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AGGREGATIBACTER ACTINOMYCETEMCOMITANS: ABRIEF REVIEW

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ABSTRACT

Aggregatibacter actinomycetemcomitans are non-motile, gram-ve, saccharolytic, capnophilic round ended rod shaped bacterium. This organism possesses a large number of virulence factors which enable it to colonise the oral cavity, destroy periodontal tissues, degrade host defences, connective tissue destruction and interfere with tissue repair. *A. actinomycetemcomitans* are isolated from localized aggressive periodontitis and refractory periodontitis. Other than periodontal infections *A. actinomycetemcomitans* are associated with systemic diseases also.

INTRODUCTION

Periodontitis is a chronic infectious disease affecting the man kind. Various types of periodontal pathogens are involved in disease initiation and progression such as *Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denticola (T.d.), Prevotella intermedia* and the list goes on expanding. Among the major pathogens *A. actinomycetemcomitans* is an opportunistic pathogen which is associated with aggressive form of periodontitis.[1] It possess diverse virulence factors including various mechanism for invading host tissue that makes it a potent pathogen in the pathology of aggressive periodontitis.[2]

History

A.actinomycetemcomitans was first isolated in 1912 from a cervicofacial actinomycotic lesion and was initially designated Bacterium *actinomycetemcomitans* by Klinger. In 1921, the organism was referred to as Bacterium comitans by Lieske and finally designated

Actinobacillus actinomycetemcomitans in 1929. The organism is called Actinobacillus because of its resemblance to ray fungus in agar medium and also owing to the short rod like or bacillary nature of individual cells. But *A. actinomycetemcomitans* is not closely related to other species in *Actinobacillus*, at the same time it is distantly related to genetics *H. influenza*, *H. aphrophilus* and *H. paraphrophilus.* Thus it can be said that *A.actinomycetemcomitans* does not fit well into the genus *Actinobacillus* nor does it fit into genus Haemophillus. Thus there is a need it should be probably placed in a new genus. Finally in 2006, a study conducted by Nørskov Lauritsen N and Kilian M resulted in the addition of the genus *Aggregatibacter* (aggregate means to come together; bacter meaning bacterial rod; together means rod shaped bacterium that aggregates with others), and placed the organism into the family pasteurellaceae.[3]

Taxonomy

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

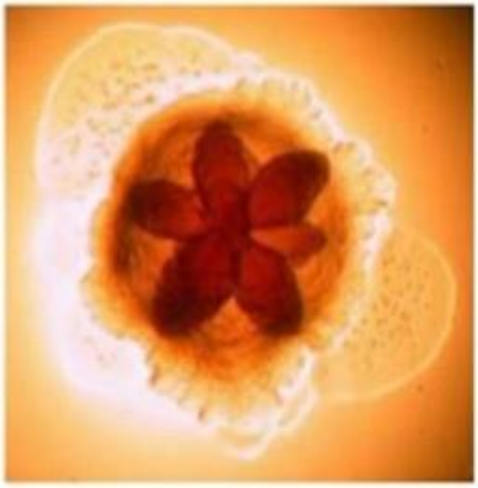
Serotypes

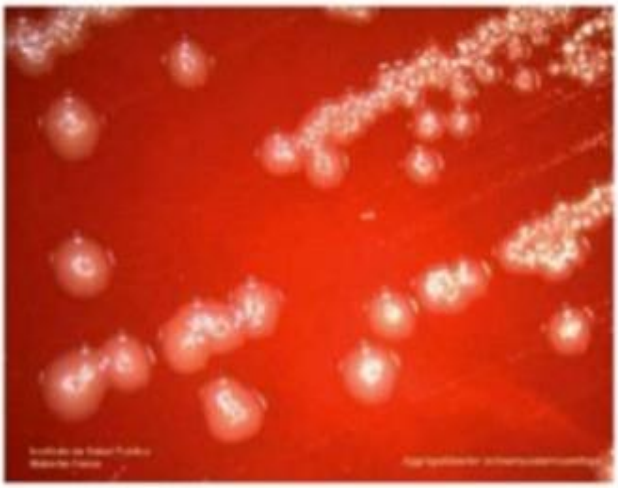
B**ased on surface polysaccharide Taichman classified AA into six serotype:-** serotype a to serotype f. Among the serotypes a, b and c are most prevalent in the oral cavity. Serotype a and c are strongly associated with periodontal health while serotype b is found in active periodontal lesions and is more frequently detected in aggressive periodontitis.[4]

