

# Virulence factors of *Aggregatibacter actinomycetemcomitans* — A status update

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## ABSTRACT

Periodontitis is a chronic infectious inflammatory disease characterized by the destruction of tooth. The contribution of bacteria to the disease progression is poorly understood probably due to the multifactorial background of this disease. *Aggregatibacter actinomycetemcomitans* is part of the normal flora in many healthy individuals, but is also a major etiologic agent in some aggressive forms of periodontitis. The genetic diversity among different isolates of *Aggregatibacter actinomycetemcomitans* is great and its ability to express and release virulence factors varies. *Aggregatibacter actinomycetemcomitans*, a pathogen not only in periodontal but also in some nonoral infections, possesses several virulence determinants which contribute to its ability to colonize the oral cavity, persist in the periodontal pocket, resist and evade host defenses, cause destruction of soft and hard tooth-supporting tissues, and interfere with host tissue repair after infection. Authors conducted a comprehensive search through PubMed/Medline databases to compile the available literature till June 2014, for the purpose of detailed insight into the bacteria. The search was designed to identify appropriate articles related to virulence factors of *Aggregatibacter actinomycetemcomitans*, and the articles were independently screened for eligibility.

**Key words:** *Aggregatibacter actinomycetemcomitans*, leukotoxin, lipopolysaccharide, juvenile periodontitis

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## INTRODUCTION

The complexity of the microbial population harbored in oral biofilms around the affected teeth and within the periodontal pockets, combined with the diversity of clinical presentations of periodontitis, has made the identification of specific microbial etiological agents very challenging. The most important gram-negative organisms are *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Bacteroides forsythus* (Bf), *Fusobacterium nucleatum* (Fn), *Capnocytophaga* species (C.sp), and *Campylobacter rectus* (Cr). Each species possesses a large number of virulence factors relevant to the periodontal disease process.<sup>[1]</sup> Dental plaque bacteria *Aggregatibacter actinomycetemcomitans* stands out as one of the most powerful periodontopathogens. It is a fastidious, facultative anaerobic, nonmotile, nonhemolytic, nonsporing, small gram-negative rod and is a prominent member of the HACEK group (*Haemophilus* spp., *Aggregatibacter*

*actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*) of pathogens. *Aggregatibacter actinomycetemcomitans* has been implicated as the causative agent of several forms of severe periodontal diseases, including localized juvenile periodontitis (LJP), early-onset periodontitis, and rapidly progressive periodontitis.<sup>[2]</sup> Authors conducted a comprehensive search through PubMed/Medline databases to compile the available literature till June 2014, for the purpose of detailed insight into the bacteria. The search was designed to identify appropriate articles related to *Aggregatibacter actinomycetemcomitans*, and the articles were independently screened for eligibility [Figures 1 and 2].

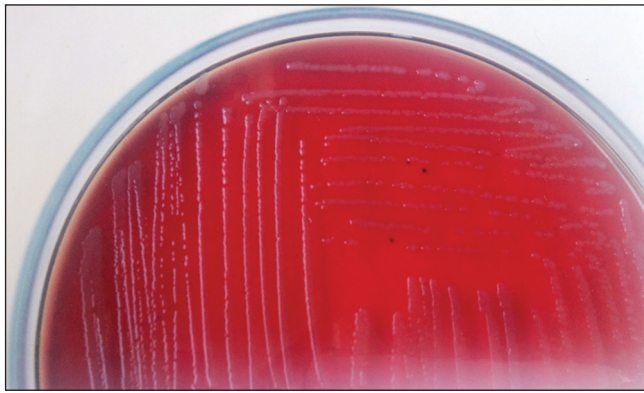
## INSIGHT

### History

*Aggregatibacter actinomycetemcomitans* was first reported in a publication by Klinger in 1912<sup>[3]</sup> who termed it as *Bacterium actinomycetemcomitans* which was changed to *Bacterium comitans* by Lieske,<sup>[4]</sup> and finally to *Actinobacillus actinomycetemcomitans* by Topley and Wilson.<sup>[5]</sup> *Actinobacillus actinomycetemcomitans* is derived from the Greek words, “actes”, meaning ray, because of the star-shaped colonies

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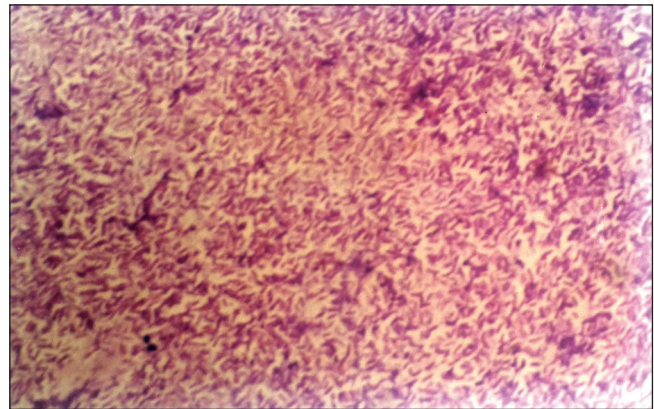


**Figure 1:** Petri dish showing colonies of *Aggregatibacter actinomycetemcomitans*

on the agar media and, mycetes, meaning fungus, because *Actinomyces* was originally thought to be a fungus. The Latin word, 'comitans', meaning in common with, or accompanying *Actinomyces* species, reflects the association of *Actinobacillus* with *Actinomyces*.<sup>[6]</sup> Kilian and Schiott<sup>[7]</sup> were the first to demonstrate that *Aggregatibacter actinomycetemcomitans* was present in dental plaque. With respect to periodontal disease, it was first implicated as the cause of juvenile periodontitis in 1976 by Newman *et al.*,<sup>[8]</sup> and Slots.<sup>[9]</sup> This disease is now called localized aggressive periodontitis (LAP). There are several lines of clinical evidence that support the association of *Aggregatibacter actinomycetemcomitans* with LAP. First, the organism is found more frequently in samples obtained from subjects with LAP as compared to healthy subjects. Second, subjects with LAP were consistently found to have elevated serum and locally produced antibody titers to *Aggregatibacter actinomycetemcomitans*. Third, several studies indicated that the treatment of subjects with LAP with the intention of reducing *Aggregatibacter actinomycetemcomitans* to undetectable levels resulted in marked clinical improvement, while a lack of clinical improvement was found to correlate with a failure to significantly reduce the level of *Aggregatibacter actinomycetemcomitans*<sup>[6]</sup> [Figure 3].

