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Review Article

Dentistry Section

Aggregatibacter Actinomycetemcomitans – A Tooth Killer?

MANOJ RAJA[1], FAJAR UMMER[2], C.P DHIVAKAR[3]

ABSTRACT

Strong evidence is available on *Aggregatibacter actinomycetemcomitans* (A.a) on its role as the causative agent of localised juvenile periodontitis (LJP), a disease characterised by rapid destruction of the tooth-supporting tissues. This organism possesses a large number of virulence factors with a wide range of activities which enable it to colonise the oral cavity, invade periodontal tissues, evade host defences, initiate connective tissue destruction and interfere with tissue repair. Adhesion to epithelial and tooth surfaces is dependent on the presence of surface proteins and structures such as microvesicles and fimbriae. Invasion has been demonstrated in vivo and in vitro. The organism has a number of means of evading host defences which include: (i) production of leukotoxin; (ii) producing immunosuppressive factors; (iv) secreting proteases capable of cleaving IgG; and (v) producing Fc-binding.

Keywords: Periodontitis, Leukotoxin, Lipopolysaccaride

INTRODUCTION

Periodontal disease results from the elaboration of noxious products by the entire plaque flora. Thus states the Non Specific Plaque Hypothesis which was proposed by Walter Loesche (1976). Later it was proposed in Specific Plaque Hypothesis, only certain plaque is pathogenic and its pathogenicity depends on presence of or increases in specific organisms. This was spurred by the recognition of *Aggegatibacter actinomycetemcomitans* as a pathogen in localized aggressive periodontitis.

Aggregatibacter actinomycetemcomitans (A.a) is an exogenous bacterium which causes true infections, transmissible among exposed individuals. It is associated with periodontitis in young individuals. It occurs in 90% of localised aggressive periodontitis and 30-50% in severe adult periodontitis. It has the ability to produce virulence factors.

This short review will introduce the reader to *Aggregatibacter actinomycetemcomitans*, an oral commensal which is also an opportunist pathogen with emphasis on its surprising but potential range of virulence factors and virulence mechanisms.

HISTORY

Bacterium *actinomycetem comitans* was described by Klinger as coccobacillary bacteria isolated together with *Actinomyces* from actinomycotic lesions of man [1]. It was reclassified as *Actinobacillus actinomycetemcomitans* by Topley & Wilson and as *Haemophilus actinomycetemcomitans* by Potts et al., [2,3]. Recent studies in 2006 involving multilocus sequence analysis by Nørskov-Lauritsen N and Kilian M have shown a phylogenetic similarity of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus* and *Haemophilus segnis*. This resulted in the addition of the genus *aggregatibacter* (aggregate, to come together; bacter, bacterial rod; *aggregatibacter*, rod shaped bacterium that aggregates with others), to the family *pasteurellaceae* in 2006 to cover gram negative, non motile, facultatively anaerobic rods or coccobacilli that were previously known as *haemophilus* (actinobacillus or bacterium) *actinomycetemcomitans* (now *aggregatibacter actinomycetemcomitans*), *haemophilus aphrophilus* and hemophilus paraphrophilus (collectively known as *aggregatibacter* aphrophilus) and *hemophilus segnis*(now *aggregatibacter* segnis) [Table/Fig-1].

Aggregatibacter actinomycetemcomitans	
Kingdom	Bacteria
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	Pasteurellales
Family	*Pasteurellaceae*
Genus	*Aggregatibacter*
Species	*Actinomycetemcomitans*

[Table/Fig-1]: *Aggregatibacter actinomycetemcomitan* scientific classification [4]

TAXONOMY OF (Aa)

The genus *aggregatibacter* is taxonomically in the family *pasteurellaceae*, order pasteurellales, class *gammaproteobacteria*, *phylum proteobacteria*.

Five sero groups of *Aggregatibacter actinomycetemcomitans* were classified by Taichman based on surface polysaccharide located on the O-side chain of lipopolysaccharide using tube agglutination studies [5].