Cultural characters

A.actinomycetemcomitans is 0.4±0.1 x1±0.4µm in size. Cells are spherical, club-shaped, or rod-shaped. Rod-shaped cells are common in agar cultures. The cells arrange as single cells, in pairs, or in piles. They are non-motile, produce no spores and do not form capsules. It grows well in 5% CO2 in air or anaerobically. On agar medium *A actinomycetemcomitans* shows three types of colony morphology. Fresh oral isolates of *A actinomycetemcomitans* are

fimbriated and form small rough surface, translucent colonies, with an internal star shaped or crossed cigar morphology that gives *A. actinomycetemcometans* its name. Rough colony morphology is due to the presence of fimbriae. Repeated sub culturing results in spontaneous change from rough to smooth colony and yields two types of colonial variants: - one is smooth surfaced and transparent, the other is smooth surfaced and opaque. The transparent smooth-surfaced variants are intermediate between the transparent rough-surfaced and opaque smooth surfaced types. Colonies of the smooth variants lack the star like inner structure and the cells do not express fimbriae.[5]





Selective media for aa

MGB media	Trypticase soy broth + Malachite green + bacitracin
TSBV media	Trypticase soy broth +bacitracin + vancomycin
A media	TSBV media + Spiramycin + fusidic acid + carbenicillin.

Biochemical characterstics

- Lack of growth on MacConkey and other enteric agars
- Catalase production: + ve
- Nitrate reduction: + ve
- Oxidase negative: + ve (occasional strains may be weakly positive)
- Urease: - ve
- Indole production: - ve
- X ,V factor requirement: - ve
- Glucose, Fructose, Mannose: strong fermentation

Surface ultrastructure of AA

The surface ultrastructure of *A. actinomycetemcomitans* includes fimbriae, vesicles, and extracellular amorphous material.

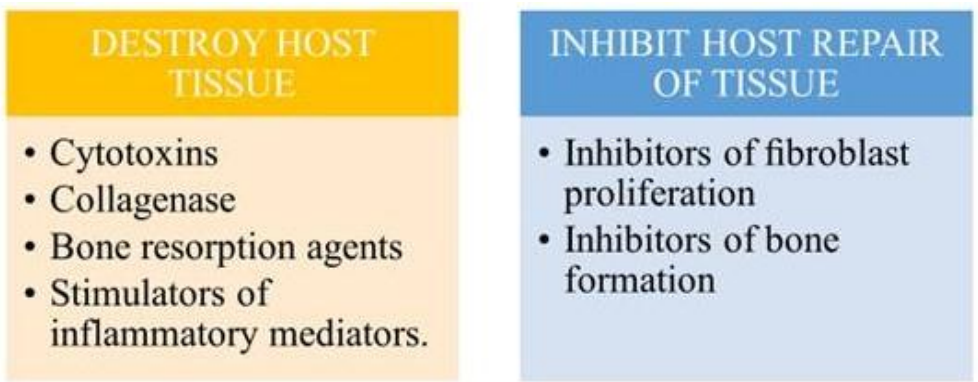
Fimbriae: - *A. actinomycetemcomitans* may exhibit peritrichous fimbriae. It is associated with bacterial colonization of host tissues. A number of studies indicate that colonial variation and fimbriation are associated with *A. actinomycetemcomitans* adhesion. Most abundant protein in a fimbria of *A. actinomycetemcomitans* is 304-a.[6]

