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# Actinobacillus actinomycetemcomitans in periodontal infections

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## ABSTRACT

**Aims**: To isolate and identify *Actino–bacillus actinomycetemcomitans* because of its importance in periodontal diseases, to show their incidence in periodontal infections, and to study their important virulence factors. **Materials and Methods**: The isolating procedure had been done through using the selective Dentaid–1 medium, with anaerobic cultivation at 37 °C for 48 hours, and different microscopic features and biochemical activities were carried out. Also the study included the detection of virulence factors that owned by this type of bacteria through various tests. **Results**: The rate of infection was 45% in patients suffering from periodontal disease and the isolated bacteria had the ability to adhere to human epithelial cells of the mouth, also had the ability to produce bacteriocins as the substantive factors for the bacterial growth among the closer species of bacteria, and the presence of capsule, which was the most important virulence factor. Susceptibility of the isolated bacteria to selected types of antibiotic showed that 100% of the isolates were sensitive to tetracycline and ciprofloxacin, 100% were resistant to erythromycin and vancomycin. **Conclusion**: *Actinohatillus actinomycetemcomitans* was very important periodontal pathogen; having a large number of pathogenic and virulence factors; ciprofloxacin is the drug of choice.

**Key Words**: *Actinohacittus actinomycetemcomitans*, periodontal diseases.

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## INTRODUCTION

Periodontal disease, like dental caries, is considered to have an infectious etiological component and a physical/mechanical component in the accumulation of plaque around the gingival margin at the cervix of tooth.(1)

*Actinobacillus actinomycetemcomitans* is a rare but recognized pathogen in medical microbiology and has been implicated in actinomycosis, abdominal and brain abscesses, septicemia and infective endocarditis.(2)

Members of genus *Actinobacillus* are Gram negative rods, coccoid to coccobacillary in shape, 0.3 to 0.5 μm and non acid–fast, often present pleomorphic coccal and long bacillary and filamentous forms.(3)

*Actinobacillus actinomycetemcomitans* possesses a large number of virulence factors, which may be relevant in the pathogenesis of periodontal diseases, such as leukotoxin, collagenase, endotoxin and fibroblast–inhibiting factor. In addition, this microorganism produces a soluble heat–labile factor that inhibits growth and proliferation of *Actinomyces viscous* and *Streptococcus sanguis* organisms closely associated with plaque formation.(4)

*Actinobacillus actinomycetemcomitans* was first described in 1912 by Klinger as *Bacterium actinomycetemcomitans*, "a bacterium associated with actinomyces", a Gram negative non motile coccobacillus, facultatively anaerobic and capnophilic, non–hemolytic, catalase positive, form rough colonies on solid media, 0.5–1 mm in diameter with slightly irregular edges.(5)

This study was designed to isolate and identify this microorganism from patients with periodontitis and gingivitis, and to find the prevalence of *Actinobacillus actinomycetemcomitans* among those patients in Mosul City.

## MATERIALS AND METHODS

*Isolation and Identification of Bacteria*: Samples were taken from the deepest periodontal pockets of patients with periodontitis or gingivitis who attending Dentistry College in Mosul City. Paper points (Diadent Co. size 40) were inserted inside the pocket, leaving it 3 minutes then transporting the paper point in aseptic conditions into vials of 4 ml nutrient broth to the laboratory.

The sample was inoculated on the selective medium–Dentaid–1, incubated in facultative anaerobic conditions at 37 ºC for 24–48 hours.[6]

The isolated colonies were identified microscopically (Gram negative coccobacilli and capsulated) and biochemically (catalase, oxidase and gelatinase tests, growth on MacConkey agar, hemolytic activity, coagulase, DNase, indole, carbohydrate fermentation and motility test).[7]

*Study of Some Virulence Factors: Bacteriocin production test:* This test was done using Double–Layered Method.[8] The activity of the toxin was examined against *Streptococcus mutans*, *Escherichia coli* and 15 isolates of *Actinobacillus actinomycetemcomitans*.

The adherence (attachment) of bacterial cell on the surfaces of human epithelial cells was carried out also.[9]

The susceptibility and resistance to selected antibiotics was done by the Bauer–Kirby Method.[10]

## RESULTS AND DISCUSSION

One hundred forty samples were collected from deep periodontal pockets of 63 patients attending the periodontal diseases section at the Dentistry College, Mosul University. Their ages ranged between 15 and 55 years. Seventy two isolates of *Actinobacillus actinomycetemcomitans* were identified (45%). Others found that 50% of the microorganisms isolated from the periodontitis cases were *Actinobacillus actinomycetemcomitans*.

