Published in final edited form as:

Expert Rev Anti Infect Ther. 2010 October ; 8(10): 1067–1070. doi:10.1586/eri.10.109.

Interspecies bacterial communication as a target for therapy in otitis media

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Keywords

biofilm; otitis media; persistence; quorum signal

It is now clear that biofilm communities are an important determinant of bacterial persistence during chronic and/or recurrent infections [1]. Epidemiological data indicate that many chronic infections such as otitis media (OM) are polymicrobial [2], and it has long been appreciated that virulence and/or resistance to treatment can be dramatically impacted by coinfection [3]. Our recent work shows that, in the context of experimental OM, coinfection promotes bacterial persistence and antibiotic resistance via interspecies communication (i.e., quorum signaling) [4]. Thus, targeting bacterial communication is a potential means for primary antimicrobial treatment or as an adjunct to antibiotic therapy.

Otitis media

Otitis media is an extremely common pediatric ailment [5], caused mostly by *Haemophilus influenzae*, *Streptococcus pneumoniae* and/or *Moraxella catarrhalis*. Most chronic OM infections are polymicrobial [2], and combinations of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* have been detected in patient samples from chronic and recurrent OM [2,6]. It is important to consider the possibility that polymicrobial infection impacts severity of disease and persistence of otopathogens. It is also well-documented that polymicrobial infection impacts treatment efficacy [3,4,7–11], thus becoming an important consideration for development of new therapeutics.

Otitis media presentations vary from acute to chronic infections, and can be highly resistant to both immune clearance and antibiotic treatment [12–14]. Chronic and recurrent OM involve persistence of bacteria within biofilm communities [15–17]. Our recent work shows

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Financial & competing interests disclosure

The work in W Edward Swords' laboratory is funded by research grants from NIH/NIDCD (DC007444 and DC10051). Chelsie Armbruster is supported by an NIH training grant (T32 AI07401, Steven Mizel, Principal Investigator). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

that polymicrobial biofilms impact bacterial persistence and antibiotic resistance [4,18]. Thus, insight into the mechanisms of biofilm formation during single species and polymicrobial infection may provide new targets for treatment of OM.

Conventional treatment of otitis media

Treatment of OM varies depending on symptoms, severity and recurrence of the infection. Acute episodes and OM with effusion are often managed through watchful waiting, prescription of antibiotics and/or use of oral steroids. Chronic OM may warrant further interventions such as myringotomy for ventilation, placement of tympanostomy tubes and/or adenoidectomy.

Antibiotic treatment of OM is extremely common [19], with β -lactams being the most frequently prescribed drugs [20]. Approximately 13% of all acute OM episodes result in treatment failure or recurrence, regardless of the treatment method [20], and the failure rate for bacteriological eradication is strikingly high for pneumococci and *H. influenzae* [21,22]. Other studies have shown that antibiotic treatment provides only minimal and transient benefit to patients experiencing OM with effusion [23]. Biofilm formation by otopathogens and polymicrobial infection also contribute to antibiotic treatment failure via secretion of β -lactamase or protection of susceptible organisms within the biofilm [4,24].

Owing to the high rate of treatment failure and the selective pressure exerted by antibiotic treatment, attention has been focused on vaccination to prevent OM and therefore reduce the need for antibiotics [25]. Despite the reduction in disease caused by certain pneumococcal serotypes, replacement of vaccine serotypes with nonvaccine serotypes and increased incidence of OM caused by other otopathogens have complicated the impact of these vaccines on carriage and chronic infection [26]. There clearly remains a pressing need for alternative and effective OM treatments that exert minimal selective pressure towards resistance.

Targeting bacterial communication

Biofilm formation by many bacterial species is controlled in part through cell density-dependent quorum signaling networks [27–30]. Signaling networks include acyl homoserine lactones (AHL) in Gram-negative bacteria, species-specific peptide autoinducers in Gram-positive bacteria, and autoinducer-2 (AI-2) in both Gram-positive and Gram-negative bacteria (see [31] for review). AHL signaling as well as peptide autoinducers generally mediate intraspecies communication. AI-2, however, is a ribose-derived signaling mediator that is commonly referred to as an interspecies signal [28,30]. Recent efforts to disrupt bacterial communication have mostly focused on inhibiting AHL and peptide autoinducer signaling [32,33], but the interspecies aspect of AI-2 makes this molecule an attractive target for treating polymicrobial infection.