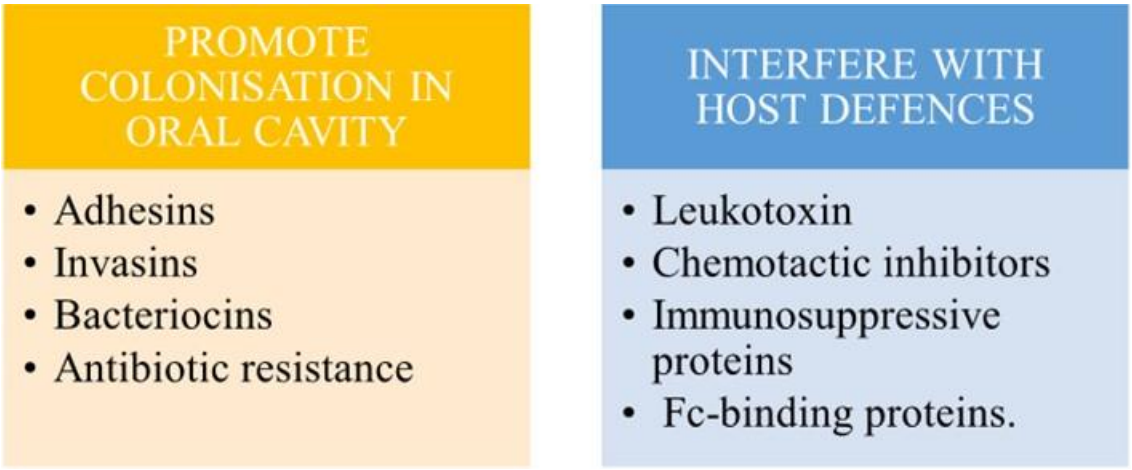
Vesicles:- A prominent feature of the surface of *A. actinomycetemcomitans* is vesicles (blebs). The surface of highly leukotoxic *A. actinomycetemcomitans* strains have abundance of extracellular vesicles, in contrast to minimally or nonleukotoxic strains, which have few or no vesicles.[7] The vesicles exhibit leukotoxic activity. The biologically active components of *A. actinomycetemcomitans* vesicles include endotoxin, bone resorption activity and a bacteriocin, termed actinobacillin. *A. actinomycetemcomitans* vesicles exhibit leukotoxic activity as well as it has adhesive properties. Vesicles function as delivery vehicles for *A. actinomycetemcomitans* toxic materials.

Extracellular amorphous material:- The material is a glyprotein, that exhibit both bone-resorbing activity and adhesive properties. Like fimbriae and vesicles, the expression of the amorphous material is determined by culture conditions. Bacteria from which the amorphous material has been removed exhibit reduced adhesion to epithelial cells.

Virulence factors

The term virulence is defined as the relative ability of an organism to cause disease or to interfere with a metabolic or physiological function of its host. *A.actinomycetemcomitans* possess a myriad of virulence factors that enhance its survival in the oral cavity.[8]





Adhesion of A. actinomycetemcomitans

The bacterial surface components involved in adhesion are called adhesins. Most *A. actinomycetemcomitans* strains that have been tested adhere strongly to epithelial cells. Binding occurs very rapidly, reaching saturation levels within 1 hr after infection. Cell surface entities that mediate adherence include fimbriae, extracellular amorphous material and extracellular vesicles. *A. actinomycetemcomitans* also produces poly-n-acetylglucosamine (PGA), a surface polysaccharide that mediates intercellular adhesion, biofilm formation and detergent resistance (Venketaraman et al. 2008).

Antibiotic resistance

A. actinomycetemcomitans were found to be resistant to tetracyclines and carried the tetracycline B resistance determinant.[9] Also this tetracycline B resistance determinant was capable of being transferred to other *A. actinomycetemcomitans* strains by conjugation. This antibiotic resistance is responsible for one of the cause for treatment failures.

Bacteriocins

Bacteriocins are lethal proteins produced by bacteria that will act against other strains and species of bacteria. These toxic agents offer a colonization advantage for the bacterium by lessening the ecological pressures associated with competition by other organisms for both nutrients and space. Actinobacillin, a bacteriocin produced by *A actinomycetemcomitans*, is active against *S. sanguis, Streptococcus uberis and A. viscosus*, has been identified and purified.[10] It has been proposed that actinobacillin may be responsible for the reciprocal relationship that occurs between *A. actinomycetemcomitans and S. sanguis or A. viscosus* in plaque and in patients with localized juvenile periodontitis.

Bone resorption

A characteristic feature of periodontal disease is the loss of supporting bone. *A.actinomycetemcomitans* initiates bone resorption by various mechanisms like lipopolysaccharide, proteolysis sensitive factor in microvesicles and surface-associated material. This surface-associated material has recently been identified as the molecular chaperone, GroEL. The chaperone appears to act in a direct way with the major bone-resorbing cell population, the osteoclast to cause the bone resorption.

Collagenase

A marked feature of periodontal disease is reduction in gingival collagen fiber density. Collagenase activity is associated with two important periodontal pathogens, *A. actinomycetemcomitans and P gingivalis.*

Cytotoxins

Fibroblasts are the major source of collagen. A heat labile cytotoxin is produced by *A. actinomycetemcomitans* which affect the viability of fibroblast. One toxin that is secreted has been identified as a 50-kDa protein that inhibits DNA synthesis in the fibroblast.

