Antimicrobial Resistance: A Dentists' Prospective

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Abstract

Antimicrobial resistance has turned from prediction to reality in a few decades and exerts a significant burden globally with a loss of approximately seventy thousand lives each year. Dentistry accounts for approximately three to eleven percent of all antibiotic prescriptions worldwide. The human oral cavity is a huge reservoir of more than 750 microbial species. Oral microbial biofilm formation such as dental plaque that initiates periodontal diseases and dental caries is the major reason for the antimicrobial therapy failure and rise in resistance against the action of antimicrobials. Biofilm bacteria embedded with a matrix of extracellular polysaccharides are more than 10,000 times less sensitive to antimicrobials than free-floating planktonic bacteria. A thorough understanding of the mechanism will provide an effective solution to combat the current problem such as developing novel antimicrobials, method that enhance the effectiveness of antibiotics by modifying the antibiotic structure or disrupting biofilm, or altering the outer membrane structure of bacterial cell walls to neutralize the resistant enzymes. Use of novel strategies e.g. nano-drug delivery system to facilitate drug entry into the cell; liposome for efficient local delivery of drug of choice in the treatment of caries, periodontitis and also for anesthetic purposes. This paper provides a review of the literature based on the studies on oral biofilm, its formation, and importance in drug resistance, mechanism of drug action and development of its drug resistance and various measures to combat it and the scope of dentists in limiting the progression of antibiotic resistance.

Keywords: *Antimicrobial resistance, Biofilm, Dental caries, periodontitis, Dentistry, Mechanism.*

Introduction

Resistance to antibiotics and adverse events associated with antibiotic use has now become a major threat to global health. It prompts the major challenge for the health providers all over the world and poses a threat to the rising mortality and morbidity. Over the first few decades the increased antibiotic-resistant gene transmission in virtually every species of bacteria for every class of antibiotics available from man and animals has been documented.[1] We are now having a huge list of multidrug-resistant bacteria with either a few or non-responsive antibiotics.[1] The possibility for a bacteria to develop resistance against antibiotics goes back to the 1930s as suggested by Dr. Flemming in his published description "discovery of penicillin".[2] The concept we have understood that "the success of antibiotics in improving morbidity and mortality rate worldwide" has created the present scenario "the resistance developed due to use of these antibiotics is responsible for increased morbidity and mortality". This is due to the microbial ability to withstand the adverse conditions involved and get transformed to protect themselves thereby deactivating the curing

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potential of antibiotics.[3] Antibiotic resistance is now a potential threat to mankind as there is a gradual rise in the number of pathogenic bacteria becoming multidrug resistance or completely resistant to various antibiotics currently available. Antimicrobial resistance has turned from prediction to reality in a few decades and exerts a significant burden globally with a loss of approximately seventy thousand lives each year.[4]

Dentistry accounts for approximately three to eleven percent of all antibiotic prescriptions worldwide.[5] The human oral cavity is a huge reservoir of more than 750 microbial species.[6] Studies suggest that the normal human flora may act as a reservoir for antibiotic-resistant genes which under the right conditions are then able to be transferred to pathogenic species.[7,8] Oral microbial biofilm formation such as dental plaque that initiates periodontal diseases and dental caries is the major reason for the antimicrobial therapy failure and rise in resistance against the action of antimicrobials.[9,10]

Microbial biofilms are formed by colonization of various unicellular organisms to form a community that is attached to any solid surface and adherence of other microorganisms within a polymeric matrix made up of exo-polysaccharide thereby forming a complex community.[11] These biofilms initiate changes within microorganisms thereby exhibiting characteristics different from their planktonic counterparts, their susceptibility to various antibiotic agents, that includes responses against immunological reactions, their interaction with the host tissues, or their physiological properties.[12] Biofilm bacteria embedded with a matrix of extracellular polysaccharides are more than 10,000 times less sensitive to antimicrobials than free-floating planktonic bacteria. Biofilm creates a protected environment for the growth and survival of microbes in a hostile environment.[13]