AI-2 influences biofilm development for many species, and in some instances can impact development of polymicrobial biofilms [34,35]. AI-2 quorum signaling influences biofilm formation and disease severity of *H. influenzae* [36,37] and *M. catarrhalis* [4], even though the latter species does not possess the genetic determinant for AI-2 production (*luxS*). *S. pneumoniae* produces AI-2, and mutation of *luxS* reduces virulence and persistence in a murine model of nasopharyngeal carriage [38,39]. In polymicrobial OM, we showed that AI-2 produced by *H. influenzae* promoted *M. catarrhalis* biofilm formation, antibiotic resistance and persistence in the chinchilla model of OM [4]. These data show that *H. influenzae* and *M. catarrhalis* utilize AI-2 to establish biofilms and persistent infections. Thus, disruption of AI-2 signaling may also disrupt biofilm formation and infection by these pathogens. Targeting bacterial communication through AI-2 quorum signaling may

therefore represent a significant advance in treatment of chronic or recurrent OM infections, particularly those of a polymicrobial nature.

As all three of the leading OM pathogens appear to utilize the AI-2 signal, one target for blocking bacterial communication would be interfering with recognition of AI-2. In *Vibrio harveyi*, sensing of AI-2 occurs through a two-component system involving LuxP and LuxQ [40], where LuxP binds AI-2 and LuxQ senses and propagates the signal through its sensor kinase and response regulator domains [41]. Outside of *Vibrio*, many species possess the Lsr ABC transporter for binding of AI-2 by LsrB and transport through a heterodimeric membrane channel [42–45]. In addition to LsrB, the ribose binding protein RbsB also binds AI-2 [46,47]. Thus, ribose transport systems may also participate in the internalization of, or response to, AI-2 and would therefore represent additional targets for disruption of AI-2 quorum signaling.

Many of the current AI-2 nucleoside analogues aimed at disrupting AI-2 transport, such as adenosine derivatives, only antagonize the *V. harveyi* LuxPQ sensor system [48]. Other AI-2 analogs have been shown to antagonize signaling in *Salmonella typhimurium* and are nontoxic toward mammalian cells, but activated signaling in *V. harveyi* in the presence of the AI-2 precursor 4,5-dihydroxy-2,3-pentanedione (DPD) [49]. Another complication in targeting AI-2 transport or sensor systems is the duration of inhibition. For alkyl-DPDs, inhibition of *V. harveyi* signaling lasted less than 2 h [50], limiting their therapeutic potential.

Another target for disrupting bacterial communication would be inhibition of AI-2 production. This strategy may also disrupt bacterial metabolism as LuxS plays an intricate role in the activated methyl cycle (see [51] for review). Research focusing on the mechanism of action of LuxS has identified key amino acid residues and catalytic intermediates that could be suitable targets for inhibiting LuxS [52]. LuxS substrate analogues of *S*-ribosyl homocysteine (SRH) acted as time-dependent inhibitors of AI-2 production in *V. harveyi*, *Bacillus subtilis* and *Escherichia coli*. It may therefore be possible to generate modified SRH analogues that permanently inhibit LuxS and AI-2 production.

Another strategy to block bacterial communication would be external quenching of AI-2. This could be accomplished through degradation or cleavage of AI-2, use of AI-2 binding proteins, or alteration of AI-2 to limit sensing of the signal. One example of AI-2 quenching in the literature is the use of *E. coli* AI-2 kinase (LsrK) for *in vitro* phosphorylation of AI-2 [53]. According to this study, the addition of LsrK and ATP to bacterial cultures generated phospho-AI-2 that could not be transported into bacterial cells, and degraded over time without stimulating quorum signaling pathways. Such strategies may prove the most effective against otopathogens as LsrK can phosphorylate DPD, the precursor of all discrete structures of AI-2, thus targeting bacterial communication regardless of the specific AI-2 structure or transport/sensor mechanisms present during the infection.

Challenges

Several challenges exist in the development of effective OM treatments targeting bacterial communication, including obstacles in specificity and delivery of the treatment, potential negative effects of AI-2 inhibition in some otopathogens, and the implications of systemic inhibition of AI-2 quorum signaling. Therapy aimed at blocking AI-2 transport systems would need to account for differences in the discrete structure of AI-2 utilized by each otopathogen. All therapeutics targeting AI-2 signaling would ideally penetrate the biofilms that form during chronic infection, and LuxS inhibitor compounds would also need to be transported into the bacterial cell to be effective.