Serotypes a, b and c are most prevalent in the oral cavity. A particular clone of serotype b with enhanced leukotoxic activity is predominantly associated with cases of localized aggressive perioodontitis [4]. Serotype c is found in healthy subjects [5] [Table/Fig-2].

MORPHOLOGICAL, BIOCHEMICAL AND GROWTH CHARACTERISTICS

Aggregatibacter actinomycetemcomitans (actis, a ray; myces , a fungus; comitans, accompanying; *actinomycetemcomitans*, accompanying an actinomycete) is a gram negative coccobacillus measuring about 0.4 ± 0.1X 0.1 ± 0.4 micrometers in size [4] [Table/Fig-3].

SURFACE ULTRASTRUCTURE

Aggregatibacter actinomycetemcomitans possess fimbriae, vesicles and extracellular amorphous materials.

FIMBRIAE

They are small filamentous cell surface appendages. They occur in a peritrichous array, measuring more than 2 *micrometers* in

Serotype	Surface polysaccharide
Serotype -a	consists of repeating dissacharide units of 0-acetyl-6-deoxy-D-talose and 6-deoxy –D-talose
Serotype -b	repeating trisaccharide units of L-rhamnase, D-fucase and N-acetyl-D-galactosamine.
Serotype – c	– repeating 0-acetyl-6-deoxy-L- talose and 6-deoxy – L – talose
Serotype – d	repeating tetrasaccharide units of D-glucose, D-mannose. And L-rhamnose
Serotype – e	2-acetamide-2-deoxy-D glycose and L-rhamnose.
Serotype – f	2-acetamide – 2-deoxy – D – galactose and L-rhamnose.
Serotype – f	2-acetamide – 2-deoxy – D – galactose and L-rhamnose.

[Table/Fig-2]: Serotypes of *Aggegatibacter actinomycetemcomitans* based on surface polysaccharide [5]

Mesophilic and capnophilic requiring 5-10% carbon dioxide for good growth.
Microaerophilic and a facultative anaerobe.
Colonies on chocolate agar are small, with a diameter of 0.5mm after 24hr, but may exceed 1-2 mm after 48h.
On primary isolation, the colonies are rough textured and adherent and have an internal, opaque pattern described as star like or like crossed cigars.
X and V factors are not required.
Capable of producing acid from glucose, fructose and maltose but not from arabinose, cellobiose,melibiose,melizitose, salicin and sorbitol.
Reduce nitrate and produce alkaline phosphatise but are negative for indole, urease, ornithine and lysine decarboxylases and arginine dihydrolase.
Oxidase negative or weakly positive and catalase positive

[Table/Fig-3]: Biochemical Properties And Growth Characteristics [4]

diameter and in bundles [6]. It forms two types of colonies; colonies with star shaped interior (star positive) or nonfimbriated strains (star negative). The most abundant protein in the fimbriae is 304-a with molecular mass 6.5k Da.

VESICLES

A.actinomycetemcomitans has numerous vesicles or blebs which are lipopolysaccharide in nature. Highly leukotoxic strains tend to have more vesicles. These vesicles contain endotoxin which has a bone resorption activitiy and a bacteriocin termed actinobacillin. These vesicles also exhibit adhesive properties and function as delivery vehicles for toxic materials [6].

A.A - CASUAL OR CAUSAL TO PERODONTAL DISEASE?

ROBERT KOCH'S POSTULATES(1884) states that for an organism to produce a disease, it must (i) occur only in that disease, (ii) be able to grow in pure culture, (iii) be recovered from the host. *Aggregatibacter actinomycetemcomitans* did not meet these needs as it did not exist in isolation in the disease.

However, it met the criteria of a potential pathogen proposed by Sigmund Socransky which states that the pathogen must, (i) be associated with a disease by increase in number of organisms at sites, (ii) be eliminated in sites after treatment and (iii) demonstrate virulence factors to cause periodontal tissue destruction [7].