The oral or dental biofilm that colonizes on the surfaces of the tooth is a complex three-dimensional structure that is formed of around 100-300 cell layers[12] and is a potential reservoir for the oral pathogenic microorganisms.[6,14] A number of gram-negative bacteria involved in periodontal infections such as *P. gingivalis, A. actinomycetemcomitans, F. nucleatum, P. intermedia, B. forsythus, S. intermedius, T. denticola*. These members are responsible for increased periodontal pocket formation as well as tooth loss.[14,15] Biofilms formed by bacteria protect themselves from antimicrobial agents and host immune responses as; (i) It protects against oxidative stress (ii) effluxes out antibiotics (iii) provides protective measures by its polysaccharide matrix and stops easy diffusion of antibiotics.[16,17]

The sequential events that occur during the formation of plaque on dental surfaces are; (1) A pellicle is formed on the tooth surface from proteins and glycoproteins present in saliva. (2) Bacterial molecules are adsorbed by the pellicle formed on the tooth surface through weak physical forces. (3) Reversible addition of oral bacteria to the tooth surface through binding between specific molecules present on the surface of the microbial cell with the complementary receptor present in the formed pellicle over the tooth surface. These constitute the primary colonizers particularly Streptococci sps and Actinomycetes sps. (4) Subsequently other microorganisms colonize to form secondary colonizers by coadhesion with primary colonizers through cell surface adhesion to the receptors present in primary colonizing organisms. This creates a rise in the microbial diversity in the plaque biofilm. (5) Bacterial proliferation takes place by multiplication resulting in the rise of biomass and formation of biofilm matrix by synthesis of exo-polymers. (6) Plaque growth and maturation takes place and all together they behave as a complex organism. There is growth and colonization of anaerobic organisms.

Due to the release of exo-polymers by the colonized microbes the penetration of the antibiotic agents is restricted into the 3-d structured plaque biofilm and also novel properties are exhibited by the slowly growing microorganisms on the biofilm surface.[18,19]

This paper provides a review of the literature based on the studies on oral biofilm, its formation, importance in drug resistance, mechanism of drug action and development of its drug resistance and various measures to combat it and the scope of dentists in limiting the progression of antibiotic resistance.

Mechanism of drug resistance by biofilms: Several groups of antimicrobial therapeutic agents (e.g:- β lactams, metronidazole, tetracyclines, quinolones) are practiced for the treatment of oral infections in addition to dentifrices and oral rinses (chlorhexidine, triclosan, fluoride)

In course of time oral microbiota develop resistance following many different mechanisms such as

1. Production of hydrolytic enzymes to inactivate antibiotics.[11]

2. Effluxing out the antibiotics taken up by the bacteria.[11]
3. Bringing conformational changes in drug targets by causing mutation in genes responsible for.[18,19]
4. Differential expression of porin proteins in biofilm cells reduced the accession of antibiotics resulting in antibiotic resistance in gram-negative bacteria.[20]
5. The formation of biofilm is controlled by quorum sensing of molecules which coordinates the gene expression to regulate the virulence factor.[21]
6. Bacteria present in biofilm adapt themselves to changed physiological conditions in response to exposure to antimicrobial agents and reduce their growth rate.[22]
7. Formation of exopolysaccharide biofilm matrix by bacteria retards the permeability of antibiotics following different mechanisms e.g: electrostatic interaction, absorption, enzymatic neutralization, hydrophobicity.[20,22]

