suggests that this may be a specific property of clarithromycin[47]. A recent study investigated the rate of penetration of different antimicrobial agents, including azithromycin and erythromycin, in agar layers containing 1% of *P. aeruginosa* alginate revealed that the two macrolide antibiotics has the best rate of penetration (100%), followed by ceftazidime and ciprofloxacin (90 to 95%)[48]. Further studies are warranted to explore antimicrobial

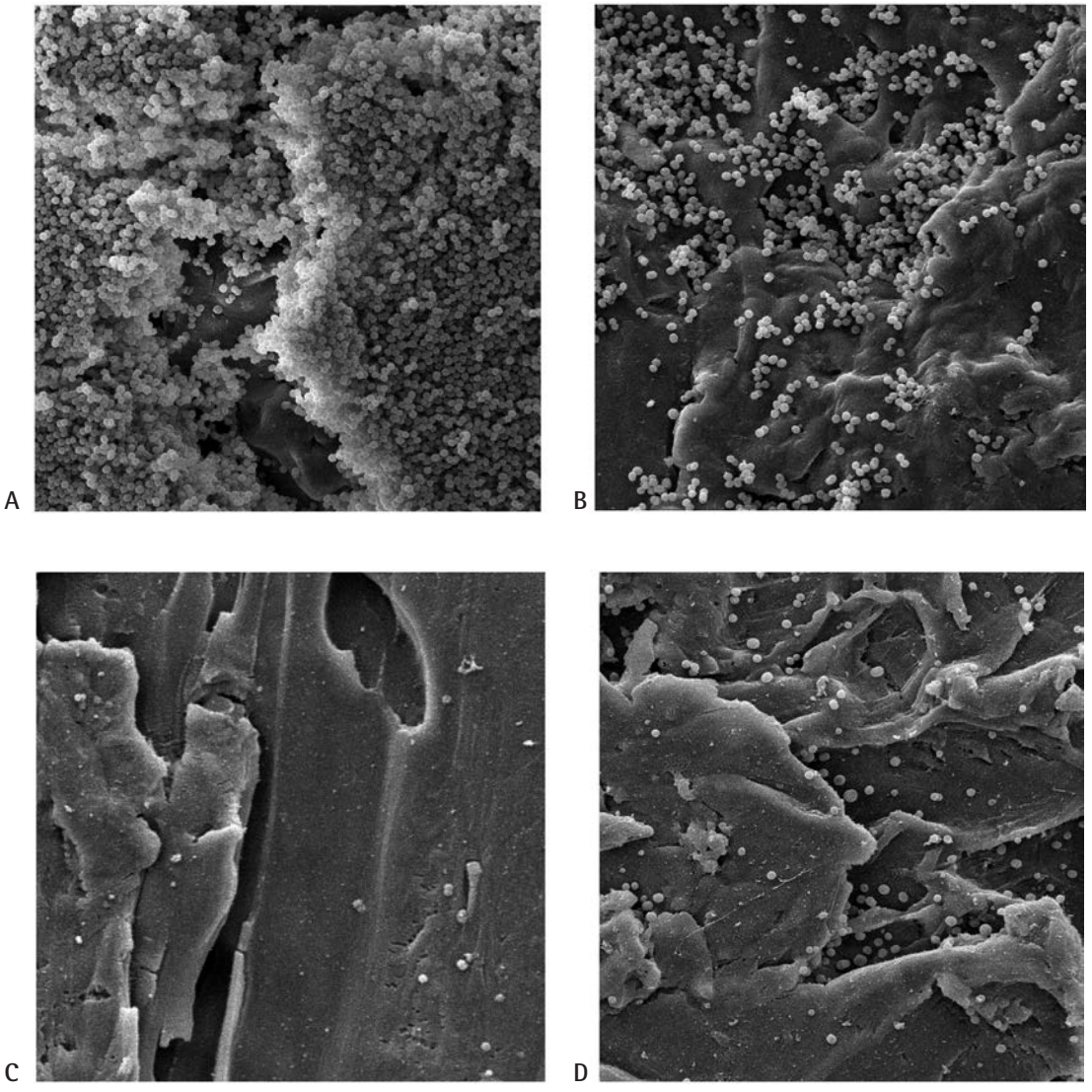

Figure 2 | **Scan Electron Microscopy imaging of the surface of the coupons to assess presence and structure of the matrix of SH1000 biofilm. Images were collected at 2,000 x magnification. A. Before any drug exposure, B. After 72 h of daptomycin exposure, C. After 72 h of daptomycin + clarithromycin exposure, D. After 72 h of moxifloxacin + clarithromycin exposure. Reproduced with permission[23]**

penetration rate, inhibition at the surface of the biofilm matrix[49] and how macrolide antibiotics could impact on these properties depending on the species.