A. actinomycetemcomitans surface-associated material has also been shown to inhibit fibroblast proliferation at low concentration. The active component of surface- associated material. designated Gapstein, is an 8-kDa protein. Gapstein inhibits cells in the G2 phase of the cell cycle.

Extracellular membranous vesicles

Almost all strains of *A. actinomycetemcomitans* examined extrude membrane vesicles from their surface. These vesicles contain leukotoxin, endotoxin, bone resorption activity and a bacteriocin. It also contains adhesins. But it is not clear what role exactly these vesicles play in the pathogenesis of *A. actinomycetemcomitans*.

Leukotoxin

Leukotoxin is considered as the most important virulence factor in the pathogenesis of localized aggressive periodontitis. It is a 114-kDa secreted lipoprotein that belongs to the RTX family of pore forming bacterial toxins. It is a heat labile toxin and is protease sensitive.[11] It can kill both lymphoid and myeloid leukocytes .Human subjects harbouring highly leukotoxic strains of *A. actinomycetemcomitans* are more likely to develop

periodontitis than subjects harbouring minimally leukotoxic strains. Some highly leukotoxic strains of *A.actinomycetemcomitans* produce about 10 – 20 times more leukotoxin than the other minimally leukotoxic strains.[12]

RTX leukotoxin is encoded by an operon consisting of 4 genes,- C, A, B and D. Ltx A encodes the leukotoxin itself and is produced as an inactive protoxin.LtxB and LtxD are involved in transporting the toxin to the surface of the cell.LtxC post-translationally activates the toxin.

Mechanism of action of leukotoxin

Two Ltx A mediated mechanisms of cell death are known to exist: Necrosis and apoptosis. The Ltx A forms pores in the target cell membrane leading to water influx and osmotic lysis. This is the case when the Ltx A is present in high concentrations. At low concentrations, Ltx A mediates cell death via apoptosis.

Fc-binding proteins

Bacterial immunoglobulin-binding proteins, or Fc receptors, are proteins that bind to the Fc portion of IgG. They interfere with complement or antibody dependent host immune mechanisms. If other proteins compete for binding to this region of PMNs, binding of the antibody may be inhibited and thereby inhibit phagocytosis.[13] Molecules on the surface of *A. actinomycetemcomitans* that are associated with capsular material and secreted into the medium bind to the Fc portion of immunoglobulin G (Tolo & Hegland).This binding inhibits the ability of opsonizing antibodies to bind polymorphonuclear leukocytes and reduces phagocytosis by 90%.

Lipopolysaccharide

Lipopolysaccharides (endotoxins) of *A. actinomycetemcomitans* have high potential to cause destruction of host cells and tissues. It causes skin necrosis (Schwartz- mann reaction), bone resorption and platelet aggregation, and it activates macrophages.[14] Low concentrations of *A. actinomycetemcomitans* lipopolysaccharide stimulate macrophages to produce interleukins and tumor necrosis factor involved in tissue inflammation and bone resorption. These lipopolysaccharides are involved in atherosclerosis and coronary heart disease.

Immunosuppressive factors

A. actinomycetemcomitans produce many factors that are capable of suppressing host defense

mechanisms. A.actinomyctemcomitans produces a 60-kDa protein, which down regulates both T and B-cell responsiveness through the activation of a subpopulation of B lymphocytes (Shenker et al. 1990). Now, this factor is known as cytolethal distending toxin (CDT), which induces apoptosis to lymphocytes. Bacteria also produce a 14-kDa protein known as suppressive factor (SF1), which suppresses cell proliferation and cytokine production. SF1 also inhibits T-cell proliferation.

Inhibitors of polymorphonuclear leukocyte function

The ability to disrupt chemotaxis permits the invading organism to survive the major challenge from the host. *A. actinomycetemcomitans* secretes a low molecular weight compound that inhibits polymorphonuclear leukocyte chemotaxis. A heat-stable protein in *A. actinomycetemcomitans* inhibits the production of hydrogen peroxide by polymorphonuclear leukocytes, and many strains are naturally resistant to high concentrations of hydrogen peroxide. Furthermore, *A. actinomycetemcomitans* has been shown to be resistant to defensins that are found in neutrophils.[15]