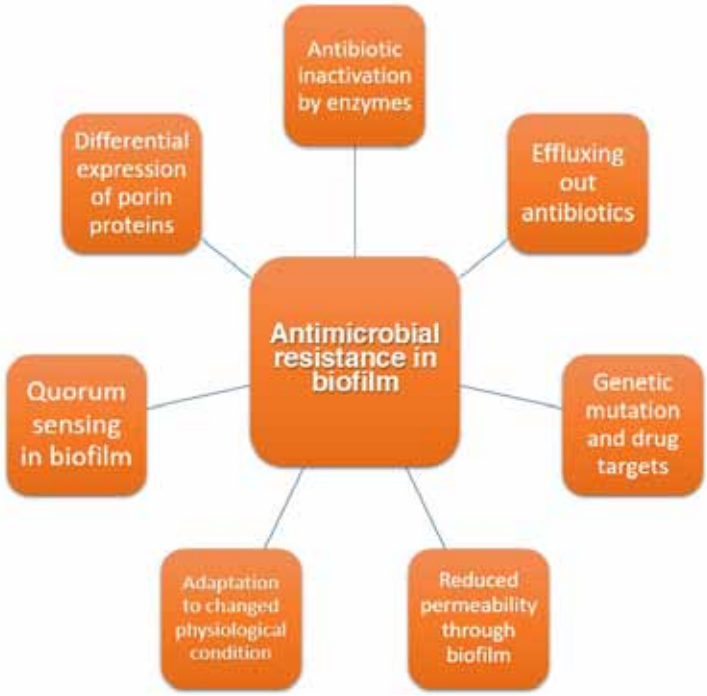

Fig 1: Different ways of development of antimicrobial resistance in biofilms

Mechanism of Antibiotics and Development of resistance: Antibiotics work by affecting things that bacterial cells have but human cells don't. Bacteria and human cells differ in the structure of their cell membranes, method of protein synthesis or DNA replication mechanisms. Some antibiotics affect the cell membrane of the bacterial cells, while some affect the protein building mechanisms, while some act on the DNA copying machinery that is specific to bacteria.

Different families of antibiotics have different ways of killing microbial pathogens.

Beta-Lactam: It generally includes antibiotics like: Penicillins, cephalosporin. Beta-Lactams act on the gram-negative microorganisms by inhibiting the synthesis of bacterial cell walls. They interrupt the links between the peptidoglycan components in the transpeptidation process of the cell wall of gram-negative bacteria. These drugs can bind to PBP (penicillin-binding proteins), the enzymes that are involved in final stages of formation of the cell wall during the growth and division of the organism, and ultimately results in incomplete cell wall formation and cell lysis.[23] Antibiotic resistance developed against beta-lactamase is due to the presence of the enzyme beta-lactamases, present in many gram-negative and some gram-positive organisms that prevent the antibiotics to bind to the specific PBPs.[24]

Tetracycline: It belongs to the broad-spectrum group of antibiotics. It possesses the ability to bind with the 30s ribosomal subunit in the mRNA translational complex resulting in inhibition of protein synthesis.[25,26] It has a wide range of activity against gram-positive and gram-negative bacteria, mycoplasmas (without cell wall), protozoan parasites, chlamydia and rickettsia. Resistance to tetracycline is primarily due to the acquisition of new genes in the bacteria.[27] Due to various side effects, including staining of the teeth, other abdominal discomfort and damage to the kidney, this drug is not in much use.

Metronidazole: It is a nitroimidazole group of antibiotics that can be prescribed in adjunct to other groups of antibiotics or alone to treat oral infections and also other inflammatory diseases.[28] It is most effective against obligate anaerobic microorganism such as *Fusobacterium, Peptostrptococcus* species, *Bacteroides, Prevotela, Clostridium.*[29,30] The nitro group of metronidazole after entering into the cell through diffusion gets reduced thereby releasing toxic products that degrade the microbial DNA and also interferes in the synthesis of new DNA.[31,32] Four genes that codes for nitroimidazole reductase, the enzyme that carries out the enzymatic reduction of nitroimidazole to its respective derivatives are found to confer some level of resistance in colonic Bacteroides species but the presence of such genes in oral species is not clear.[33,34]