### Taxonomy

*Actinobacillus actinomycetemcomitans* is a member of the genus *Actinobacillus* that belongs to the family Pasteurellaceae.<sup>[10]</sup> Phylogenetic analysis of the three Pasteurellaceae genera; *Actinobacillus*, *Haemophilus*, and *Pasteurella*; based on 16S rRNA gene sequences, revealed that *Actinobacillus actinomycetemcomitans* is closely related to *Haemophilus aphrophilus* and *Haemophilus paraaphrophilus* and that these three species together with *Haemophilus segnis* formed a single phylogenetic cluster. Due to their phylogeny and their typical phenotypic characteristics — the autoaggregation, the species *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraaphrophilus* and *Haemophilus segnis* were recently reclassified to a novel genus "Aggregatibacter" by Nørskov-Lauritsen and Kilian<sup>[11]</sup> [Figure 4].



**Figure 2:** Photomicrograph showing gram-negative rods of *Aggregatibacter actinomycetemcomitans*

### Serotypes

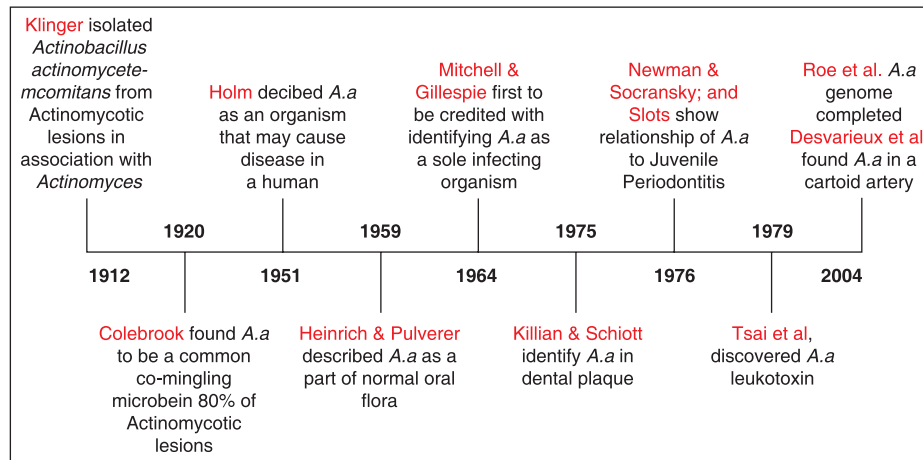
Serological investigations of *Aggregatibacter actinomycetemcomitans* have identified specific serotypes and bacterial antigens which may be important in the etiology of periodontal disease. Up to now seven serotypes have been designated (a through g), leaving 3-8% of clinical isolates non-serotypeable.<sup>[12-14]</sup> Serotypes a, b, and c are most prevalent in the oral cavity. Healthy subjects frequently carry serotype c strains. While periodontally healthy subjects harbor *Aggregatibacter actinomycetemcomitans* serotypes a and b in approximately equal prevalence, serotype b is increased in LJP, implicating the serotype b antigen as particularly important in the pathogenesis of this periodontal disease.<sup>[15]</sup>

### Biofilm characteristics

Bacteria have naturally developed different mechanisms to survive in nutrient scarce environments such as biofilms. For instance, slow-growing *Aggregatibacter actinomycetemcomitans* utilizes lactic acid, and inhibits its glucose uptake. *In vitro* biofilm co-culture studies suggest that *Aggregatibacter actinomycetemcomitans* can avoid competition with fast-growing *Streptococcus gordonii* bacteria by using lactate, a secondary product of the metabolism performed by *Streptococcus gordonii*.<sup>[16]</sup> The presence of H<sub>2</sub>O<sub>2</sub> (another metabolite produced by *Streptococcus gordonii*) in proximity to *Aggregatibacter actinomycetemcomitans* induces the expression of catalase and the serum protein H binding protein ApiA. As a result of this sensing, the *Aggregatibacter actinomycetemcomitans* biofilm become more resistant to toxic H<sub>2</sub>O<sub>2</sub> through the action of catalase, and the biofilm can inhibit serum complement activity by binding to serum protein H. The polymicrobial interaction between these two oral bacteria, thus influences biofilm resistance to innate host immunity.<sup>[17]</sup>

### Virulence factors

*Aggregatibacter actinomycetemcomitans* has been shown to possess a myriad of virulence factors that



**Figure 3:** Important discoveries about *Aggregatibacter actinomycetemcomitans*

Scientific Classification
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pasteurellales
Family: Pasteurellaceae
Genus: <i>Aggregatibacter</i>
Species: <i>actinomycetemcomitans</i>
Binomial name:
<i>Aggregatibacter actinomycetemcomitans</i>

**Figure 4:** Taxonomy of *Aggregatibacter actinomycetemcomitans*

enhance its survival in the oral cavity and enable it to circumvent the host's protective strategies. Virulence factors can be broadly categorized into three groups:

1. Factors promoting the colonization and persistence in the oral cavity:
  - A. Adhesins.
  - B. Invasins.
  - C. Bacteriocins.
  - D. Antibiotic resistance.
2. Factors that interfere with the host's defenses:
  - A. Leukotoxin.
  - B. Lipopolysaccharides (LPSs).
  - C. Chemotactic inhibitors.
  - D. Cytolethal distending toxin (CDT).
  - E. Immunosuppressive proteins.
  - F. Fc-binding proteins.
3. Factors that destroy host tissues:
  - A. Cytotoxins.
  - B. Heat shock proteins (HSPs).
  - C. Collagenase.
  - D. Bone resorption agents.

## Factors promoting the colonization and persistence in the oral cavity

### Adhesins

The bacterial surface components involved in adhesion to a specific substrate are called adhesins. These are proteinaceous structures found on the surface of the bacterial cell which interact and bind to specific receptors in saliva, on the surface of tooth, on extracellular matrix proteins, and on epithelial cells.<sup>[18]</sup>

Cell surface entities mediating adherence include fimbriae, extracellular amorphous material, and extracellular vesicles.