Penetration of epithelial cells

A.actinomycetemcomitans can penetrate the gingival epithelium. A number of factors are listed that may contribute to host cell invasion. These include tad (tight adherence) gene locus which mediates adhesion, OmpA1 which is associated with the entry of *A.actinomycetemcomitans* into gingival epithelial and Omp100 which promotes invasion of *A.actinomycetemcomitans* into human gingival keratinocytes. It has also been told that cytolethal distending toxin of the bacteria can cause disruption of the epithelial barrier and promote tissue invasion.[16]

Role of *A.actinomycetemcomitans* in periodontitis

Localized aggressive periodontitis is a periodontal condition in adolescents that exhibits rapid destruction of periodontal tissue, which slows with time. *A. actinomycetemcomitans* has been suggested as the organism causing localized aggressive periodontitis. Large numbers of *A.actinomycetemcomitans* are routinely isolated from lesions of localized aggressive periodontitis (97 % of cases).[17]

Socransky criteria applied to explain role of *A.actinomycetemcomitans* in periodontal diseases:-

Association	Increased in localized aggressive periodontitis Increased in some lesions of chronic periodontitis Detected in the tissues of localized aggressive periodontitis lesions
Elimination	Suppressed (or) eliminated in successful therapy Found in recurrent lesions
Host response	Increased serum and local antibody levels in localized aggressive periodontitis
Animal studies	Capable of inducing disease in gnotobiotic rats
Virulence factors	A. actinomycetemcomitans produces several potentially pathogenic substances, including a leukotoxin

Generalized aggressive periodontitis and rapidly progressive periodontitis have been frequently associated with the detection of *A. actinomycetemcomitans* along with *P. gingivalis and B. forsythus*.[17]

The association between *A. actinomycetemcomitans* and chronic periodontitis seems to be less evident than observed in aggressive periodontitis and the frequency of isolation varies from 20 to 75%.[18]

A. actinomycetemcomitans is also associated with refractory periodontitis.[19] this may be due to the difficulty in eradicating these bacteria from the subgingival area because of its invasive capability.

Aggregatibacter actinomycetemcomitans in non oral infections

Cardiovascular infections

Endocarditis represents the most frequent non oral *A.actinomycetemcometans* infection. In 1972, Stauffer & Goldman documented the first case of prosthetic valve endocarditis due to *A. actinomycetemcomitans*.[19] Since then, several reports on *A actinomycetemcomitans* prosthetic valve endocarditis have been published.[20]

Intracranial infection

Intracranial abscesses may originate from dental and periodontal infections. Martin et al. and Fabiani et al reported on brain abscesses due to *A. actinomycetemcomitans*.[20]

Thoracic infections

A. actinomycetemcomitans in association with *Actinomyces israelii* can cause lung infection. Legum et al. found that dissemination of *A. actinomycetemcomitans* and *Actinomyces israelii*

from the lung can cause secondary infection of the skin, subcutaneous tissue and bone.[20]

Miscellaneous Infections caused by AA

Skin infections associated with the coinfection of *A. actinomycetemcomitans* and *Actinomyces israelii* or with A. actinomycetemcomitans alone have been reported. *A. actinomycetemcomitans* has also been recovered from urinary tract infection and submandibular space abscess.[20]

Treatment

In 1983, Slots & Rosling showed that scaling and root planing alone was unable to remove *A.actinomycetemcomitans* from localized juvenile periodontitis lesions. Systemic use of tetracycline and metronidazole-amoxicillin (250 mg each) showed striking results but not always guaranteed. Periodontal surgery also fails to control the level of subgingival *A. actinomycetemcomitans.* Modified Widman flap surgery may suppress A. actinomycetemcomitans in about 50% of localized juvenile periodontitis lesions.Tuan et al. found that an apically positioned flap with osseous recontouring is more effective. Resective types of periodontal surgery are more effective due to the excision of *A. actinomycetemcomitans i*nfected gingival tissue and achieve pocket depth reduction to levels that permits maintainenace of adequate oral hygiene measures. So for complete treatment of *A.actinomycetemcomitans* mediated periodontal infections, the treatment plan should include scaling and root planning with a surgical procedure with or without osseous recontouring along with systemic and local antibiotics.[21]

CONCLUSION

A.actinomycetemcomitans plays an important role in the pathology of periodontal disease. Various virulent factors produced by the organism are responsible for its pathogenicity in periodontal disease as well as well as systemic disease. Its ability to penetrate into deeper tissue complicates the treatment. So a combination of treatment therapy is required for its complete elimination.

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