Quinolones: These are the chemotherapeutic bactericidal drugs eradicating bacteria by interfering with DNA replication. It acts on the DNA gyrase, a type-II topoisomerase which is essential for the replication of DNA and also recombination following repair.[1] Quinolones bind to DNA gyrase and DNA resulting in the formation of a stable complex that prevents the DNA gyrase for recombination of cut strands of DNA. Resistance to quinolones is found in a variety of anaerobic organisms are either due to alterations in DNA gyrase or for reduction in antibiotic accumulation.[35]

Fluoride: This is an anion of fluorine used extensively as a constituent in mouthwashes, toothpaste and oral supplements as dental caries preventive agents.[36,37]The fluoride ions interact with the mineral content of the tooth and induces remineralization and also prevents demineralization caused by cariogenic bacteria.[38] Studies suggest that fluoride acts on the enzyme enolase in the glycolytic pathway resulting in growth inhibition and reduction in the production of acid from oral streptococci such as *S.mutans*.[39,40] Administration of fluoride is restricted owing to the development of dental and skeletal fluorosis along with fluoride resistance found in oral bacteria.[41]

Chlorhexidine: It is the most effective antiseptic agent, is a cationic poly biguanide with hydrophilic and hydrophobic properties that acts as both supragingival and mucosal plaques[42,43]due to its dicationic nature, it can bind reversibly to the negatively charged molecules in the oral cavity and persist for a long duration after its application.[43] Chlorhexidine is active against a group of organisms including gram-positive and gram-negative bacteria aerobes, facultative anaerobes, and yeasts by causing damage to the inner cytoplasmic membrane.[42,44] Not only it inhibits plaque formation, but it also reduces plaque adhesion to the tooth surface by blocking the acidic groups of glycoproteins that are present in saliva. It brings about ultra-structural alterations within the oral biofilm by being adsorbed on to extracellular polysaccharide or competing with agglutination of calcium ions in plaque resulting in a reduction in the adhesion of bacteria to the tooth surface.[45]

Triclosan: It is a non-ionic phenolic molecule used majorly in dentifrices having a broad-spectrum antimicrobial effect against gram-positive and gram-negative microorganisms.[46] A numerous randomized, controlled clinical trials summarized the efficacy of triclosan as a plaque reducing agent and also exhibits anti-inflammatory properties for improving gingival health.[46-49]

Role of dentists in reducing antibiotic resistance: As health care provider**s,** dentists should be aware of their contribution towards developing antibiotic resistance and limit their role in exacerbating it. Studies suggest that about 66% of the prescribed antibiotics are not clinically indicated.[4] The pathophysiology of the pulpal diseases should be emphasized and understood and inadvertent use of antibiotics in the treatment of acute endodontic infections to relieve pain without intervening endodontic treatment should be avoided. Antibiotics are ineffective in eliminating the pathogens in the root canal system as there is reduced blood circulation in the necrotic or infected pulp. Surgical or endodontic intervention is required to alleviate the symptoms and infection.[50] Antibiotics should be prescribed for localized infection in those with systemic involvement.

Dentists should follow some guidelines[51, 52]

1. Accurate diagnosis should be obtained before prescribing any antibiotics
2. Obtaining the involved microbial analysis presenting the infection and prescribing the suitable antibiotic
3. Rather than adopting broad-spectrum antibiotics one should prefer the narrowest spectrum antibiotic.
4. Avoid prescribing antibiotics if it can be managed without.
5. Should not prescribe for unknown or improperly understood infections
6. Should thoroughly review the medical history of the patient to avoid adverse effects of a specific group of antibiotics if any.
7. Should not be biased by the demand of patient or pressure due to competitive healthcare providers
8. Should be avoided in infections due to fungus or virus or traumatic aphthae ulcers
9. Prescribed when systemic spread of bacterial infection is evident.
10. Should advise patients for proper dosage and duration of prescribed antibiotics.
11. Should update self about appropriate oral infection management from conferences, journals, continuing dental education (CDE) programs.
12. Should discourage and educate the patients not to avail antibiotics over the counter.