Fimbriae — Also referred to as “attachment pili”.

Location — They are long filamentous polymeric protein structures that can be found projecting from the external surfaces of many gram-negative bacteria.

Structure — *Aggregatibacter actinomycetemcomitans* fimbriae occur in peritrichous arrays, may be more than 2 µm in length and 5 nm in diameter and often occur in bundles.<sup>[19]</sup>

Mechanism of virulence — Fimbriae carry curlin proteins and adhesins which attach them to the substratum so that the bacteria can withstand shear forces and obtain nutrients. Recently, it was reported that the most abundant protein in a fimbria preparation of *Aggregatibacter actinomycetemcomitans* 304 was a protein with a molecular mass of 6.5 kDa termed Flp, exhibits some amino acid sequence similarity to type-IV pilin. Strains possessing fimbriae adhere three- to fourfold better than non-fimbriated variants of *Aggregatibacter actinomycetemcomitans*.<sup>[20]</sup>

### Vesicles

Location — A bleb-like structure which are LPS in nature, originate from and are continuous with the outer membrane.

Structure and composition — Enclosed by lipid bilayer, vesicles can form naturally, for example, during endocytosis.



Alternatively, they may be prepared artificially, in which case they are called liposomes. If there is only one phospholipid bilayer, they are called unilamellar vesicles; otherwise they are called multilamellar.

**Mechanism of virulence** — Vesicles are involved in metabolism, transport, buoyancy control, and enzyme storage. They can also act as chemical reaction chambers.

*Aggregatibacter actinomycetemcomitans* vesicles exhibit leukotoxic activity. Other biologically active components of *Aggregatibacter actinomycetemcomitans* vesicles are endotoxin, bone resorption activity, and a bacteriocin, termed actinobacillin. *Aggregatibacter actinomycetemcomitans* vesicles also exhibit adhesive properties; this observation prompted the hypothesis that vesicles function as delivery vehicles for *Aggregatibacter actinomycetemcomitans* toxic materials.<sup>[21]</sup>

### Extracellular amorphous material

**Location** — Associated with the surface of *Aggregatibacter actinomycetemcomitans* cells is an amorphous material that frequently embeds adjacent cells in a matrix.

**Composition and mechanism of virulence** — The material is most likely a glycoprotein, and has been shown to exhibit both bone-resorbing activity and adhesive properties. *Aggregatibacter actinomycetemcomitans* strains, which normally exhibit low levels of adhesion, exhibit increased levels of adhesion when suspended in extracellular amorphous material, a phenomenon termed conveyed adhesion. A recent report has stated that serotype-specific epitopes of *Aggregatibacter actinomycetemcomitans* are located on the amorphous material of the cell surface.<sup>[22]</sup>

### Invasins

**Location** — Invasion of *Aggregatibacter actinomycetemcomitans* is a rapid mechanism involving the formation of cell-surface “craters” or apertures with lip-like rims. These invasins occur as indentations on the cell surface, as well as in membrane ruffles where they appear to be entering into the epithelial cells.

**Mechanism of virulence** — Invasion is a dynamic process with bacteria appearing in the host cell cytoplasm within 30 min. The cells of *Aggregatibacter actinomycetemcomitans* bind through adhesins, to surface receptors on gingival cells — transferrin receptor. The epithelial cell membrane ruffles and efface and invaginations engulf the bacteria, which then become internalized within a membrane vesicle. Invasion can be actin-dependent or actin-independent process. The bacterial cells destroy the membrane vesicles by secretion of phospholipase C, releasing the bacteria into the cytoplasm where they grow and divide rapidly. Bacteria become localized at membrane protrusions through which

they enter adjoining epithelial cells in a microtubule-dependent process.<sup>[23]</sup>

### Bacteriocins

**Location** — Bacteriocins are [HYPERLINK “http://en.wikipedia.org/wiki/Toxin”](http://en.wikipedia.org/wiki/Toxin) \o “Toxin” toxins produced by [HYPERLINK “http://en.wikipedia.org/wiki/Bacteria”](http://en.wikipedia.org/wiki/Bacteria) \o “Bacteria” bacteria to inhibit the growth of closely related bacterial strains. In *Aggregatibacter actinomycetemcomitans* they are usually associated with both the bacterial cell surface and extracellular vesicles.

**Structure and composition** — Bacteriocins are a heterogeneous group of particles with different morphological and biochemical entities. They range from a simple protein to a high molecular weight complex of proteins.

**Mechanism of virulence** — These toxic agents can confer a colonization advantage for the bacterium by lessening the ecological pressures associated with competition by other organisms for both nutrients and space. Lima et al.,<sup>[24]</sup> isolated a bacteriocin named actinomycetemcomitin from *Aggregatibacter actinomycetemcomitans* P (7-20) strain that is active against *Peptostreptococcus anaerobius* ATCC 27337. Actinobacillin, a bacteriocin that is active against *Streptococcus sanguis*, *Streptococcus uberis*, and *Actinomyces viscosus*, has been identified and purified. Actinobacillin results in alteration in cell permeability of target bacteria causing leakage of DNA, RNA, and other intercellular molecules required for growth. It has been proposed that actinobacillin may be responsible for the clinical observation that a reciprocal relationship occurs between *Aggregatibacter actinomycetemcomitans* and *Streptococcus sanguis* and/or *Actinomyces viscosus* in plaque and in patients with LJP.<sup>[24]</sup>

### Antibiotic resistance

Antibiotic resistance is a form of [HYPERLINK “http://en.wikipedia.org/wiki/Drug\\_resistance”](http://en.wikipedia.org/wiki/Drug_resistance) \o “Drug resistance” drug resistance whereby some subpopulations of a [HYPERLINK “http://en.wikipedia.org/wiki/Microorganism”](http://en.wikipedia.org/wiki/Microorganism) \o “Microorganism” microorganism, are able to survive after exposure to one or more [HYPERLINK “http://en.wikipedia.org/wiki/Antibiotic”](http://en.wikipedia.org/wiki/Antibiotic) \o “Antibiotic” antibiotics.