Therapeutic potential of macrolides in the treatment of biofilm related infections

Since clinicians became aware of the anti-biofilm properties of macrolides against *Pseudomonas* biofilms, there has been a widespread use of azithromycin and clarithromycin in biofilm-associated infections caused by *Pseudomonas* spp. like cystic fibrosis or diffuse panbronquilitis. Randomized controlled trials have demonstrated respiratory benefits of long-term therapy with macrolides[50,51]. The beneficial effect of macrolide therapy was evident only in patients infected with *P. aeruginosa* suggesting that the beneficial effect was not related to the immunomodulatory properties of macrolides[52]. The same conclusion was reported very recently in a meta-analysis of randomized controlled trials of azithromycin (7 trials) and clarithromycin (1 trial) for the treatment of cystic fibrosis, where macrolide therapy was associated with improvement of lung function, especially for *P. aeruginosa*-colonized cystic fibrosis patients[53].

However, despite that much evidence regarding macrolide therapy for biofilm-associated infections of *P. aeruginosa*, there is no data about macrolide therapy for biofilms-associated infections of *Staphylococci* and therefore no consistent recommendations can be made regarding its therapeutic potential in the treatment of this type of infections. However, because of the potential benefit, and safety of macrolides, as shown in randomized controlled trials of long term macrolide therapy[51,53], clinicians should consider the addition to macrolides when treating medical-device associated infections.

CONCLUSIONS

Because of their well-described ability to disrupt alginate-based biofilms, macrolides have been extensively studied against *Pseudomonas*. In contrast, little is known regarding their interaction with staphylococcal biofilms and potential clinical interest in the treatment of Staphylococci infection associated with biofilms. There is growing evidences of the anti-biofilm activity of macrolides, especially clarithromycin against mature staphylococcal biofilms. Although the mechanism is not completely elucidated, inhibition of hexose-production is suggested in an MIC independent manner, similarly to what has already been described in gram-negative rods. Further investigations, especially regarding the genetic pathways (*agr*, *SigB*, *SarA*, *ica,* etc…) involved in the biofilm production and how antimicrobial therapy is affecting these genetic components are warranted. As *Staphylococci* are responsible of most of the medical devices-associated infections, clinical application of the anti-biofilm effect of macrolides could be of paramount importance in the treatment of those difficult-to-treat infections.