Due to the interspecies nature of AI-2 signaling, targeting AI-2 has implications beyond the common otopathogens. Disruption of AI-2 can promote virulence or virulence-related phenotypes for *Staphylococcus aureus* and *Streptococcus pyogenes* [54,55], species found within the upper airways that occasionally cause OM. Another consideration for interference with AI-2 signaling concerns the potential impact on the normal microbiota. AI-2 is produced by avirulent species within the oral and gastrointestinal microbiota [56,57]. There is limited information concerning AI-2 quorum signaling in these populations, but the possibility exists that AI-2 signaling may be critical for the establishment or maintenance of normal microbiota and defense against pathogens. Thus, it would be ideal to engineer an AI-2 inhibitor that remains localized to the site of infection.

Despite the challenges in manipulating bacterial communication, AI-2 remains an intriguing target for disruption of chronic, recurrent and polymicrobial infections. Limiting bacterial communication may be sufficient to reduce or eliminate key species and make biofilms more susceptible to immune clearance or antibiotics. Thus, disruption of polymicrobial biofilms in this manner represents a potential means to reduce antibiotic treatment failure.

References

1. Costerton JW, Cheng KJ, Geesey GG, et al. Bacterial biofilms in nature and disease. *Annu Rev Microbiol.* 1987; 41(1):435–464. [PubMed: 3318676]
2. Hendolin PH, Markkanen A, Ylikoski J, Wahlfors JJ. Use of multiplex PCR for simultaneous detection of four bacterial species in middle ear effusions. *J Clin Microbiol.* 1997; 35(11):2854–2858. [PubMed: 9350746]
3. Maddocks JL, May JR. 'Indirect pathogenicity' of penicillinase-producing enterobacteria in chronic bronchial infections. *Lancet.* 1969; 1(7599):793–795. [PubMed: 4180358]
4. Armbruster CE, Hong W, Pang B, et al. Indirect pathogenicity of *Haemophilus influenzae* and *Moraxella catarrhalis* in polymicrobial otitis media occurs via interspecies quorum signaling. *MBio.* 2010; 1(3):e00102–e00110. [PubMed: 20802829]
5. Finkelstein JA, Davis RL, Dowell SF, et al. Reducing antibiotic use in children: a randomized trial in 12 practices. *Pediatrics.* 2001; 108(1):1–7. [PubMed: 11433046]
6. Rayner MG, Zhang Y, Gorry MC, Chen Y, Post JC, Ehrlich GD. Evidence of bacterial metabolic activity in culture-negative otitis media with effusion. *JAMA.* 1998; 279(4):296–299. [PubMed: 9450714]
7. Wardle JK. *Branhamella catarrhalis* as an indirect pathogen. *Drugs.* 1986; 31(Suppl 3):93–96. [PubMed: 3732085]
8. Brook I. Direct and indirect pathogenicity of *Branhamella catarrhalis*. *Drugs.* 1986; 31(Suppl 3):97–102. [PubMed: 3488202]
9. Maddocks JL. Indirect pathogenicity. *J Antimicrob Chemother.* 1980; 6(3):307–309. [PubMed: 6967479]
10. Hol C, Van Dijke EE, Verduin CM, Verhoef J, van Dijk H. Experimental evidence for *Moraxella*-induced penicillin neutralization in pneumococcal pneumonia. *J Infect Dis.* 1994; 170(6):1613–1616. [PubMed: 7996007]
11. Budhani RK, Struthers JK. Interaction of *Streptococcus pneumoniae* and *Moraxella catarrhalis*: investigation of the indirect pathogenic role of β -lactamase-producing moraxellae by use of a continuous-culture biofilm system. *Antimicrob Agents Chemother.* 1998; 42(10):2521–2526. [PubMed: 9756750]
12. Pichichero ME. Recurrent and persistent otitis media. *Pediatr Infect Dis J.* 2000; 19(9):911–916. [PubMed: 11001126]
13. St Geme JW. Molecular and cellular determinants of non-typeable *Haemophilus influenzae* adherence and invasion. *Cell Microbiol.* 2002; 4(4):191–200. [PubMed: 11952636]
14. Grevers G. Challenges in reducing the burden of otitis media disease: an ENT perspective on improving management and prospects for prevention. *Int J Pediatr Otorhinolaryngol.* 2010; 74(6):572–577. [PubMed: 20409595]

15. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999; 284:1318–1322. [PubMed: 10334980]
16. Bakaletz LO. Bacterial biofilms in otitis media: evidence and relevance. *Pediatr Infect Dis J*. 2007; 26(10 Suppl):S17–S19. [PubMed: 18049376]
17. Post JC, Hiller NL, Nistico L, Stoodley P, Ehrlich GD. The role of biofilms in otolaryngologic infections: update 2007. *Curr Opin Otolaryngol Head Neck Surg*. 2007; 15(5):347–351. [PubMed: 17823552]
18. Weimer KED, Armbruster CE, Juneau RA, Hong W, Pang B, Swords WE. Coinfection with *Haemophilus influenzae* promotes pneumococcal biofilm formation during experimental otitis media and impedes the progression of pneumococcal disease. *J Infect Dis*. 2010; 202(7):1068–1075. [PubMed: 20715928]
19. Grijalva CG, Nuorti JP, Griffin MR. Antibiotic prescription rates for acute respiratory tract infections in US ambulatory settings. *JAMA*. 2009; 302(7):758–766. [PubMed: 19690308]
20. Grubb MS, Spaugh DC. Treatment failure, recurrence, and antibiotic prescription rates for different acute otitis media treatment methods. *Clin Pediatr (Phila)*. 2010 (Epub ahead of print). 10.1177/0009922810370363
21. Dagan R, Leibovitz E. Bacterial eradication in the treatment of otitis media. *Lancet Infect Dis*. 2002; 2(10):593–604. [PubMed: 12383609]
22. Pelton SIMD, Leibovitz EMD. Recent advances in otitis media. *Pediatric Infectious Disease Journal*. 2009; 28(10):S133–S137. Supplement, Childhood respiratory (diseases), management in an era of antibiotic resistance. [PubMed: 19918136]
23. Williams RL, Chalmers TC, Stange KC, Chalmers FT, Bowlin SJ. Use of antibiotics in preventing recurrent acute otitis media and in treating otitis media with effusion. A meta-analytic attempt to resolve the brouhaha. *JAMA*. 1993; 270(11):1344–1351. [PubMed: 8141875]
24. Anderson GG, O'Toole GA. Innate and induced resistance mechanisms of bacterial biofilms. *Curr Top Microbiol Immunol*. 2008; 322:85–105. [PubMed: 18453273]
25. Schuerman L, Borys D, Hoet B, Forsgren A, Prymula R. Prevention of otitis media: now a reality? *Vaccine*. 2009; 27(42):5748–5754. [PubMed: 19666154]
26. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med*. 2001; 344(6):403–409. [PubMed: 11172176]
27. Hardie KR, Heurlier K. Establishing bacterial communities by 'word of mouth': LuxS and autoinducer 2 in biofilm development. *Nat Rev Microbiol*. 2008; 6(8):635–643. [PubMed: 18536728]
28. Jayaraman A, Wood TK. Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. *Annu Rev Biomed Eng*. 2008; 10:145–167. [PubMed: 18647113]
29. Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol*. 2001; 55:165–199. [PubMed: 11544353]
30. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*. 2005; 21:319–346. [PubMed: 16212498]
31. Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. *Annu Rev Genet*. 2009; 43:197–222. [PubMed: 19686078]
32. Njoroge J, Sperandio V. Jamming bacterial communication: new approaches for the treatment of infectious diseases. *EMBO Mol Med*. 2009; 1(4):201–210. [PubMed: 20049722]
33. Landini P, Antoniani D, Burgess JG, Nijland R. Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. *Appl Microbiol Biotechnol*. 2010; 86(3):813–823. [PubMed: 20165945]
34. Alexander HR, Robert JP Jr, David SB, et al. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. *Mol Microbiol*. 2006; 60(6):1446–1456. [PubMed: 16796680]
35. Rickard AH, Campagna SR, Kolenbrander PE. Autoinducer-2 is produced in saliva-fed flow conditions relevant to natural oral biofilms. *J Appl Microbiol*. 2008; 105(6):2096–2103. [PubMed: 19120655]