Conclusion

The scale and speed of increasing antibiotic resistance has become a global concern where the solution is required at every level. A thorough understanding of the mechanism will provide an effective solution to combat the current problem such as developing novel antimicrobials, method that enhance the effectiveness of antibiotics by modifying the antibiotic structure or disrupting biofilm, or altering the outer membrane structure of bacterial cell walls to neutralize the resistant enzymes. Use of novel strategies e.g. nano-drug delivery system to facilitate drug entry into the cell; liposome for efficient local delivery of drug of choice in the treatment of caries, periodontitis, and also for anesthetic purposes.

Acknowledgement: I am thankful to Dean, IDS and President SOA deemed to be University for their kind support and encouragement throughout.

Conflict of Interests: None

Ethical Permission: Not required as it is a review paper.

Funding: Nil

References

1. Marilyn CR. Antibiotic resistance mechanism in bacteria of oral and upper respiratory origin. International Journal of Antimicrobial Agents 9, 1998; 255-267
2. Fleming A. On the antibacterial action of cultures of a Penicil- lium, with special reference to their use in the isolation of B.influenzae. Br J ExpPathol 1929;10:226–36
3. Smitha S et al. Think before you prescribe: How dentistry contributes to antibiotic resistance. Australian Dental Journal,2019; 65(1): 21-29
4. Patrick A, Kandiah T. Resistance to change: how much longer will our antibiotics work? Fac Dent J 2018; 9: 103– 111.
5. Teoh L, Stewart K, Marino R, McCullough M. Antibiotic resistance and relevance to general dental practice in Australia. Aust Dent J 2018; 63: 414– 421.
6. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. Trends Microbiol 2005; 13: 589-95.
7. Roberts MC. Gene transfer in the urogenital and respiratory tract. In: Levy SB, Miller RV, editors. Gene Transfer in the Environment. New York: McGraw-Hill, 1989:347–75
8. Cohen M. Epidemiology of drug resistance: Implications for apost-antimicrobial era. Science 1992;257:1050–5
9. G. Karibasappa and A. Sujatha, "Antibiotic resistance–a concern for dentists," IOSR Journal of Dental and Medical Sciences, vol. 13, pp. 112–118, 2014. View at:
10. S. Godreuil, N. Leban, A. Padilla et al., "Aedesin: structure and antimicrobial activity against multidrug resistant bacterial strains," PLoS One, vol. 9, Article ID e105441, 2014.
11. Smith AW. Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems. Advanced Drug Del Rev 2005; 57: 1539-50.