- Identification of *Actinobacillus actinomycetemcomitans*: The bacterial growth on Dentaid–1 medium at 37 ºC after 48 hours give rounded, convex and white colonies, 1 mm in diameter with slightly irregular edges, and attached firmly to the agar surface as in Figure (1). The microbial cells were Gram negative, capsulated. The isolated colonies were identified as shown in Table (1), which coincides with others.[3, 11, 12]

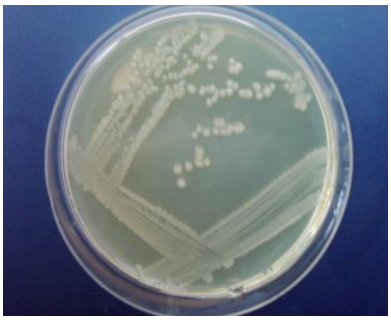

Figure (1): Colonies of *Actinobacillus actinomycetemcomitans* on Dentaid–1 agar

Table (1): Results of biochemical test of *Actinobacillus actinomycetemcomitans*

| Test Name | Result* | Test Name | Result |
|---|---|---|---|
| **Catalase** | +ve | **Carbohydrate Fermentation:** | |
| **Oxidase** | Variable 38% +ve | 1. Glucose | +ve |
| **Coagulase** | +ve | 2. Galactose | +ve |
| **DNase** | +ve | 3. Mannitol | Variable 22% +ve |
| **Gelatinase** | –ve | 4. Fructose | +ve |
| **Indole** | –ve | 5. Sucrose | –ve |
| **Capsule** | +ve | 6. Lactose | –ve |
| **Motility** | –ve | 7. Maltose | –ve |
| **Growth on MacCokey** | –ve | | |

* +ve result: > 95% of the isolates gave positive result.

- The Virulence Factors: Fifteen isolates were selected from the 72 isolates of *Actinobacillus actinomycetemcomitans* to examine their ability to produce the bacteriocins and the symbols f1, f2, f3, ……f15 were given to these isolates. The diameter of the inhibition zones due to antibacterial effect of bacteriocin from each producer isolate against the *Streptococcus mutans*, *Escherichia coli* and each of the fifteen isolates was shown in Table (2).

Table (2): The diameters of the inhibition zones for bacteriocin production test (in mm.s)

| Producer Strains | Affected Strains | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** | **F10** | **F11** | **F12** | **F13** | **F14** | **F15** | ***Strept. Mutans*** | ***E. coli*** |
| **F1** | – | 30 | 51 | 42 | 32 | 45 | 40 | 50 | 55 | 49 | 59 | 51 | 35 | 37 | 40 | – | 54 |
| **F2** | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **F3** | 20 | 23 | – | 40 | 36 | 35 | 35 | 40 | 45 | 25 | 51 | 50 | 40 | 30 | 50 | – | 20 |
| **F4** | 12 | 25 | 21 | – | 40 | 26 | 43 | 36 | 34 | 50 | 30 | 50 | 51 | 42 | 35 | – | 42 |
| **F5** | 20 | 28 | 15 | 20 | – | 22 | 30 | 45 | 29 | 30 | 29 | 25 | 26 | 30 | 40 | – | 21 |
| **F6** | 21 | 35 | 45 | 32 | 45 | – | 27 | 30 | 25 | 38 | 30 | 28 | 40 | 45 | 51 | – | 28 |
| **F7** | 25 | 40 | 45 | 30 | 25 | 40 | – | 33 | 44 | 41 | 36 | 20 | 40 | 31 | 43 | – | 39 |
| **F8** | 40 | 50 | 30 | 23 | 36 | 30 | 20 | – | 31 | 42 | 35 | 42 | 50 | 29 | 37 | – | 33 |
| **F9** | 10 | 40 | 26 | 30 | 23 | 25 | 46 | 40 | – | 48 | 50 | 31 | 45 | 50 | 42 | – | 30 |
| **F10** | 10 | 30 | 20 | 11 | 20 | 15 | 20 | 35 | 20 | – | 25 | 24 | 39 | 36 | 30 | – | 23 |
| **F11** | 20 | 45 | 27 | 30 | 32 | 25 | 35 | 40 | 35 | 35 | – | 40 | 40 | 49 | 50 | – | 16 |
| **F12** | 30 | 35 | 25 | 40 | 32 | 43 | 36 | 40 | 40 | 30 | 42 | – | 30 | 40 | 37 | – | 24 |
| **F13** | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| **F14** | 45 | 30 | 47 | 40 | 41 | 35 | 49 | 40 | 40 | 35 | 40 | 45 | 50 | – | 40 | – | 25 |
| **F15** | 25 | 22 | 30 | 39 | 32 | 42 | 43 | 25 | 25 | 40 | 50 | 38 | 45 | 30 | – | – | 23 |