LOCALIZED JUVENILE PERIODONTITIS (LJP)

LJP is genetic or hereditary with a rapid destruction of tissues around incisors and first molars. Serotype b strains of *A.actinomycetemcomitans* are found associated with this disease [8]. Large numbers (97%) are isolated from LJP lesions whereas from healthy sites it is low. Large numbers found in periodontal pockets are related to low immune response in the host. A wide range of virulence factors are produced by the organisms. Rapid bone loss is the hallmark of the disease. Young adults (25-30 years) are affected. *A.actinomycetemcomitans* is isolated either singly or in combination with other bacteria [9].

EXTRA ORAL INFECTIONS

Serious extra oral infections are caused by *A.actinomycetemcomitans* due to its ability to reduce oxygen in the tissues. Brain meninges, septicemia, UTI, osteomyelitis, endocarditis and abscesses are common [10].

A. actinomycetemcomitans mediated endocarditis is a chronic syndrome with fever, chills, anorexia, weight loss, heart murmur and night sweats. *Aggregatibacter actinomycetemcomitans* expresses a Human Shock Protein like molecule, HSP 60 that cross reacts with antibodies to human HSP 60 [11].

CULTURE MEDIUM

MGB (trypticase soy broth) with malachite green and bacitracin was the earliest media used to culture (A.a). It was then followed by medium with trypticase soy agar, serum with bacitracin and vancomycin (TSBV). Exclusive growth of A.a was found in a particular culture medium which contained TSBV, spiramycin, fucidic acid and carbencillin. RPMI – 1640 and Dulbecco's modified Eagle medium are used now with a generation time of 246 and 346 min [12].

VIRULENCE FACTORS

The putative virulence factors of *A. actinomycetemcomitans* can be subdivided into those that: (i) modulate inflammation, (ii) induce tissue destruction and (iii) inhibit tissue repair.

a. Virulence factors which modulate immune system

A. actinomycetemcomitans appears to employ multiple products to inactivate or evade immune defences. The most actively studied gene product of the organism is a leukotoxin and a member of the RTX (repeats in toxin) family whose cellular receptor is the integrin, LFA-1, thus accounting for its selective effect on leucocytes (although only those from primates) [13-15].

Almost all the RTX leukotoxins are secreted except LtxA toxin of *A. actinomycetemcomitans* which is thought to be entirely cell associated; either bound to cell surface-associated nucleic acids [16] or within membranous vesicles which bud from bacterium's surface [17,18]. This affirms to the possibility that the bacterium itself is toxic to the target cells. The apoptosis of the target cells in response to *A.actinomycetemcomitans* leukotoxin is by a mechanism involving mitochondrial perturbation [19]. Injection of *A. actinomycetemcomitans* into mice has been claimed to induce immunosuppression and sonicates of this organism suppressed the IgG response to sheep red blood cells in mice [20,21].

It has also been proposed that *A.actinomycetemcomitans* can produce super antigens which have the ability to bring about T cell apoptosis by binding to T cell receptors [22,23]. *A. actinomycetemcomitans* has been reported to produce a number of, as yet unidentified, proteins with cell cycle-inhibitory activity causing arrest in the G2 phase of the cell cycle. These proteins range in molecular mass from the 8-kDa protein termed gapstatin to 60 kDa and all the way up to 80 kDa- [24-27]. One cell cycle-modulatory protein with immunosuppressive function that has recently been identified as being produced by *A. actinomycetemcomitans* is cytolethal distending toxin (CDT) [28,29].

Fc binding protein termed as Omp34 identical with OmpA of *E. coli*, a protein implicated in the virulence of this organism is another immunomodulatory virulence factor of *A. actinomycemcomitans* [30-31]. *A. actinomycetemcomitans* produces a 65-kDa macromolecule able to bind to the IL-10 receptor and henceforth can modulate monocyte/macrophage function as IL- 10 is considered to be a major macrophage de-activating cytokine [32].