**Location** — Poor permeability of the outer membrane is responsible for the antimicrobial resistance in gram-negative organisms.<sup>[25]</sup>

**Mechanism of virulence** — Approximately 30% of oral *Aggregatibacter actinomycetemcomitans* are resistant to benzylpenicillin. New or altered penicillin-binding proteins on the bacterial cell surface may account for the nonenzymatic penicillin resistance of *Aggregatibacter actinomycetemcomitans*, as has been observed among strains of *Haemophilus influenza*.

Tetracyclines, as an adjunct to mechanical debridement, are antibiotics frequently employed in treating LJP. In a recent study, 82% of 19 clinical isolates of *Aggregatibacter actinomycetemcomitans* were resistant to tetracyclines and carried the tetB resistance determinant. Moreover, the tetB determinant was capable of being transferred by conjugation to other *Aggregatibacter actinomycetemcomitans* strains and to *Haemophilus influenza*. These data suggest that antibiotic resistance in *Aggregatibacter actinomycetemcomitans* is on the rise and likely to be responsible for treatment failures in the future.<sup>[26]</sup>

## Factors that interfere with the host defenses

### Leukotoxin

One of the most studied virulence factors of *Aggregatibacter actinomycetemcomitans* is leukotoxin. This toxin is a 116 kDa protein produced by 56% of strains isolated from LJP patients.

**Location** — It is a proteinaceous toxin secreted from the cell membrane of *Aggregatibacter actinomycetemcomitans*.

**Structure and composition** — Leukotoxin is a member of the RTX family of toxins that produce pore-forming hemolysins. The leukotoxin operon consists of four coding genes designated *ltxC*, *ltxA*, *ltxB*, and *ltxD* and an upstream promoter gene.

*ltxA* encodes the structure of the toxin.

*ltxC* encodes for components required for posttranslational acylation of the toxin.

*ltxB* and *ltxD* encodes for transport of the toxin to the bacterial outer membrane.

Leukotoxin consists of 1,055 amino acids encoded by the leukotoxin gene in the leukotoxin operon.<sup>[27]</sup>

*Aggregatibacter actinomycetemcomitans* strains that produce high levels of leukotoxin appear to be a clonal type (JP2 phenotype) and are associated with aggressive forms of periodontitis in those of Afro-Caribbean descent.<sup>[28]</sup>

**Mechanism of virulence** — Leukotoxin is not only species-specific but also cell-specific. The toxin binds to neutrophils, monocytes, and a subset of lymphocytes; and forms pores in the membranes of these target cells overwhelming their ability to sustain osmotic homeostasis, resulting in cell death.

**Interaction with polymorphonuclear leukocytes (PMNs)** — Leukotoxin has shown to efficiently cause death of human PMNs through extracellular release of proteolytic enzymes from both primary and secondary granules, along with activation and release of matrix metalloproteinase-8, which can contribute to periodontal tissue destruction.

**Interaction with lymphocytes** — The ability of leukotoxin to induce apoptosis in lymphocytes might impair the acquired immune response of periodontal infections. A shift in the balance between Th-1 and Th-2 subsets of T-cells is found in periodontal inflammation, with the Th-2 cells to associate with chronic periodontitis. Its ability to affect the lymphocytes also indicates a possible role of this molecule in Th-1/Th-2/Th-17 differentiation, important in inflammatory pathogenesis.

**Interaction with monocytes/macrophages** — Leukotoxin causes the activation of caspase-1, which is a cytosolic cysteine proteinase that specifically induces activation and secretion of the proinflammatory cytokines interleukin-1 $\beta$  and 18, which result in monocyte/macrophage lysis by incorporation in a cytosolic multimer complex named the inflammasome [Figure 5].

### LPS

LPSs are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond.

**Location** — They are found in the outer membrane of gram-negative bacteria, act as endotoxins, and elicit strong immune responses in animals.

