Original Article

Antimicrobial susceptibility pattern of oral isolates of *Aggregatibacter actinomycetemcomitans*

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Abstract

Background: *Aggregatibacter actinomycetemcomitans* is involved in the etiology of localized aggressive periodontitis (LAP), a condition that frequently requires supplemental antibiotic therapy. Information on antimicrobial susceptibility pattern and guidelines for oral antibiotic therapy are not available on Indian patients.

Aim: The main aim of the present study was to screen clinical isolates on a panel of antibiotics commonly used for oral/systemic therapy.

Materials and Methods: The study included 40 strains of *A. actinomycetemcomitans* isolated from patients with LAP. The subgingival plaque was plated onto Trypticase Soy Serum Bacitracin Vancomycin Agar medium and incubated for 72 h, and suspected colonies were confirmed by phenotypic tests. Each isolate was tested against a panel of 12 antibiotics using MIC gradient strip test. ATCC strains of *A. actinomycetemcomitans* serotype A and C were used as standards. Performance and interpretation of the test were done according to the manufacturers' instructions. Distribution of MICs among isolates ($n = 40$) were used to calculate concentrations inhibiting 50% (MIC_{50}) and 90% (MIC_{90}) of strains.

Results: Moxifloxacin, cefotaxime and ceftriaxone showed excellent activity with 100% growth inhibition followed by amoxicillin, amoxiclav and doxycycline (>90% activity). The bacterial strains were moderately susceptible to cefuroxime, cefazolin and tetracycline but displayed poor susceptibility to clindamycin and azithromycin. All isolates were resistant to metronidazole.

Conclusion: The isolates of *A. actinomycetemcomitans* displayed a high level of resistance to azithromycin and clindamycin. Development of resistance against tetracycline also appears to be significant. Variable resistance among the different members of the cephalosporin group is a factor to be investigated further since susceptibility profile against these antibiotics and interpretative criteria for oral bacteria are not available.

Keywords: *Aggregatibacter actinomycetemcomitans,* cephalosporins, enzyme test, MIC50, MIC90

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INTRODUCTION

Aggregatibacter actinomycetemcomitans is a tiny, nonmotile, facultatively anaerobic, fastidious Gram-negative coccobacillus that requires an atmosphere with 5%–10% CO_2 for its growth.[1] It occurs as a commensal in the human oral cavity and can be recovered from up to 20% of healthy individuals by the culture of the subgingival plaque.[2] Investigations over several years have convincingly shown the involvement of this organism in the etiology of localized aggressive periodontitis (LAP), a disease that mainly affects younger subjects leading to extensive periodontal tissue destruction and tooth loss.[1,3-6] It is also known to produce several nonoral infections and as a part of the HACEK group is frequently isolated from patients with bacterial endocarditis.[7]

In patients with aggressive periodontitis, adjunctive systemic therapy of antibiotics is known to offer better relief when used in combination with root planing and surgical intervention. Despite numerous research studies about the role of antibiotics in the treatment of periodontal diseases, clinicians are still not clear about what to prescribe for their patients.[8] One main reason for such a dilemma could be nonavailability of antimicrobial susceptibility pattern of oral bacterial pathogens. In addition, the susceptibility/resistance profile of an organism is largely dependent on the panel of antimicrobial agents most commonly used in that geographical area.[9]

So far, there are no reports on the antimicrobial susceptibility pattern of *A. actinomycetemcomitans* from India isolated from the oral cavity of our subjects. Keeping this in mind, we undertook the present study, wherein oral isolates of *A. actnomycetemcomitans* were tested against a panel of 12 antibiotics which are either recommended for treating oral infections and/or most commonly used in hospital settings in our area. The testing was performed with commercially available gradient diffusion test strips. This study is part of a research project titled "Determination of Antimicrobial susceptibility pattern and induced metronidazole resistance and prevalence of drug resistance genes in oral Gram-negative anaerobes" funded by the Indian Council of Medical Research, New Delhi.