Virulence Factors That Modulate Immune System	Leukotoxin of RTX(repeats in toxin) family.
	Super antigen producing T cell apoptosis.
	Cell cycle modulatory protein called as cytolethal distending toxin.
	Fc binding protein termed as Omp34.
	Monocyte/macrophage modulating protein and neutrophil chemotaxis inhibitor
Virulence factors inducing tissue destruction	Lipopolysaccaride(LPS) present on the bacterial cell wall
	Secreted proteins like cell stress protein.

[Table/Fig-4]: Virulence Factors [13-37]

A. actinomycetemcomitans has also been reported to produce a low molecular mass inhibitor of neutrophil chemotaxis to FMLP [33] [Table/Fig-4].

b. Virulence factors inducing tissue destruction

Tissue destructive virulence factors of *A.actinomycetemcomitans* include lipopolysaccaride(LPS) present on the bacterial cell wall and the secreted proteins like cell stress protein. LPS is reported to stimulate bone resorption in vitro and in vivo [34-36]. But it's considered to be a less significant cytokine inducer than the secreted protein. A cell stress protein, chaperonin 60 is considered to b a potent bone degrading molecule bystimulating bone resorption by acting as an osteoclast 'growth factor' [37] [Table/Fig-4].

VIRULENCE MECHANISMS

a. Adhesion

Bacterial adhesion which facilitates colonization is the key virulence mechanism [38]. Bacterial components involved in Adhesion are called adhesins. They are proteinaceous structures found on cell surfaces. They bind with specific receptors in the saliva, tooth, extra cellular matrix and epithelial cells. *A.actinomycetemcomitans* forms chains which cannot be broken up by sublethal sonication. Surface entities like vesicles mediate aggregation. *A.actinomycetemcomitans* adheres to the gingival crevice epithelium. Strains with fimbriae adhere three to four folds better. *A.actinomycetemcomitans* binds to collagen I,II,III and V but not IV. It also binds to fibronectin but not fibrinogen.

The tight auto-adhesion of *A. actinomycetemcomitans* has been described is due to the expression of long, bundled fibrils composed of a 6.5-kDa subunit protein,Flp-1 (fimbrial low-mol. wt protein) which has been reported to be glycosylated [39-46].

Bacteriocins are proteins produced by bacteria that are lethal for other strains and species of bacteria. These agents confer colonization by lessening ecological pressures. This is an advantage for the bacterium.

b. Invasion

It has been affirmed that many bacteria have the ability to invade host cells and *A. actinomycetemcomitans* is one among them [37]. Studies of invasion *A. actinomycetemcomitans* reveals that 25% of *A.actinomycetemcomitans* isolates are invasive [47,48] [Table/Fig-5]. *A. Actinomycetemcomitans* penetrate and survive within eukaryotic cells. They penetrate gingival epithelium. They occur in specific intracellular locations like the epithelial wall, enlarged intracellular pocket spaces and the epithelial side of basal lamina in connective tissue and alveolar bone. It has been observed that microfilaments and microtubules for intracellular movement [49]. The process of intracellular movement and the cell spreading could be inhibited by agents that interfered with microtubule dynamics, suggesting that this bacterium when internalized interacts closely with the microtubules of the host cell [50].

It has been suggested that the transferrin and integrin receptors are involved in the adhesion of the bacteria to host cells [51].