REFERENCES

1. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 1999; 37:1771-6.
2. Frank KL, Reichert EJ, Piper KE, Patel R. In vitro effects of antimicrobial agents on planktonic and biofilm forms of *Staphylococcus lugdunensis* clinical isolates. Antimicrob Agents Chemother 2007;5 1:888-95.
3. Jefferson KK. What drives bacteria to produce a biofilm? Fems Microbiol Lett 2004; 236:163-73.
4. Gunther F, Wabnitz GH, Stroh P, et al. Host defence against *Staphylococcus aureus* biofilms infection: phagocytosis of biofilms by polymorphonuclear neutrophils (PMN). Mol Immunol 2009; 46:1805-13.
5. Schommer NN, Christner M, Hentschke M, Ruckdeschel K, Aepfelbacher M, Rohde H. *Staphylococcus epidermidis* uses distinct mechanisms of biofilm formation to interfere with phagocytosis and activation of mouse macrophage-like cells 774A.1. Infect Immun 2011; 79:2267-76.
6. Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. Clin Orthop Relat Res 2005:7-11.
7. Rosenthal VD, Maki DG, Salomao R, et al. Device-associated nosocomial infections in 55 intensive care units of 8 developing countries. Ann Intern Med 2006; 145:582-91.
8. Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med 2008; 59:415-28.
9. Cataldo MA, Petrosillo N, Cipriani M, Cauda R, Tacconelli E. Prosthetic joint infection: recent developments in diagnosis and management. J Infect 2010; 61:443-8.
10. El Helou OC, Berbari EF, Lahr BD, et al. Efficacy and safety of rifampin containing regimen for staphylococcal prosthetic joint infections treated with debridement and retention. Eur J Clin Microbiol Infect Dis 2010; 29:961-7.
11. Forrest GN, Tamura K. Rifampin combination therapy for nonmycobacterial infections. Clin Microbiol Rev 2010; 23:14-34.
12. Perlroth J, Kuo M, Tan J, Bayer AS, Miller LG. Adjunctive use of rifampin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. Arch Intern Med 2008; 168:805-19.
13. Moran E, Byren I, Atkins BL. The diagnosis and management of prosthetic joint infections. J Antimicrob Chemother 2010; 65 Suppl 3:iii45-54.
14. Cobo J, Miguel LG, Euba G, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. Clin Microbiol Infect 2010; 17:1632-7.
15. Kobayashi H. Biofilm disease: Its clinical manifestation and therapeutic possibilities of macrolides. Am J Med 1995; 99:S26-S30.
16. Ichimiya T, Takeoka K, Hiramatsu K, Hirai K, Yamasaki T, Nasu M. The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa* in vitro. Chemotherapy 1996; 42:186-91.
17. Yanagihara K, Tomono K, Imamura Y, et al. Effect of clarithromycin on chronic respiratory infection caused by *Pseudomonas aeruginosa* with biofilm formation in an experimental murine model. J Antimicrob Chemother 2002; 49:867-70.
18. Takeoka K, Ichimiya T, Yamasaki T, Nasu M. The in vitro effect of macrolides on the interaction of human polymorphonuclear leukocytes with *Pseudomonas aeruginosa* in biofilm. Chemotherapy 1998; 44:190-7.
19. Schultz MJ. Macrolide activities beyond their antimicrobial effects: macrolides in diffuse panbronchiolitis and cystic fibrosis. J Antimicrob Chemoth 2004; 54:21-8.
20. Yasuda H, Ajiki Y, Koga T, Yokota T. Interaction between Clarithromycin and Biofilms Formed by Staphylococcus-Epidermidis. Antimicrob Agents Chemother 1994; 38:138-41.
21. Fujimura S, Sato T, Kikuchi T, Zaini J, Gomi K, Watanabe A. Efficacy of clarithromycin plus vancomycin in mice with implant-related infection caused by biofilm-forming *Staphylococcus aureus*. J Orthop Sci 2009; 14:658-61.
22. Fujimura S, Sato T, Mikami T, Kikuchi T, Gomi K, Watanabe A. Combined efficacy of clarithromycin plus cefazolin or vancomycin against *Staphylococcus aureus* biofilms formed on titanium medical devices. Int J Antimicrob Agents 2008; 32:481-4.
23. Parra-Ruiz J, Vidaillac C, Rose WE, Rybak MJ. Activities of high-dose daptomycin, vancomycin, and moxifloxacin alone or in combination with clarithromycin or rifampin in a novel in vitro model of *Staphylococcus aureus* biofilm. Antimicrob Agents Chemother 2010; 54:4329-34.
24. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8:623-33.
25. Abee T, Kovacs AT, Kuipers OP, van der Veen S. Biofilm formation and dispersal in Gram-positive bacteria. Curr Opin Biotechnol 2011; 22:172-9.

26. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science 2002; 295:1487.
27. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 2009; 14:2535-54.
28. Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Curr Opin Microbiol 2007; 10:644-8.
29. Tielen P, Rosenau F, Wilhelm S, Jaeger KE, Flemming HC, Wingender J. Extracellular enzymes affect biofilm formation of mucoid *Pseudomonas aeruginosa*. Microbiology 2010; 156:2239-52.
30. Otto M. Staphylococcal biofilms. Curr Top Microbiol Immunol 2008; 322:207-28.
31. O'gara JP. ica and beyond: biofilm mechanisms and regulation in Staphylococcus epidermidis and *Staphylococcus aureus*. Fems Microbiol Lett 2007; 270:179-88.
32. Palmer J, Flint S, Brooks J. Bacterial cell attachment, the beginning of a biofilm. J Ind Microbiol Biotechnol 2007; 34:577-88.
33. Dunne WM, Jr. Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 2002; 15:155-66.
34. Christner M, Franke GC, Schommer NN, et al. The giant extracellular matrix-binding protein of *Staphylococcus epidermidis* mediates biofilm accumulation and attachment to fibronectin. Mol Microbiol 2010; 75:187-207.
35. Fey PD, Olson ME. Current concepts in biofilm formation of *Staphylococcus epidermidis*. Future Microbiol 2010; 5:917-33.
36. Novick RP, Geisinger E. Quorum sensing in staphylococci. Annu Rev Genet 2008;42:541-64.
37. Boles BR, Horswill AR. Agr-mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathog 2008; 4:e1000052.
38. Lauderdale KJ, Malone CL, Boles BR, Morcuende J, Horswill AR. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. J Orthop Res 2009; 28:55-61.
39. Ichimiya T, Takeoka K, Hiramatsu K, Hirai K, Yamasaki T, Nasu M. The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa* in vitro. Chemotherapy 1996; 42:186-91.
40. Skindersoe ME, Alhede M, Phipps R, et al. Effects of antibiotics on quorum sensing in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2008; 52:3648-63.
41. Starner TD, Shrout JD, Parsek MR, Appelbaum PC, Kim G. Subinhibitory concentrations of azithromycin decrease nontypeable *Haemophilus influenzae* biofilm formation and Diminish established biofilms. Antimicrob Agents Chemother 2008; 52:137-45.
42. Fujimaki K, Ikeda Y, Takahata M, Yasuda T. Characteristics of biofilm formed by *Pseudomonas aeruginosa* in vitro. Chemotherapy (Tokyo) 1992; 40:886-93.
43. Yasuda H, Ajiki Y, Koga T, Kawada H, Yokota T. Interaction between Biofilms Formed by *Pseudomonas aeruginosa* and Clarithromycin. Antimicrob Agents Chemother 1993; 37:1749-55.
44. Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrob Agents Chemother 2000; 44:3357-63.
45. Wang Q, Sun FJ, Liu Y, Xiong LR, Xie LL, Xia PY. Enhancement of biofilm formation by subinhibitory concentrations of macrolides in icaADBC-positive and -negative clinical isolates of Staphylococcus epidermidis. Antimicrob Agents Chemother 2010; 54:2707-11.
46. Sano M, Hirose T, Nishimura M, Takahashi S, Matsukawa M, Tsukamoto T. Inhibitory action of clarithromycin on glycocalyx produced by MRSA. J Infect Chemother 1999; 5:10-5.
47. Presterl E, Hajdu S, Lassnigg AM, Hirschl AM, Holinka J, Graninger W. Effects of azithromycin in combination with vancomycin, daptomycin, fosfomycin, tigecycline, and ceftriaxone on Staphylococcus epidermidis biofilms. Antimicrob Agents Chemother 2009; 53:3205-10.
48. Abdi-Ali A, Mohammadi-Mehr M, Alaei YA. Bactericidal activity of various antibiotics against biofilm-producing Pseudomonas aeruginosa. Int J Antimicrob Agents 2006; 27:196-200.
49. Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrob Agents Chemother 2000; 44:1818-24.
50. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in, patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa* - A randomized controlled trial. J Am Med Assoc 2003; 290:1749-56.
51. Fauroux B, Clement A, Tamalet A, Leroux E, Ravilly S, Jais JP. Long term effects of azithromycin in patients with cystic fibrosis: a double blind, placebo controlled trial. Thorax 2006; 61:895-902.
52. Saiman L, Anstead M, Mayer-Hamblett N, et al. Effect of Azithromycin on Pulmonary Function in Patients With Cystic Fibrosis Uninfected With *Pseudomonas aeruginosa* A Randomized Controlled Trial. J Am Med Assoc 2010; 303:1707-15.
53. Wang R, Cai Y, Chai D, Bai N, Liang BB, Liu YN. Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systematic review. J Antimicrob Chemother 2011; 66:968-78.
54. Herscu G, Wilson SE. Prosthetic infection: lessons from treatment of the infected vascular graft. Surg Clin North Am 2009; 89:391-401, viii.
55. von Eiff C, Jansen B, Kohnen W, Becker K. Infections associated with medical devices: pathogenesis, management and prophylaxis. Drugs 2005; 65:179-214.
56. Richards HK, Seeley HM, Pickard JD. Efficacy of antibiotic-impregnated shunt catheters in reducing shunt infection: data from the United Kingdom Shunt Registry. J Neurosurg Pediatr 2009; 4:389-93.
57. O'Connor S, Andrew P, Batt M, Becquemin JP. A systematic review and meta-analysis of treatments for aortic graft infection. J Vasc Surg 2006; 44:38-45.
58. Huber TS, Carlton LM, O'Hern DG, et al. Financial impact of tertiary care in an academic medical center. Ann Surg 2000; 231:860-8.
59. Zeitler DM, Budenz CL, Roland JT, Jr. Revision cochlear implantation. Curr Opin Otolaryngol Head Neck Surg 2009; 17:334-8.
60. Yamasaki O, Akiyama H, Toi Y, Arata J. A combination of roxithromycin and imipenem as an antimicrobial strategy against biofilms formed by Staphylococcus aureus. J Antimicrob Chemother 2001; 48:573-7.
61. Furuhata M, Iwamura M, Baba S, Inoue M. Combined effect of clarithromycin and imipenem/cilastatin against urinary biofilm infection after pyeloplasty. Int J Urol 2003; 10:228-30.
62. Peck KR, Kim SW, Jung SI, et al. Antimicrobials as potential adjunctive agents in the treatment of biofilm infection with Staphylococcus epidermidis. Chemotherapy 2003; 49:189-93.