MATERIALS AND METHODS

The present study was carried out in the Central Research Laboratory of our Institution after obtaining approval from the Institutional Ethics Committee. A total of 40 strains of *A. actinomycetemcomitans* isolated from clinically and radiologically confirmed patients with LAP were included in the study. Written informed consent was obtained from each patient before the collection of the clinical material for testing. Subgingival plaque specimens from each participant were collected with sterile curette and placed in a vial of reduced transport fluid and sent to the laboratory. The vials were then vortexed briefly to break the plaque and plated onto Dentaid[10] and Trypticase soy Serum Bacitracin Vancomycin Agar prepared as per the instructions of the original authors.[11] The plates were incubated in an atmosphere of 5% Co_2 at 37°C for 72 h. The identity of the strains was confirmed by characteristic colony characters and various phenotypic tests such as catalase, oxidase, indole and fermentation of glucose, xylose, maltose and mannitol.[12] The isolates were then stored at −80°C in glycerol broth till tested.

Antimicrobial susceptibility testing was performed on each isolate using E-test gradient diffusion method (Ezy strip, Hi-Media). The antimicrobial agents used in the study included amoxicillin, amoxicillin/clavulanic acid (2:1) (amoxy-clav), tetracycline, doxycycline, clindamycin, azithromycin, moxifloxacin, cefazolin, ceftriaxone, cefuroxime, cefotaxime and metronidazole. Inocula of test strains were prepared in thioglycollate broth to a concentration of 0.5 MacFarland standard and inoculated onto brucella blood agar plates supplemented with hemin and menadione. One Ezy test strip of the respective antibiotic was placed in the center of the plate and the plates were then incubated anaerobically in a gas-pak jar at 37°C overnight. MICs were determined according to the manufacturer's instructions [Figure 1]. Two serotypes of *A. actinomycetemcomitans* serotype A (ATCC 29523) and serotype C (ATCC 43719) was used as standard strains in the assays.

Data analysis

The interpretative criteria for the susceptibility of anaerobes were applied to determine the breakpoints for

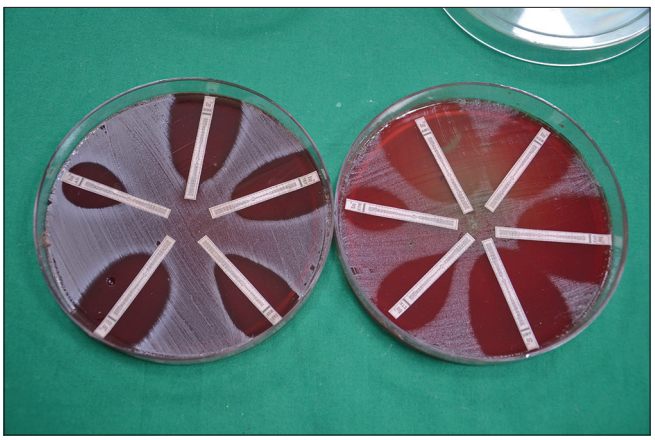

Figure 1: E-test showing zone of inhibition on blood agar

ampicillin, amoxyclav, ceftriaxone, cefotaxime, tetracycline, moxifloxacin, clindamycin and metronidazole.[13] Since guidelines for doxycycline, azithromycin, cefazolin and cefuroxime were not available for anaerobic bacteria, interpretative criteria for facultative anaerobic organisms were applied to these antibiotics[13] [Table 1].

Distribution of MICs among isolates (n = 40) were used to calculate concentrations inhibiting 50% (MIC_{50}) and 90% (MIC_{90}) of strains.

RESULTS

Both the standard strains of *A. actinomycetemcomitans* used in the study were susceptible to all the antibiotics tested except metronidazole. All clinical isolates were susceptible to moxifloxacin, cefotaxime and ceftriaxone. Only one strain had shown intermediate sensitivity to moxifloxacin, which persisted even after repeat testing.