**Structure and composition** — It comprises three parts:

1. O antigen
2. Core oligosaccharide
3. Lipid A

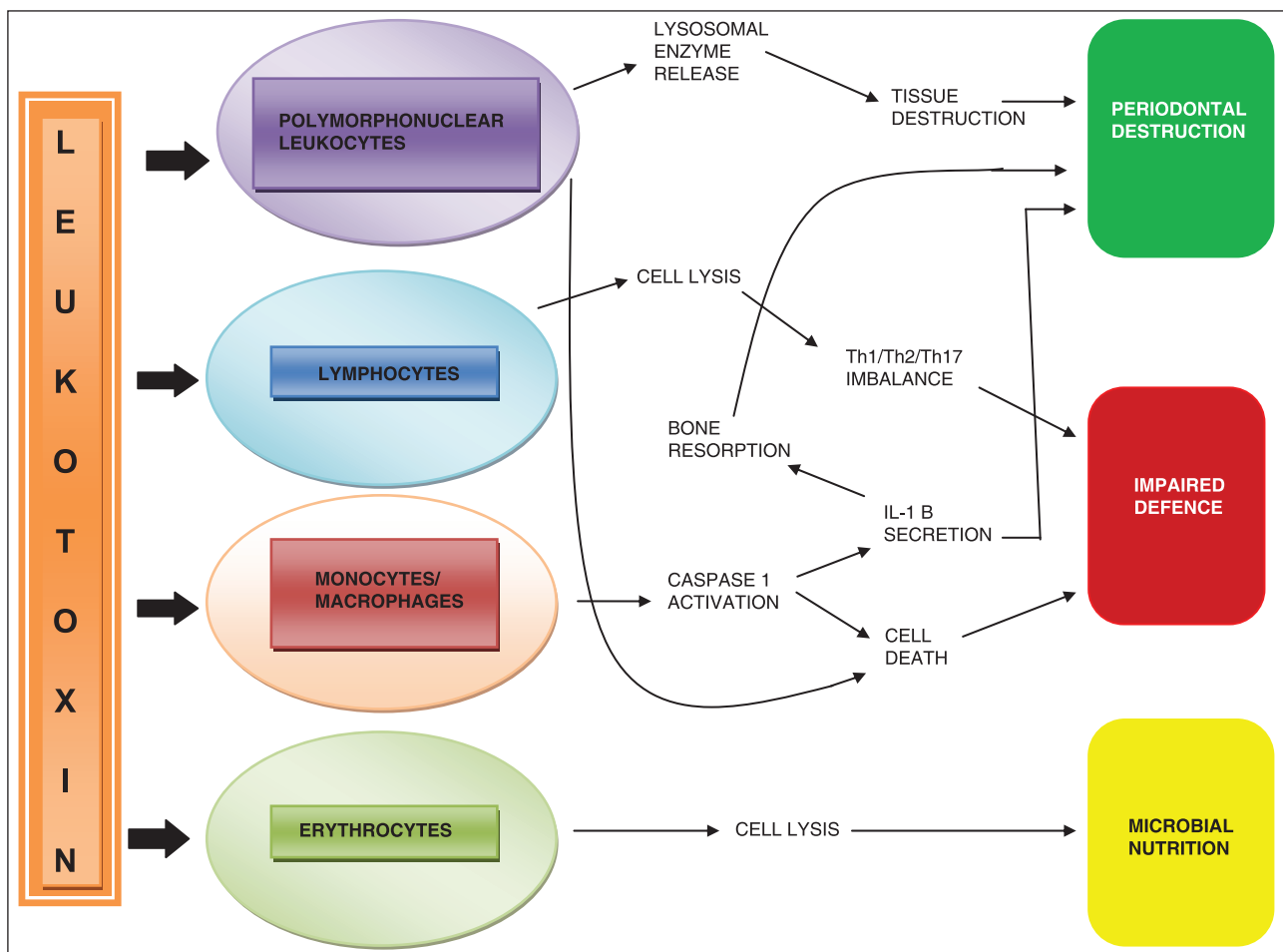
**O antigen** — A repetitive [HYPERLINK “http://en.wikipedia.org/wiki/Glycan”](http://en.wikipedia.org/wiki/Glycan) \o “Glycan” [glycan HYPERLINK “http://en.wikipedia.org/wiki/Polymer”](http://en.wikipedia.org/wiki/Polymer) \o “Polymer” polymer is attached to the core oligosaccharide, comprising the outermost domain of the LPS molecule.

**Mechanism of virulence** — O antigen is the basis of antigenic variation among many gram-negative pathogens which guarantees the existence of multiple serotypes.

**Core oligosaccharide** — The core domain contains an oligosaccharide component that attaches directly to [HYPERLINK “http://en.wikipedia.org/wiki/Lipid\\_A”](http://en.wikipedia.org/wiki/Lipid_A) \o “Lipid A” lipid A and commonly contains [HYPERLINK “http://en.wikipedia.org/wiki/Sugar”](http://en.wikipedia.org/wiki/Sugar) \o “Sugar” sugars.

**Mechanism of virulence** — It can allow organisms to adhere to epithelial tissues and provide protection from damaging reactions with antibody and complements.

**Lipid A** — It is a [HYPERLINK “http://en.wikipedia.org/wiki/Phosphorylated”](http://en.wikipedia.org/wiki/Phosphorylated) \o “Phosphorylated” phosphorylated [HYPERLINK “http://en.wikipedia.org/wiki/Glucosamine”](http://en.wikipedia.org/wiki/Glucosamine) \o “Glucosamine” glucosamine [HYPERLINK “http://en.wikipedia.org/wiki/Disaccharide”](http://en.wikipedia.org/wiki/Disaccharide) \o “Disaccharide” disaccharide



**Figure 5:** Effect of *Aggregatibacter actinomycetemcomitans* leukotoxin on human blood cells causing periodontal inflammation and tissue destruction

decorated with multiple HYPERLINK "[http://en.wikipedia.org/wiki/Fatty\\_acids](http://en.wikipedia.org/wiki/Fatty_acids)" \o "Fatty acids" fatty acids.

**Mechanism of virulence** — It exerts its toxic effects when released from multiplying cells, or when the bacteria are lysed. In monocytes and macrophages it results in production of interleukin-1, 6, and 8, tumor necrosis factor- $\alpha$ , and platelet-activating factor (PAF); activation of the complement; and coagulation cascade.<sup>[29]</sup>

LPS may also contribute to destruction of periodontal connective tissue by activating the pathways that lead to stimulation of matrix metalloproteinases and plasminogen activator. Recently, *Aggregatibacter actinomycetemcomitans* LPS has shown to induce foam cell formation and cholesteryl ester accumulation in murine macrophages which suggests that it also has proatherogenic activity.<sup>[30]</sup>

#### Chemotactic inhibitor

**Mechanism of virulence** — Disruption or inhibition of neutrophil chemotaxis is advantageous for *Aggregatibacter actinomycetemcomitans*. Van Dyke *et al.*,<sup>[17]</sup> conducted a study to examine the ability of major bacterial species to inhibit

peripheral blood neutrophil chemotaxis. *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*, which were not chemotactic by themselves, inhibited binding of chemotactic peptide suggesting that *in vitro* chemotaxis inhibition was mediated by nonchemotactic components that compete for the chemotactic factor receptor on the neutrophil.<sup>[31]</sup>

#### CDT

**Location** — CDT is a cell cycle-modulatory protein with immunosuppressive function. The toxin is either secreted freely or associated with the membrane of the producing bacteria.

**Structure and composition** — CDT is a tripartite structure encoded by a locus of three genes, *cdtABC*. The toxin itself is encoded by *cdtB*, while *cdtA* and *cdtC* appear to encode proteins that mediate interaction between the CDT complex and the host cell surface.