Author	Review of literature
Klinger et al., (1912)	Described Bacterium actinomycetem -comitans as coccobacillary bacteria isolated together with *Actinomyces* from actinomycotic lesions of man1
Topley & Wilson (1929)	Reclassified as *Actinobacillus actinomycetemcomitans*
Nørskov-Lauritsen N and Kilian (2006)	Performed mutilocus sequence analysis and found phylogenetic similiarity between Actinobacillus actinomycetemcomitns , *Haemophilus aphrophilus* and *Haemophilus segnis* and reclassified them into the genus *aggregatibacter*
Faveri M (2009)	Evaluated subgingival microflora of in localized and generalized aggressive patients and found that proportions of *Aggregatibacter actinomycetemcomitans* were elevated in shallow and intermediate pockets of localized aggressive subjects.
Lally ET (1999)	Described the interaction of *aggregatibacter actinomycetemcomitans* leukotoxin of RTX family with target cells.
Pickett CL (1999)	Reviewed on cytolethal distending toxin which modulates the cell cycle.
White PA (1999)	Isolated the major outer membrane protein(omp) of *A. Actinomycetemcomitans* from localized juvenile periodontitis patient and named it as omp34, and its corresponding gene has been named omp34.
Iino Y, Hopps RM (1984)	The bone-resorbing activities in tissue culture of lipopolysaccharides from the bacteria *Actinobacillus actinomycetemcomitans*, Bacteroides gingivalis and Capnocytophaga ochracea isolated from human mouth were assessed and found that LPS could be important in mediating bone loss in chronic periodontitis
Kagermeier AS, London J (1985)	Studied the adhesion Of *Actinobacillus actinomycetemcomitans* to human epithelial cells and the findings were consistent with the hypothesis that a protein(s) is required for bacterial adhesion and that host components may play a role in modulating adhesion to epithelial cells.
Inouye T et al., (1990)	Three colonial variants of *actinobacillus actinomycetemcomitans* which are transparent rough(TR), transparent smooth(TS), and opaque smooth(os) surfaced colonies were described in relation to their fimbriation.
Lepin G et al.,	Studied the invasiveness of actinobacillus actinimycetemcomitans strains in localized juvenile periodontitis patients and found that five isolates were invasive.
Meyer DH	Studied the invasion process of *actinobacillus actinomycetemcomitans* with microscopy and viable quantitative assays and observed that the invasion occurred rapidly within 30mins of infection.

[Table/Fig-5]: Review of literature

Unique aspects of the behaviour of *A. actinomycetemcomitans* are its rapid exit from cells after invasion, its ability to move from one cell to another and its capacity to divide rapidly within host cells [52].

CONCLUSION

A.a. is a highly non motile gram negative coccobacillus with a vast array of potential virulence factors and mechanisms. Though it was initially named as *Actinobacillus actinomycetemcomitans*, it was found that the bacterium is more similar to *haemophilus* than actinobacillus and hence it was reclassified under *aggregatibacter* as *aggregatibacter actinomycetemcomitans*. Scientific data clearly underlines its etiological role in localized aggressive periodontitis. This review also tries to throw light on the virulence abilities of this pathogen like immune evasion mechanisms like production of leukotoxin, cell cycle modulatory protein and immunomodulatory protein like Fc binding proteins. It also brings about tissue destruction by other novel mechanisms like binding to host matrices and invading host cells. Still, a lot is still to be understood and established. With the advent of newer technological methodologies and genome information, we would be able to understand not only how *A. actinomycetemcomitans* produces such profound but local pathology like periodontal infections but also its role in systemic pathology.