Amoxicillin, amoxyclav and doxycycline showed very good effect inhibiting the growth of more than 90% strains [Table 2]. Tetracycline was slightly less effective with 5 strains showing intermediate susceptibility and 2 strains were resistant. Cefuroxime and cefazolin could exert inhibitory action on 77.5% and 75% of strains, respectively.

The test strains showed only moderate susceptibility to azithromycin (30%) and clindamycin (40%). On the other hand, all the isolates showed complete resistance to metronidazole.

When the susceptibility pattern of isolates studied was compared with MIC_{50} and MIC_{90} results, it could be seen that moxifloxacin, amoxicillin, amoxyclav and ceftriaxone had MIC_{90} of 1 ug/ml showing excellent efficacy. On the other hand, MIC_{90} values of azithromycin, tetracycline, clindamycin and cefuroxime were quite high, falling between 8 ug/ml and 64 ug/ml [Table 3]. Other antibiotics except metronidazole showed moderate MIC_{90} values.

DISCUSSION

Numerous investigations in the past two decades have established the definitive role played by *A. actinomycetemcomitans* in the etiology of aggressive periodontitis.[3] Studies have also shown that mechanical therapy alone cannot eliminate all the major periodontal pathogens such as *A. actinomycetemcomitans* from diseased sites mainly due to the inability of the periodontal instruments to access the deeper part of the gingival sulcus.[14] Hence in patients with aggressive periodontitis, supplemental antibiotic therapy is recommended by many clinicians.[8] However unfortunately, neither proper guidelines nor antimicrobial susceptibility pattern of *A. actinomycetemcomitans* are available from our country for adequate antimicrobial therapy.

The most common drugs used as part of the periodontal therapy include amoxicillin, amoxicillin-clavulanic acid, tetracycline, azithromycin, clindamycin, moxifloxacin and metronidazole.[8] However, keeping in mind, the most common antibiotics prescribed for systemic illnesses, we

Table 1: Susceptibility/resistance interpretative criteria for antibiotics included in the study

Antibiotic	Sensitive (S) (ug/ml)	Intermediate (I) (ug/ml)	Resistant (R) (ug/ml)
Amoxicillin	<0.5	1	>2
Amoxicillin-clavulinic acid	<4/2	8/4	>16/8
Azithromycin	<4	8	>16
Clindamycin	<2	4	>8
Cefazolin	<2	4	>8
Cefotaxime	<16	32	>64
Ceftriaxone	<16	32	>64
Cefuroxime	<4	8	>16
Doxycycline	<4	8	>16
Metronidazole	<8	16	>32
Moxifloxacin	<2	4	>8
Tetracycline	<4	8	>16

Table 2: The susceptibility pattern of *Aggregatibacter actinomycetemcomitans* isolates studied

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin	36 (90)	2 (5)	2 (5)
Amoxicillin-clavulanic acid	37 (92.5)	1 (2.5)	2 (5)
Azithromycin	25 (62.5)	3 (7.5)	12 (30)
Clindamycin	15 (37.5)	9 (22.5)	16 (40)
Cefazolin	30 (75)	5 (12.5)	5 (12.5)
Cefotaxime	40 (100)	0	0
Ceftriaxone	40 (100)	0	0
Cefuroxime	31 (77.5)	6 (15)	3 (7.5)
Doxycycline	38 (95)	2 (5)	0
Metronidazole	0	0	40 (100)
Moxifloxacin	39 (97.5)	1 (2.5)	0
Tetracycline	33 (82.5)	5 (12.5)	2 (5)

Table 3: Minimum inhibitory concentration$_{50}$, minimum inhibitory concentration$_{90}$ values and mean of range for each antibiotic tested