12. Sbordone L, Bortolaia C. Oral microbial biofilms and plaquerelated diseases: microbial communities and their role in the shift from oral health to disease. Clin Oral Invest 2003; 7: 181-8.
13. IndulataKanwar, Abhishek K Sah, Preeti K Suresh. Biofilm-mediated Antibiotic-resistant Oral Bacterial Infections: Mechanism and Combat Strategies. Current pharmaceutical design 2017; 23,2084-2095
14. Chung JH, Kim YK, Kim KH, et al. Synthesis, characterization, biocompatibility of hydroxyapatite natural polymers nanocomposites for dentistry applications. Artif Cells NanomedBiotechnol 2014; 11: 18.
15. Dimitris NT, Purnima SK. Etiology and pathogenesis of periodontal diseases. Dent Clin Nam 2005; 491-516.
16. B. Kouidhi, T. Zmantar, K. Mahdouani, H. Hentati, and A. Bakhrouf, "Antibiotic resistance and adhesion properties of oral enterococci associated with dental caries," BMC Microbiology, vol. 11, p. 155, 2011
17. N. Kaur, P. Sahni, A. Singhvi, M. K. Hans, and A. S. Ahluwalia, "Screening the drug resistance property among aerobic pathogenic microorganisms of dental caries in north-western Indian population: a preliminary study," Journal of Clinical and Diagnostic Research, vol. 9, pp. Zc05–Zc08, 2015.
18. Melo WCMA, Perussi JR. Strategies to overcome biofilm resistance, microbial pathogens and strategies for combating them: science, technology and education. In Méndez-Vilas A, Ed. 2013:179-87.
19. Marsh PD. Dental plaque as a biofilm: the significance of pH in oral health and caries. 2009; 30(2): 76-87.
20. Kouidhi B, Qurashi YM, Chaieb K. Drug resistance of bacterial dental biofilm and the potential use of natural compounds as alternative for prevention and treatment. Microbial Pathogen 2015; 80: 39-49.
21. Wu H, Moser C, Wang HZ, et al. Strategies for combating bacterial biofilm infections. Int J Oral Sci 2015; 7: 1-7.
22. Mah TFC, Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9: 34-9.
23. Ghuysen J-M. Molecular structures of penicillin-binding proteins and b-lactamases. Trends Microbiol Virulence Infect Pathogen 1994;2:372–80.
24. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for b-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995;39:1211–33.
25. S. Jeong and I. R. Paeng, "Sensitivity and selectivity on aptamer-based assay: the determination of tetracycline residue in bovine milk," The Scientific World Journal, vol. 2012, Article ID 159456, 10 pages, 2012.
26. S. D. Patil, R. Sharma, S. Srivastava, N. K. Navani, and R. Pathania, "Downregulation of yidC in Escherichia coli by antisense RNA expression results in sensitization to antibacterial essential oils eugenol and carvacrol," PLoS One, vol. 8, Article ID e57370, 2013.
27. Roberts MC. Tetracycline resistance determinants: Mechanisms of action, regulation of expression, genetic mobility, and distri-bution. FEMS Microbiol Rev 1996;19:1–24
28. S. A. Dingsdag and N. Hunter, "Metronidazole: an update on metabolism, structure-cytotoxicity and resistance mechanisms," Journal of Antimicrobial Chemotherapy, vol. 73, no. 2, pp. 265–279, 2018.
29. N. Dione, S. Khelaifia, J.-C. Lagier, and D. Raoult, "The aerobic activity of metronidazole against anaerobic bacteria," International Journal of Antimicrobial Agents, vol. 45, no. 5, pp. 537–540, 2015.
30. R. Ghotaslou, H. BannazadehBaghi, N. Alizadeh, M. Yekani, S. Arbabi, and M. Y. Memar, "Mechanisms of Bacteroidesfragilis resistance to metronidazole," Infection, Genetics and Evolution, vol. 64, pp. 156–163, 2018.
31. Church DL, Bryant RD, Sim V, Lishley EJ. Metonidazole susceptibility and the presence of hydrogenase in pathogenic bacteria. Anaerobe 1996;2:147–53.
32. Greenstein G. The role of metronidazole in the treatment of periodontal disease. J Periodontol 1993;64:1–15.
33. Reysset G, Trinh S, Carlier J-P, Sebald M. Base genetiques de la resistance aux 5-nitroimidazoles des Bacteroides spp. Med Mal Infect 1996;26:S213–9.

34. Carlier J-P, Sellier N, Rager M-Nm, Reysset G. Metabolism of a 5-nitroimidazole in susceptible and resistant isolgenic strains of Bacteroidesfragilis. Antimicrob Agents Chemother 1997;41:1495–9.

35. Wexler HM, Molitoris E, Finegold SM. In vitro actirities of three of the newer quinolones against anaerobic bacteria. An-timicrob Agents Chemother 1992;36:239–43.