36. Armbruster CE, Hong W, Pang B, et al. LuxS promotes biofilm maturation and persistence of nontypeable *Haemophilus influenzae* *in vivo* via modulation of lipooligosaccharides on the bacterial surface. *Infect Immun*. 2009; 77(9):4081–4091. [PubMed: 19564381]
37. Daines DA, Bothwell M, Furrer J, et al. *Haemophilus influenzae* luxS mutants form a biofilm and have increased virulence. *Microb Pathog*. 2005; 39(3):87–96. [PubMed: 16099134]
38. Joyce EA, Kawale A, Censini S, Kim CC, Covacci A, Falkow S. LuxS is required for persistent pneumococcal carriage and expression of virulence and biosynthesis genes. *Infect Immun*. 2004; 72(5):2964–2975. [PubMed: 15102809]
39. Stroehrer UH, Paton AW, Ogunniyi AD, Paton JC. Mutation of luxS of *Streptococcus pneumoniae* affects virulence in a mouse model. *Infect Immun*. 2003; 71(6):3206–3212. [PubMed: 12761100]
40. Bassler BL, Wright M, Silverman MR. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol Microbiol*. 1994; 13(2):273–286. [PubMed: 7984107]
41. Henke JM, Bassler BL. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J Bacteriol*. 2004; 186(20):6902–6914. [PubMed: 15466044]
42. Pereira CS, McAuley JR, Taga ME, Xavier KB, Miller ST. *Sinorhizobium meliloti*, a bacterium lacking the autoinducer-2 (AI-2) synthase, responds to AI-2 supplied by other bacteria. *Mol Microbiol*. 2008; 70:1223–1235. [PubMed: 18990189]
43. Taga ME, Semmelhack JL, Bassler BL. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol Microbiol*. 2001; 42(3):777–793. [PubMed: 11722742]
44. Xavier KB, Bassler BL. Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J Bacteriol*. 2005; 187(1):238–248. [PubMed: 15601708]
45. Shao H, Lamont RJ, Demuth DR. Autoinducer 2 is required for biofilm growth of *Aggregatibacter (Actinobacillus) actinomycetemcomitans*. *Infect Immun*. 2007; 75(9):4211–4218. [PubMed: 17591788]
46. Shao H, James D, Lamont RJ, Demuth DR. Differential interaction of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* LsrB and RbsB proteins with autoinducer 2. *J Bacteriol*. 2007; 189(15):5559–5565. [PubMed: 17526716]
47. James D, Shao H, Lamont RJ, Demuth DR. The *Actinobacillus actinomycetemcomitans* ribose binding protein RbsB interacts with cognate and heterologous autoinducer 2 signals. *Infect Immun*. 2006; 74(7):4021–4029. [PubMed: 16790775]
48. Brackman G, Celen S, Baruah K, et al. AI-2 quorum-sensing inhibitors affect the starvation response and reduce virulence in several *Vibrio* species, most likely by interfering with LuxPQ. *Microbiology*. 2009; 155(Pt 12):4114–4122. [PubMed: 19778962]
49. Lowery CA, Park J, Kaufmann GF, Janda KD. An unexpected switch in the modulation of AI-2-based quorum sensing discovered through synthetic 4,5-dihydroxy-2,3-pentanedione analogues. *J Am Chem Soc*. 2008; 130(29):9200–9201. [PubMed: 18576653]
50. Lowery CA, Abe T, Park J, et al. Revisiting AI-2 quorum sensing inhibitors: direct comparison of alkyl-DPD analogues and a natural product fimbrolide. *J Am Chem Soc*. 2009; 131(43):15584–15585. [PubMed: 19824634]
51. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. Making ‘sense’ of metabolism: autoinducer-2, LUXS and pathogenic bacteria. *Nat Rev Micro*. 2005; 3(5):383–396.
52. Gopishetty B, Zhu J, Rajan R, et al. Probing the catalytic mechanism of S-ribosylhomocysteinase (LuxS) with catalytic intermediates and substrate analogues. *J Am Chem Soc*. 2009; 131(3):1243–1250. [PubMed: 19099445]
53. Roy V, Fernandes R, Tsao C-Y, Bentley WE. Cross species quorum quenching using a native AI-2 processing enzyme. *ACS Chem Biol*. 2009; 5(2):223–232. [PubMed: 20025244]
54. Siller M, Janapatla R, Pirzada Z, Hassler C, Zinkl D, Charpentier E. Functional analysis of the group A streptococcal luxS/AI-2 system in metabolism, adaptation to stress and interaction with host cells. *BMC Microbiol*. 2008; 8(1):188. [PubMed: 18973658]
55. Zhao L, Xue T, Shang F, Sun H, Sun B. *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. *Infect Immun*. 2010; 78(8):3506–3515. [PubMed: 20498265]

56. Duan K, Dammel C, Stein J, Rabin H, Surette MG. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol.* 2003; 50(5): 1477–1491. [PubMed: 14651632]
57. Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB. Bacteria–host communication: the language of hormones. *Proc Natl Acad Sci USA.* 2003; 100(15):8951–8956. [PubMed: 12847292]