Antibiotic	MIC_{50} values (ug/ml)	MIC_{90} values (ug/ml)	Range (ug/ml)
Amoxicillin	0.5	1	0.064-16
Amoxicillin-clavulanic acid	0.75	1	0.064-8
Azithromycin	4	16	0.125-64
Cefazolin	1	4	0.5-24
Cefotaxime	0.5	2	0.038-8
Ceftriaxone	0.125	1	0.094-1
Cefuroxime	2	8	0.2-48
Clindamycin	4	64	1->256
Doxycycline	1	2	0.064-8
Metronidazole	>256	>256	64-256
Moxifloxacin	0.125	0.5	0.047-4
Tetracycline	2	8	0.125-16

MIC: Minimum inhibitory concentration

have made additions to this panel that include doxycycline, cefazolin, cefuroxime, cefotaxime and ceftriaxone. Even though *A. actinomycetemcomitans* is a facultative anaerobe, the antimicrobial susceptibility testing procedure adopted is that of anaerobic bacteria. There are three different methods for this purpose that include-agar dilution, broth microdilution and MIC gradient method by E-test strips.[9] In the present study, we have used gradient MIC test strips for antimicrobial testing of *A. actinomycetemcomitans* since the results are comparable to that of agar dilution method which is considered as the "gold standard."

Several studies have examined the effect of different periodontal therapies on clinical and microbiological parameters in LAP.[8,15] To the best of our knowledge, there are no publications about antimicrobial susceptibility pattern of *A. actinomycetemcomitans* from India using the MIC gradient method. In our study, the isolates studied showed high level of susceptibility to amoxicillin (90%) and amoxyclav (92.5%). Other investigators have shown varying results with moderate-to-high susceptibility to amoxicillin and usually excellent efficacy of amoxyclav.[16-19]

We found high level of resistance to metronidazole among our isolates (100%) with MIC_{50} values of >256 and moderate resistance to clindamicin and azithromycin. This is in accordance to the results of several other studies.[16-18,20] In the present study, doxycycline had a very good inhibitory effect (95%) on *A. actinomycetemcomitans* compared to tetracycline (82.5%). Investigators from several countries have demonstrated rising level of resistance in *A. actinomycetemcomitans* whereas doxycycline is said to be having excellent activity, even against biofilms of this organism.[16,21-23]

Fluoroquinolones are known to be having very good action against oral bacteria including *A. actinomycetemcomitans*. Among various drugs in this group, moxifloxacin has been approved by the FDI.[8] Almost all the investigators have shown that moxifloxacin has excellent activity against oral microbes, a finding similar to our results.[17-20] In our study, all the strains were highly susceptible to this drug with MIC_{50} and MIC_{90} values of <1 ug/ml except one strain which showed intermediate susceptibility (4 ug/ml). The readings were similar even on repeated testing. This aspect should be looked into.

For the first time, we have tested the activity of different cephalosporins on *A. actinomycetemcomitans* strains. We found the results to be highly variable. While cefotaxime and ceftriaxone showed very good efficacy (100%), with MIC_{50} values of <1 ug/ml, cefazolin (75%) and cefuroxime (77.5%) displayed moderate activity. Even though cefoxitin is the preferred drug from this group to treat anaerobes,[24] we chose to include the cephalosporins most commonly prescribed in our area for systemic/nonoral conditions. The findings clearly show that cefotaxime and ceftriaxone which belong to the 3rd generation of cephalosporins and have a broader range of activity have superior effect in comparison to cefazolin (1st generation) and cefuroxime (2nd generation) in bacterial growth inhibition.

CONCLUSION

The data presented here demonstrate the level of resistance of *A. actinomycetemcomitans* to different commonly prescribed drugs. It appears that moxifloxacin, amoxy-clav, amoxicillin and doxycycline have definite benefits over the other antibiotics. Moderate level of resistance shown against clindamycin and azithromycin indicate the limited efficacy of these drugs in treatment of aggressive periodontitis. The MIC gradient method of testing, even though expensive, has the advantage of ease of performance and interpretation and can be applied to even a single isolate at a time. We feel more such studies should be taken up from other parts of our country to get information on effect of geographical distribution on resistance pattern of *A. actinomycetmcomitans*.

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Conflicts of interest

There are no conflicts of interest.

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