**Mechanism of virulence** — The active subunit, *cdtB*, exhibits DNase I activity. While the role of *cdtA* and *cdtC* is less clear, both proteins possess putative mucin-like carbohydrate-

binding domains that predict interaction with the host cell surface. CdtB is transported into the nucleus where it causes DNA damage through its DNase activity resulting in apoptosis, through caspase activation. *Aggregatibacter actinomycetemcomitans* Cdt (AaCdt) disrupts macrophage function by inhibiting phagocytic activity as well as affecting the production of interleukins 1- $\beta$ , 6, and 8.<sup>[32,33]</sup> It was found that AaCdt is largely responsible for the inhibition of proliferation of human periodontal ligament cells and gingival fibroblasts. In human gingival fibroblasts, AaCdt is able to stimulate the production of receptor activator of nuclear factor- $\kappa$ B ligand which may be involved in pathological bone resorption, characteristic of LAP.<sup>[34]</sup>

### Immunosuppressive factors

**Mechanism of virulence**—*Aggregatibacter actinomycetemcomitans* has been shown to elaborate many factors capable of suppressing the host defense mechanisms. A 60-kDa protein secreted by *Aggregatibacter actinomycetemcomitans* has been purified and shown to inhibit IgG and IgM synthesis by human lymphocytes. It is believed that it affects immunoglobulin production by activating B cells that downregulate the ability of B and T cells to respond to mitogens. In addition, leukotoxin impairs the ability of lymphocytes to respond to mitogens by inhibiting DNA, RNA, protein, IgG, and IgM synthesis.<sup>[35]</sup>

### Fc binding proteins

**Location** — Fc binding proteins are found to be associated with the bacterial cell surface and are released in soluble form during bacterial growth.

**Structure** — One of the protein sequence exhibits significant homology with the N termini of outer membrane protein A (OmpA) of *Escherichia coli* and related OmpA-like proteins from other gram-negative bacteria.

**Mechanism of virulence** — Fc region of an antibody molecule is important in the binding of the antibody to specific receptors on PMNs. If other proteins compete for binding to this region of PMNs, binding of the antibody may be inhibited and, thereby, inhibit phagocytosis. Tolo and Hegland demonstrated that molecules on the surface of *Aggregatibacter actinomycetemcomitans* that are associated with capsular material and secreted into the medium bind to the Fc portion of IgG; the binding inhibits the ability of opsonizing antibodies to bind PMNs and reduces phagocytosis by 90%. It is believed that the Fc receptors also play a role in complement activation.<sup>[36]</sup>

### Factors that destroy host tissues

#### Cytotoxins

**Location** — *Aggregatibacter actinomycetemcomitans* surface associated material produces heat-labile cytotoxins which inhibit human fibroblast proliferation.

**Mechanism of virulence** — One toxin that is secreted into the supernatant has been isolated and identified as a 50-kDa protein that inhibits DNA synthesis in the fibroblast. Another surface-associated material cytotoxin, designated Gapstein, is an 8-kDa protein. The inhibition of fibroblast growth may be expressed as a decrease in collagen synthesis which is manifested as a loss of collagen in certain forms of juvenile periodontitis.<sup>[37]</sup>

#### HSPs

**Location** — Certain HSPs have been found in the surface-associated material that are molecular [HYPERLINK “http://en.wikipedia.org/wiki/Chaperone\\_\(protein\)”](http://en.wikipedia.org/wiki/Chaperone_(protein)) \o “Chaperone (protein)” chaperones and play a critical role in protein folding, intracellular trafficking of proteins, and coping with proteins denatured by heat and other stresses.

**Mechanism of virulence** — *Aggregatibacter actinomycetemcomitans* strains have shown the presence of HSPs, including GroEL-like (HSP60) and DnaK-like (HSP70) proteins. Protein homologous to GroEL-like HSP is osteolytic. Purified native GroEL-like HSP from *Aggregatibacter actinomycetemcomitans* promotes epithelial cell proliferation at lower HSP concentrations, but has a toxic effect on epithelial cells at higher HSP concentrations.<sup>[38]</sup>

#### Collagenase

**Mechanism of virulence** — Collagenases are zinc endopeptidases/extracellular proteolytic enzymes secreted by bacteria that digest nearly all collagen fibers in their insoluble triple helical form. Collagenase activity is associated with two important periodontal pathogens, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, which results in reduction in collagen density induced in periodontal disease.<sup>[39]</sup>

#### Bone resorption agents

**Mechanism of virulence** — There is evidence that components of *Aggregatibacter actinomycetemcomitans* like LPSs, proteolysis-sensitive factor in microvesicles, surface associated materials, etc., inhibit osteoblast proliferation and synthetic activity. They cause activation of bone resorption and induction of osteoclast proliferation. *Aggregatibacter actinomycetemcomitans* LPS, is a very effective bone resorption mediator, has been shown to cause the release of calcium from fetal long bones in the  $^{45}\text{Ca}^{2+}$  fetal bone resorption assay.<sup>[40]</sup>

Clonal variations in the virulence factors of *Aggregatibacter actinomycetemcomitans* — O-antigenic part of LPS differs between serotypes, but differences among the biological functions is not known. Serotype b is associated with high production of leukotoxin; whereas, serotype c is associated with decreased production of CDT.<sup>[41]</sup>



## Therapeutic modalities

Slots and Ting<sup>[2]</sup> had reviewed the effectiveness of various therapies to suppress or remove subgingival *Aggregatibacter actinomycetemcomitans*. Scaling and root planing alone was unable to remove *Aggregatibacter actinomycetemcomitans* from LJP lesions and periodontal surgery also often fails to control subgingival *Aggregatibacter actinomycetemcomitans* effectively. Modified Widman flap surgery may suppress *Aggregatibacter actinomycetemcomitans* to below detectable levels in about 50% of LJP lesions and may be even less effective in adult periodontitis lesions.<sup>[42]</sup> Some studies have however indicated that apically positioned flap surgery or gingivectomy is capable of controlling subgingival *Aggregatibacter actinomycetemcomitans*. Tetracycline has been used for periodontal infections, but lacks bactericidal activity for most strains and the clinical response has been variable. Systemic use of amoxicillin and metronidazole has been effective in treating *Aggregatibacter actinomycetemcomitans*-associated LJP and adult periodontitis and 8 days therapy with 250 mg tid (tds) of amoxicillin and metronidazole is the current recommended (adult dosage) therapy. Azithromycin and clarithromycin have been shown to be more active *in vitro*, than Erythromycin and synergy has been demonstrated with either  $\beta$ -lactams or ciprofloxacin in combination with metronidazole or its metabolite. Because of their excellent bactericidal activity against *Aggregatibacter actinomycetemcomitans*, fluoroquinolones are likely to find a role in the treatment of periodontitis, albeit in combination with antibiotics having activity against anaerobes.<sup>[43]</sup> More recently local drug delivery and host modulation with subantimicrobial doses of doxycycline are under research for the treatment of LJP.