The diameter of the inhibition zones varied from 10 to 59 mm. Thirteen of the isolates were toxin producers, and only two (α2 and α13) were not. This means that not all *Actinobacillus actinomycetemcomitans* were toxin producers, and the production of bacteriocins was unstable character[13] and affect only the related strains of *Actinobacillus actinomycetemcomitans* and other Gram negative bacteria, but not the Gram positive *Streptococcus mutans*.[8]

The examination of adherence ability of *Actinobacillus actinomycetemcomitans* isolates to human epithelial cells from the oral cavity gave a positive result as in Figure (2).

The pilli found on the whole surface of the cell play an important role in its attachment and colonization on oral epithelial cells.[14, 15]

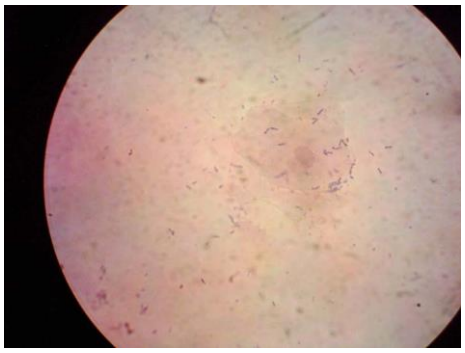

Figure (2): Adherence to oral human epithelial cells of *Actinobacillus actinomycetemcomitans* cells

Also the lipopolysaccharide from the cell wall might be an intermediate component during the adherence;[16,17] as well as the important role of the capsule in adherence mechanism.[18]

The susceptibility of the isolated bacteria to antibiotics showed 100% of the isolates were resistant to erythromycin and vancomycin, and sensitive to tetracycline and ciprofloxacin at the same time. The susceptibility towards ampicillin, clindamycin, flucoxacillin and doxycycline was variable (Table 3 and Figure 3). The same results were found by other study,[19] which suggest that the resistance of these bacteria to some antibiotics might be due to the poor permeability of cell membrane.

Table (3): Percentages of the susceptibility to antibiotics of *Actinobacillus actinomycetemcomitans* isolates

| The Used Antibiotics | Concentration (µg/disc) | Percentage of Sensitive Isolates | Percentage of the Intermediate Sensitive Isolates | Percentage of Resistant Isolates |
|---|---|---|---|---|
| **Ampicillin** | 10 | 72 | – | 28 |
| **Clindamycin** | 30 | 58 | 6 | 36 |
| **Ciprofloxacin** | 30 | 100 | – | – |
| **Tetracycline** | 30 | 100 | – | – |
| **Vancomycin** | 30 | – | – | 100 |
| **Flucoxacillin** | 30 | 40 | 44 | 16 |
| **Erythromycin** | 15 | – | – | 100 |
| **Doxycycline** | 30 | 96 | 2 | 2 |

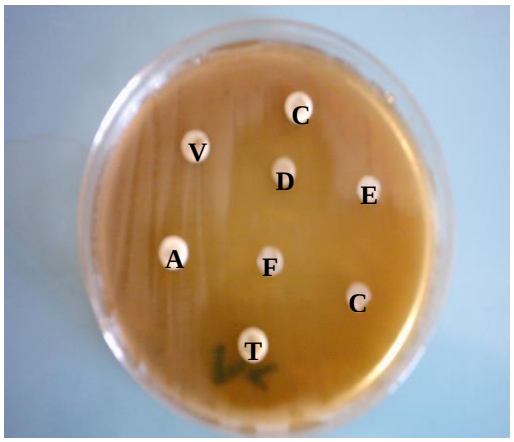


Figure (3): Susceptibility of *Actinobacillus actinomycetemcomitans* to antibiotics

## CONCLUSION

*Actinobacillus actinomycetemcomitans* was an important periodontal pathogen found in 45% of periodontitis cases, has an important and selective features that give it the superiority in these infections over other oral microorganisms; for example, production of enzymes, toxins presence of pilli, capsule and its resistance to number of antibiotics.

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