REFERENCES

[1] Klinger R. Untersuchungen über menschliche Aktinomykose. *Zentralbl Bakteriol*. 1912;62:191–200 (in German).
[2] Topley WWC & Wilson GS. The Principles of Bacteriology and Immunity, 1929: pp. 1–587. London: Edward Arnold.
[3] Potts TV, Zambon JJ & Genco RJ. Reassignment of *Actinobacillus actinomycetemcomitans* to the genus *Haemophilus* as *Haemophilus actinomycetemcomitans*. *comb nov Int J Syst Bacteriol.* 1985;35:337–41.
[4] Nørskov-Lauritsen N, 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-dependent and V factor-independent isolates". *Int J Syst Evol Microbiol*. 2006 ; 56(9):2135–46.
[5] Zambon JJ, Christersson LA, Slots J. *Actinobacillus actinomycetemcomitans* in human periodontal disease: Prevalence in patient groups and distribution of biotypes and serotypes within families. *J Periodontol.* 1983; 54(12):707-11.
[6] Holt SC, Tanner AC, Socransky SS. Morphology and ultrastructure of oral strains of *actinobacillus actinomycetemcomitans* and *haemophilus aphrophilus*. *Infect Immun.* 1980; 30: 588-600.
[7] Sigmund S. Socransky. Criteria for the infectious agents in dental caries and periodontal disease. *Journal of Clinical Periodontology.* 1979; 7(6):16–21.
[8] Shinichi Arakawa1, Takuma Nakajima1, Hiroaki Ishikural. Novel ApoptosisInducing Activity in Bacteroides forsythus: A Comparative Study with Three Serotypes of *Actinobacillus actinomycetemcomitans*. *Infect. Immun.* 2000; 68 (8): 4611-5.
[9] Faveri M, Figueiredo LC, Duarte PM, Mestnik MJ, Mayer MP, Feres M. Microbiological profile of untreated subjects with localized aggressive periodontitis. *J Clin Periodontol.* 2009; 36(9):739-49.
[10] Janette C Rahamat-Langendoen, Marit G Avan Vonderen. Brain abscess associated with *Aggregatibacter actinomycetemcomitans*: case report and review of literature. *Journal Of Clinical Periodontology.* 2011; 38(8):702-06.
[11] Gaoyan Tang, Todd Kitten, Cindy L. Munro. A Potential virulence determinant of *Aggregatibacter actinomycetemcomitans* in Infective Endocarditis. *Infect Immun.* 2008; 76(6):2316–24.
[12] Rurenga P, Raangs E, Singadji Z, Wekema-Mulder G, Veloo AC, Van Winkelhoff AJ. Evaluation of three selective media for isolation of *Aggregatibacter actinomycetemcomitans*. *J Periodontal* Res. 2013;48(5):549-52.
[13] Lally ET, Hill RB, Kieba IR, Korostoff J. The interaction between RTX toxins and target cells. *Trends Microbiol.* 1999; 7: 356–61.
[14] Narayanan SV, Nagaraja TG, Chengappa MM, Stewart GC. Leukotoxins of gram-negative bacteria. *Vet Microbiol.* 2002; 84:337-39.
[15] Lally ET, Kieba IR, Sato A, et al. RTX toxins recognize integrin on the surface of human target cells. *J Biol Chem.* 1997; 272:30463–69.
[16] Ohta H, Hara H, Fukui K, Kurihara H, Murayama Y, Kato K. Association of Actinobacillus actinomycetemcomitan leukotoxin with nucleic acids on the bacterial cell surface. *Infect Immun.* 1993; 61: 4878–84.
[17] Berthold P, Forti D, Kieba IR, Rosenbloom J, Taichman NS, Lally ET. Electron immunocytochemical localization of *Actinobacillus actinomycetemcomitans* leukotoxin. *Oral Microbiol Immunol.* 1992;7:24–27.
[18] Kato S, Kowashi Y, Demuth DR. Outer membrane-like vesicles secreted by *Actinobacillus actinomycetemcomitans* are enriched in leukotoxin. *Microb Pathog.* 2002; 32:1–13.
[19] Korostoff J, Yamaguchi N, Miller M, Kieba I, Lally ET. Perturbation of mitochondrial structure and function plays a central role in *Actinobacillus actinomycetemcomitans* leukotoxin- induced apoptosis. *Microb Pathog.* 2000;29:267–78.