Vaccination — Development of vaccine against this pathogen has also been tried using its different antigens. A synthetic oligopeptide was prepared based on the amino acid sequence of *Aggregatibacter actinomycetemcomitans* fimbriae which was found to be effective in a rabbit model, ensuring inhibition of adhesion and its subsequent colonization.<sup>[44]</sup> Apart from this, subcutaneous and intranasal immunization of mice with capsular serotype b-specific polysaccharide antigen (SPA) has given positive results.<sup>[45]</sup> Mice immunized with antisurface associated material from *Aggregatibacter actinomycetemcomitans* exhibited a rise in protective antibody levels acting as an opsonin. These modalities of immunization have not been able to be incorporated as a sole or complete 'vaccine' against periodontal disease for use in the human population as yet, due to the multifactorial and polymicrobial nature of periodontal disease, which requires a sophisticated vaccine design regimen targeting multiple pathogenic species for which extensive research is underway.<sup>[46]</sup>

## CONCLUSION

There may be as many as 300-400 different bacterial species which can inhabit the human oral cavity. It is difficult to pinpoint a single of these species as the etiologic agent in a specific periodontal disease, especially since it may not be possible to fulfill the classic criteria for a bacterial pathogen as formulated by Koch. However, data originating from the microbiologic, immunologic, histopathologic studies, etc., indicate that *Aggregatibacter actinomycetemcomitans* is important in the etiology of LJP. *Aggregatibacter actinomycetemcomitans* is a bacterium with an array of diverse potential virulence characteristics, including multiple immune evasion mechanisms, any one of which may play a crucial role in the local tissue pathology of LAP. Our understanding of this organism still lags behind that of enteric pathogens, largely because methods for genetic manipulation have only just become available. We live in exciting times, where the rapid development of high-throughput technologies such as parallel DNA sequencing, proteomics, metabolomics, transcriptomics, etc., through which the discovery and exploration of the molecular genomics of *Aggregatibacter actinomycetemcomitans* is being carried out in order to provide more insight into the evolution of the association between the bacteria and their hosts, so as to aid in the prevention and treatment of destructive periodontal disease.

## REFERENCES

- Henderson B, Ward JM, Ready D. *Aggregatibacter (Actinobacillus) actinomycetemcomitans*: A triple A\* periodontopathogen. *Periodontol* 2000 2010;54:78-105.
- Slots J, Ting M. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: Occurrence and treatment. *Periodontol* 2000 1999;20:82-121.
- Klinger R. Studies on human actinomycosis *Centralblatt Bacteriol* 1912;62:191-200.
- Lieske R. Morphology and Biology of the Strahlenpilze. Leipzig, GDR: Borntraeger 1921;1:415.
- Topley WW, Wilson GS. The principles of bacteriology and immunity. London: Edward Arnold and Co. 1929;1:1-587.
- Fine DH, Kaplan KB, Kachlany SC, Schreiner HC. How we got attached to *Actinobacillus actinomycetemcomitans*: A model for infectious diseases. *Periodontol* 2000 2006;42:114-57.
- Kilian M, Schiott C. Haemophila and related species in the human oral cavity. *Arch Oral Biol* 1975;20:791-6.
- Newman MG, Socransky SS, Savitt ED, Propas DA, Crawford A. Studies of the microbiology of periodontosis. *J Periodontol* 1976;47:373-9.
- Slots J. The predominant cultivable organisms in juvenile periodontitis. *Scand J Dent Res* 1976;84:1-10.
- Olsen I, Shah HN, Gharbia SE. Taxonomy and biochemical characteristics of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Periodontol* 2000 1999;20:14-52.
- Norskov-Lauritsen, Kilian M. Reclassification of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter*



- aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor - dependant and V - factor independent isolates. *Int J Syst Evol Microbiol* 2006;56:2135-46.
12. Zambon JJ, Slots J, Genco RJ. Serology of oral *Actinobacillus actinomycetemcomitans* and serotype distribution in human periodontal disease. *Infect Immun* 1983;41:19-27.
  13. Saarela M, Asikainen S, Alaluusua S, Pyhälä L, Lai CH, Jousimies-Somer H. Frequency and stability of mono- or poly-infection by *Actinobacillus actinomycetemcomitans* serotypes a, b, c, d or e. *Oral Microbiol Immunol* 1992;7:277-9.
  14. Kaplan JB, Perry MB, MacLean LL, Furgang D, Wilson ME, Fine DH. Structural and genetic analyses of O polysaccharide from *Actinobacillus actinomycetemcomitans* serotype f. *Infect Immun* 2001;69:5375-84.
  15. Takada K, Saito M, Tsuzukibashi O, Kawashima Y, Ishida S, Hirasawa M. Characterization of a new serotype g isolate of *Aggregatibacter actinomycetemcomitans*. *Mol Oral Microbiol* 2010;25:200-6.
  16. Brown SA, Whiteley M. A novel exclusion mechanism for carbon resource partitioning in *Aggregatibacter actinomycetemcomitans*. *J Bacteriol* 2007;189:6407-14.
  17. Ramsey MM, Whiteley M. Polymicrobial interactions stimulate resistance to host innate immunity through metabolite perception. *Proc Natl Acad Sci U S A* 2009;106:1578-83.
  18. Meyer DH, Fives-Taylor PM. Characteristics of adherence of *Actinobacillus actinomycetemcomitans* to epithelial cells. *Infect Immun* 1994;62:928-35.
  19. Rosan B, Slots J, Lamont RJ, Listgarten MA, Nelson GM. *Actinobacillus actinomycetemcomitans* fimbriae. *Oral Microbiol Immunol* 1988;3:58-63.
  20. Inouye T, Tanimoto I, Ohta H, Kato K, Murayama Y, Fukui K. Molecular characterization of low-molecular-weight component protein, Flp, in *Actinobacillus actinomycetemcomitans* fimbriae. *Microbiol Immunol* 1998;42:253-8.
  21. Nowotny A, Behling UH, Hammond B, Lai CH, Listgarten M, Pham PH, et al. Release of toxic microvesicles by *Actinobacillus actinomycetemcomitans*. *Infect Immun* 1982;37:151-4.
  22. Fives-Taylor P, Meyer D, Mintz K. Characteristics of *Actinobacillus actinomycetemcomitans* invasion of and adhesion to cultured epithelial cells. *Adv Dent Res* 1995;9:55-62.
  23. Lamont R, Yilmaz O. In or out: The invasiveness of oral bacteria. *Periodontol* 2000 2002;30:61-9.
  24. Stevens RH, Lillard SE, Hammond BF. Purification and biochemical properties of a bacteriocin from *Actinobacillus actinomycetemcomitans*. *Infect Immun* 1987;55:692-7.
  25. Walker CB, Pappas JD, Tyler KZ, Cohen S, Gordon JM. Antibiotic susceptibilities of periodontal bacteria. *In vitro* susceptibilities to eight antimicrobial agents. *J Periodontol* 1985;56:s67-74.
  26. Zambon JJ, DeLuca C, Slots J, Genco RJ. Studies of leukotoxin from *Actinobacillus actinomycetemcomitans* using the promyelocytic RL-60 cell line. *Infect Immun* 1983;40:205-12.
  27. Schreiner H, Li Y, Cline J, Tsiagbe VK, Fine DH. A Comparison of *Aggregatibacter actinomycetemcomitans* (Aa) virulence traits in a rat model for periodontal disease. *PLoS One* 2013;8:e69382.
  28. Johansson A. *Aggregatibacter actinomycetemcomitans* Leukotoxin: A powerful tool with capacity to cause imbalance in the host inflammatory response. *Toxins (Basel)* 2011;3:242-59.
  29. Todar K. Todar's online textbook of bacteriology. Available from: <http://www.textbookofbacteriology.net/17/11/2014>.
  30. Saglie FR, Simon K, Merrill J, Koeffler HP. Lipopolysaccharide from *Actinobacillus actinomycetemcomitans* stimulates macrophages to produce interleukin - 1 and tumor necrosis factor mRNA and protein. *Oral Microbiol Immunol* 1990;5:256-62.
  31. Van Dyke TE, Bartholomew E, Genco RJ, Slots J, Levine MJ. Inhibition of neutrophil chemotaxis by soluble bacterial products. *J Periodontol* 1982;53:502-8.
  32. Ando-Sugimoto ES, da Silva MP, Kawamoto D, Chen C, DiRienzo JM, Mayer MP. The cytolethal distending toxin of *Aggregatibacter actinomycetemcomitans* inhibits macrophage phagocytosis and subverts cytokine production. *Cytokine* 2014;66:46-53.
  33. DiRienzo JM. Breaking the gingival epithelial barrier: Role of the *Aggregatibacter actinomycetemcomitans* cytolethal distending toxin in oral infectious disease. *Cells* 2014;3:476-99.
  34. Saiki K, Gomi T, Konishi K. Deletion and purification studies to elucidate the structure of the *Actinobacillus actinomycetemcomitans* cytolethal distending toxin. *J Biochem* 2004;136:335-42.
  35. Rabie G, Lally ET, Shenker BJ. Immunosuppressive properties of *Actinobacillus actinomycetemcomitans* leukotoxin. *Infect Immun* 1988;56:122-7.
  36. Letzelter C, Croue F, Pianezzi B, Roques C, Soleilhavou JP. Supernatant cytotoxicity and proteolytic activity of selected oral bacteria against human gingival fibroblasts *in vitro*. *Arch Oral Biol* 1998;43:15-23.
  37. Mayer MP, Bueno LC, DiRienzo JM. Cytolethal distending toxin (CDT) of *Actinobacillus actinomycetemcomitans*. *J Dent Res* 1998;77:6-80.
  38. Schett G, Metzler B, Kleindienst R, Moschen I, Hattmannsdorfer R, Wolf H, et al. Salivary anti-hsp65 antibodies as a diagnostic marker for gingivitis and a possible link to atherosclerosis. *Int Arch Allergy Immunol* 1997;114:246-50.
  39. Lawson DA, Meyer TF. Biochemical characterization of *Porphyromonas (Bacteroides) gingivalis* collagenase. *Infect Immun* 1992;60:1524-9.
  40. Fives-Taylor PM, Meyer DH, Mintz KP, Brissette C. Virulence factors of *Actinobacillus actinomycetemcomitans*: *Periodontol* 2000 1999;20:136-67.
  41. Jain R, Mittal K, Kapoor S. Virulence factors of *Aggregatibacter actinomycetemcomitans* - A review. *J Pharm Biomed Sci* 2013;34:1693-8.
  42. Nieminen A, Asikainen S, Torkko H, Kari K, Uitto VJ, Saxen L. Value of some laboratory and clinical measurements in the treatment plan for advanced periodontitis. *J Clin Periodontol* 1996;23:572-81.
  43. Pavieic MJ, van Winkelhoff AJ, Pavieit-Ternming A, de Graaff J. Metronidazole susceptibility factors in *Actinobacillus actinomycetemcomitans*. *J Antimicrob Chemother* 1995;35:263-9.
  44. Harano K, Yamanaka A, Okuda K. An antiserum to a synthetic fimbrial peptide of *Actinobacillus actinomycetemcomitans* blocked adhesion of the microorganism. *FEMS Microbiol Lett* 1995;130:279-85.
  45. Takamatsu MN, Yamaguchi N, Kawasaki M, Yamashita Y, Takehara T, Koga T. Immunogenicity of *Actinobacillus actinomycetemcomitans* serotype b-specific polysaccharide-protein conjugate. *Oral Microbiol Immunol* 1996;11:220-5.
  46. Hermijnajeng E, Asmara W, Yuswanto A, Barid I, Sosroseno W. Protective humoral immunity induced by surface-associated material from *Actinobacillus actinomycetemcomitans* in mice. *Microbes Infect* 2001;3:997-1003.

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