36. G. D. Slade, R. S. Bailie, K. Roberts-Thomson et al., "Effect of health promotion and fluoride varnish on dental caries among Australian aboriginal children: results from a community-randomized controlled trial," Community Dentistry and Oral Epidemiology, vol. 39, no. 1, pp. 29–43, 2011

37. V. C. Marinho, L. Y. Chong, H. V. Worthington, and T. Walsh, "Fluoride mouthrinses for preventing dental caries in children and adolescents," The Cochrane Database of Systematic Reviews, vol. 7, no. 7, p. Cd002284, 2016.

38. M. A. R. Buzalaf, J. P. Pessan, H. M. Honório, and J. M. Ten Cate, "Mechanisms of action of fluoride for caries control," Fluoride and the Oral Environment, vol. 22, pp. 97–114, 2011.

39. Z. Tong, L. Zhou, W. Jiang et al., "An in vitro synergetic evaluation of the use of nisin and sodium fluoride or chlorhexidine against Streptococcus mutans," Peptides, vol. 32, no. 10, pp. 2021–2026, 2011.

40. J. Pietkiewicz, A. Bronowicka-Szydełko, K. Dzierzba, R. Danielewicz, and A. Gamian, "Glycation of the muscle-specific enolase by reactive carbonyls: effect of temperature and the protection role of carnosine, pirydoxamine and phosphatidylserine," The Protein Journal, vol. 30, no. 3, pp. 149–158, 2011.

41. Y. Liao, J. Chen, B. W. Brandt et al., "Identification and functional analysis of genome mutations in a fluoride-resistant Streptococcus mutans strain," PLoS One, vol. 10, Article ID e0122630, 2015.

42. S. Balagopal and R. Arjunkumar, "Chlorhexidine: the gold standard antiplaque agent," Journal of Pharmaceutical Sciences and Research, vol. 5, pp. 270–274, 2013.

43. Strydonck DA, Slot DE, Velden U, Weijden F. Chlorhexidine mouthwash reduces plaque and gingivitis. Evidence-Based Dentistry, 2013, 17-18.

44. F. Cieplik, N. S. Jakubovics, W. Buchalla, T. Maisch, E. Hellwig, and A. Al-Ahmad, "Resistance toward chlorhexidine in oral bacteria-is there cause for concern?" Frontiers in Microbiology, vol. 10, p. 587, 2019.

45. T. Rema, P. Medihala, J. R. Lawrence et al., "Proteomic analyses of chlorhexidine tolerance mechanisms in Delftiaacidovorans Biofilms," mSphere, vol. 1, no. 1, pp. e00017–15, 2016.

46. Blinkhorn A, Bartold PM, Cullinan MP, et al. Is there a role for triclosan/copolymer toothpaste in the management of periodontal disease. Br Dental J 2009; 207: 117-25.

47. Davies RM, Ellwood RP, Davies GM. The effectiveness of toothpaste containing Triclosan and polyvinyl-methyl ether maleic acid copolymer in improving plaque control and gingival health. A systematic review. J ClinPeriodontol 2004; 31: 1029-33.

48. Biesbrock AR, Bartizek RD, Gerlach RW. Oral hygiene regimens, plaque control, and gingival health: a two-month clinical trial with antimicrobial agents. J Clin Dent 2007; 18: 103-7.

49. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc 2006; 137: 1649-57.

50. Patrick A, Kandiah T. Resistance to change: how much longer will our antibiotics work? Fac Dent J 2018; 9: 103– 111.

51. Segura-EgeaJJ,GouldK,Sen BH, JonassonP,Cotti E, Mazzoni A, et al. Antibiotics in endodontics: a review. International Endodontic Journal. 2017;50(12):1169-1184

52. Thornhill MH, Dayer M, Lockhart PB, et al. Guidelines on prophylaxis to prevent infective endocarditis. Br Dent J 2016; 220: 51– 6.

53. Marie T. F,Addressing Antibiotic Resistance in Dentistry: "What can WE do?" 2017, https://blogs.cdc.gov/safehealthcare/addressing-antibiotic-resistance-in-dentistry-what-can-we-do/