[20] Chen PB, Davern LB, Neiders ME, Reynolds HS, Zambon JJ. Analysis of in vitro lymphoproliferative responses and antibody formation following the subcutaneous injection of *Actinobacillus actinomycetemcomitans* and Wolinella recta in a murine model. *Oral Microbiol Immunol*. 1991;6: 12–16.
[21] Kuritai Ochiai T, Ochiai K, Ikeda T. Immunosuppressive effects induced by *Actinobacillus actinomycetemcomitans*: Effect on immunoglobulin production and lymphokine synthesis. *Oral Microbiol Immunol*. 1992;7: 338–43.
[22] Nalbant A, Zadeh HH. Evidence for apoptosis of the majority of T cells activated in vitro with *Actinobacillus actinomycetemcomitans*. *Oral Microbiol Immunol.* 2000; 15: 290–98.
[23] Zadeh HH, Nalbant A, Park K. Large-scale early in vitro response to *Actinobacillus actinomycetemcomitans* suggestssuperantigenic activation of T-cells. *J Dent Res.* 2001; 80: 356–62.
[24] White PA, Wilson M, Nair SP, Kirby AC, Reddi K, Henderson B. Characterization of an antiproliferative surface-associated protein from *Actinobacillus actinomycetemcomitans* which can be neutralized by sera from a proportion of patients with localized juvenile periodontitis. *Infect Immun.* 1995;63:2612–18.
[25] White PA, Pater M, Nair S, et al. Control of the human cell cycle by a bacterial protein, gapstatin. *Eur J Cell Biol.* 1998; 77: 228–38.
[26] Helgeland K, Nordby O. Cell cycle-specific growth inhibitory effect on human gingival fibroblasts of a toxin isolated from the culture medium of *Actinobacillus actinomycetemcomitans*. *J Periodont Res.* 1993;28:161–65.
[27] Ohguchi M, Ishisaki A, Okahashi N, et al. *Actinobacillus actinomycetemcomitans* toxin induces both cell cycle arrest in the G2/M phase and apoptosis. *Infect Immun.* 1998; 66:5980–87.
[28] Pickett CL, Whitehouse CA. The cytolethal distending toxin family. *Trends Microbiol.* 1999;7:292–97.
[29] De Rycke J, Oswald E. Minireview. Cytolethal distending toxin (CDT): a bacterial weapon to control host cell proliferation? FEMS Microbiol Lett. 2001; 203:141–48.
[30] White PA, Nair SP, Kim M-J, Wilson M, Henderson B. Molecular characterization of an outer membrane protein of*Actinobacillus actinomycetemcomitans* belonging to the OmpA family. *Infect Immun.* 1998; 66: 369–72.
[31] Weiser JN, Gotschlich EC. Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of Escherichiacoli K-1. *Infect Immun.* 1991;59:2252–8.
[32] Kato T, Okudo K. *Actinobacillus actinomycetemcomitans* possesses an antigen binding to anti-human IL-10 antibody. *FEMS Microbiol Lett.* 2001; 204: 293–7.
[33] Van Dyke TE, Bartholomew E, Genco RJ, Slots J, Levine MJ.Inhibition of neutrophil chemotaxis by soluble bacterial products. *J Periodontol.* 1992; 53:502–8.
[34] Iino Y, Hopps RM. The bone-resorbing activities in tissue culture of lipopolysaccharides from the bacteria *Actinobacillus actinomycetemcomitans*, Bacteroides gingivalis and Capnocytophaga ochracea isolated from human mouths. *Arch Oral Biol.* 1984; 29: 59–63.
[35] Ishihara Y, Nishihara T, Maki E, Noguchi T, Koga T. Role of interleukin-1 and prostaglandin in in vitro bone resorptioninduced by *Actinobacillus actinomycetemcomitans* lipopolysaccharide. *J Periodont Res.* 1991; 26: 155–60.
[36] Nishida E, Hara Y, Kaneko T, Ikeda Y, Ukai T, Kato I. Boneresorption and local interleukin-1 alpha and interleukin-1 betasynthesis induced by *Actinobacillus actinomycetemcomitans*and Porphyromonas gingivalis lipopolysaccharide. *J PeriodontRes.* 2001;36:1–8.
[37] Reddi K, Meghji S, Nair SP, et al. The Escherichia coli chaperonin 60 (groEL) is a potent stimulator of osteoclast formation. J Bone Miner Res. 1998;13:1260–66.
[38] Wilson M, McNab R, Henderson B. Bacterial disease mechanisms: an introduction to cellular microbiology. *Cambridge University Press.* 2002.
[39] Kagermeier AS, London J. *Actinobacillus actinomycetemcomitans* strains Y4 and N27 adhere to hydroxyapatite by distinctive mechanisms. *Infect Immun.* 1985; 47: 654–58.
[40] Rosan B, Slots J, Lamont RJ, Listgarten MA, Nelson GM. *Actinobacillus actinomycetemcomitans* fimbriae. *Oral Microbiol Immunol.* 1988; 3: 58–63.
[41] Fine DH, Furgang D, Kaplan J, Charlesworth J, Figurski DH. Tenacious adhesion of *Actinobacillus actinomycetemcomitans* strain CU1000 to salivary coated hydroxyapatite. *Arch Oral Biol.* 1999; 44: 1063–76.
[42] Fine DH, Furgang D, Schreiner HC, et al. Phenotypic variation in *Actinobacillus actinomycetemcomitans* during laboratory growth: implications for virulence. *Microbiology.* 1999; 145: 1335–47.
[43] Kachlany SC, Planet PJ, Bhattacharjee MK, et al. Nonspecific adherence by *Actinobacillus actinomycetemcomitans* requires genes widespread in bacteriA. *J Bacteriol.* 2000;182:6169–76.
[44] Inouye T, Ohta H, Kokeguchi S, Fukui K, Kato K. Colonial variation and fimbriation of *Actinobacillus actinomycetemcomitans*. *FEMS Microbiol Lett.*1990; 57: 13–17.
[45] Inoue 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: 253–58.
[46] Inoue T, Ohta H, Tanimoto I, Shingaki R, Fukui K Heterogeneous post translational modification of *Actinobacillus actinomycetemcomitans* fimbrillin. *Microbiol Immunol*. 2000;44:715–18.
[47] Meyer DH, Sreenivasan PK, Fives-Taylor PM. Evidence for invasion of a human oral cell line by *Actinobacillus actinomycetemcomitans*. *Infect Immun.* 1991; 59: 2719–26.
[48] Lepine G, Caudry S, DiRienzo JM, Ellen RP. Epithelial cell invasion by *Actinobacillus actinomycetemcomitans* strains from restriction fragment length polymorphism groups associated with juvenile periodontitis or carrier status. *Oral Microbiol Immunol*. 1998; 13: 341–47.
[49] Oelschlaeger TA, Hacker J. Bacterial invasion into eukaryotic cells. New York, *Kluwer Academic*. 2000xxxvii,687p.
[50] Meyer DH, Rose JE, Lippman JE, Fives-Taylor PM. Microtubules are associated with intracellular movement and spread of the periodontopathogen *Actinobacillus actinomycetemcomitans*. *Infect Immun.* 1999; 67:6518–25.
[51] Meyer DH, Mintz KP, Fives-Taylor PM. Models of invasion of enteric and periodontal pathogens into epithelial cells: a comparative analysis. *Crit Rev Oral Biol Med.* 1997; 8: 389–95.
[52] Meyer DH, Lippman JE, Fives-Taylor PM. Invasion of epithelial cells by *Actinobacillus actinomycetemcomitans*: a dynamic multistep process. I*nfect Immun.* 1996; 64: 2988–97.

PARTICULARS OF CONTRIBUTORS:
1. Reader, Department of Periodontics, Karpaga Vinayaga Institute of Dental Sciences, Chennai, India.
2. Reader, Department of Periodontics, MES Dental College, Perintalmanna, India.
3. Senior Lecturer, Department of Periodontics, karpagavinayaga Institute of Dental Sciences, Chennai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Manoj Raja,
Flat no:2, Ground Floor, Krishna Apartments,6/60 Pulla Avenue,
Shenoy Nagar, Chennai- 30, India.
Phone : 9840966563, E-mail : msabitha@hotmail.com

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