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In-vitro evaluation of Antimicrobial potency of commercially available drugs against Dentinal Caries microbes

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Abstract

Bacterial infections are common in dental practice and use of antibiotics for their treatment is also frequent. But their rampant use leads to antimicrobial resistance; a global growing issue affecting both developed and developing countries. Determining the susceptibility profile of potential pathogens is therefore necessary. Efficacy of natural phyto-chemicals isolated from plants and oral hygiene products against Streptococcus mutans; the principal dental pathogen associated with dental caries and other cariogenic bacteria isolated using the method of pure culturing has been evaluated. However, the literature lacks studies evaluating the efficacy of antibiotics against the microflora responsible for dental caries. Therefore, commercially used drugs were screened for their in-vitro antimicrobial potency against the microbial community obtained from dentinal caries lesion using Kirby-Bauer disc diffusion method. The polymicrobial growth showed susceptibility towards all the tested antibiotics except in few samples where Ampicillin/Cloxacillin, Cefixime/Clavulanic acid, Cephalexin, Cefixime and Ampicillin/ Sulbactam showed no zone of inhibition; suggesting possible resistance to such antibiotics in these patients. The comprehensive data obtained may support the polymicrobial etiology of dental caries. Such study may further allow investigation on the spatial distribution of pathogenic, antibiotic resistant bacteria among patients suffering from dental caries.

Keywords: Dental caries, antibiotic susceptibility, Kirby-Bauer disc diffusion method.

Introduction

The preference of micro-organisms to live as surface adherent communities forms the basis of what is called a biofilm[1]. Compared to their planktonic counterparts, such microbial communities are self- sustainable, resistant to immune defense mechanisms of host and have increased tolerance to antimicrobial agents[2]; the root cause of several bacterial infections perceived in day-to-day clinical practice.

The oral cavity is a habitat to diverse microflora that colonizes various mucosal surfaces including teeth. Certain bacterial species have been implicated in oral diseases such as dental caries and periodontitis, which are among the most common biofilm (dental plaque) dependent bacterial infections in humans[3]. Although *Streptococcus mutans* (*S. mutans*) is established as the key pathogen responsible for dental caries; other species by their ability to produce acids can also be implicated in the disease; therefore pointing towards the polymicrobial nature of dental caries.

Odontogenic infections resulting from widespread caries and its sequelae are common in dental practice; thus antibiotic use prescribed for their treatment is also frequent that may lead to antimicrobial resistance. Determination of susceptibility pattern of microbes towards the antimicrobials used is essential; guiding the clinicians in selecting the best drug to combat such infections.

Many studies have been undertaken to evaluate the efficacy of commercially available drugs, natural phyto-chemicals isolated from plants used in traditional medicine and oral hygiene products against *S. mutans*; the principal dental pathogen associated with dental caries and other cariogenic bacteria isolated using the method of pure culturing[4-10]. However, there is lack of literature on studies evaluating the efficacy of antibiotics against the microflora responsible for dental caries.

With this aforementioned problem in mind, commercially used drugs in dentistry were screened for their *in-vitro* antimicrobial potency against the microbial community obtained from dentinal caries lesion using Kirby-Bauer disc diffusion method.

Material and Method

The following antibiotic discs were purchased from HiMedia Laboratories, Mumbai, India: Ampicillin/ Cloxacillin (Ax^{10}), Norfloxacin (Nx^{10}), Doxycycline Hydrochloride (DO^{30}), Ofloxacin (OF^{5}), Gatifloxacin (GAF^{5}), Cefixime/Clavulanic acid ($CMC^{5/10}$), Amoxycillin/Clavulanic acid (AMC^{30}), Gentamicin (GEN^{10}), Cephalexin (CN^{30}), Tetracycline (TE^{30}),

Ceftriaxone (CTR^{30}), Cefixime (CFM^{5}), Amikacin (AK^{30}), Lomefloxacin (LOM^{10}), Ampicillin/Sulbactam ($A/S^{10/10}$), Ampicillin (AMP^{10}), Erythromycin (E^{15}), Ciprofloxacin (CIF^{5}) and Levofloxacin (LE^{5}). All the other chemicals used were of analytical grade and manufactured in India.

The study was approved by the Institutional Review Board of Madurai Kamaraj University at Madurai, Tamil Nadu (India) on activities involving human subjects.

Thirty adult patients were enrolled in the study with their informed consent. Patients were included if they had a carious lesion that had spread into the dentine (confirmed radiographically) with cavitation. The superficial, necrotic layer was removed and discarded, and the samples were sequentially hand excavated with sterile, spoon excavator and collected at a level that represented the middle of the dentine lesion. The thirty dentinal caries samples were collected and subjected to further analysis.

Lesion samples from dentinal caries were inoculated on Luria-Bertani (LB) broth. Following overnight incubation at 37^0C on shaker at 180 rpm, the presence of turbidity in LB broth indicated bacterial growth. As per routine procedure in microbiology, the obtained bacterial growth was neither isolated further to get individual micro-organisms using pure culturing method nor characterized on the basis of biochemical tests. Antimicrobial potency of commercially available drugs against the obtained polymicrobial growth was evaluated using Kirby-Bauer disc diffusion method[11].

About 25 ml of the Mueller Hinton agar medium (HiMedia M173-500G) was cooled to 45^0C and poured into sterile petri plates and allowed to solidify completely. The entire surface of the agar plate was seeded with the polymicrobial growth using sterilized spreader. The plates were allowed to dry before antibiotic disc application. To prevent condensation, antibiotic discs were allowed to warm to ambient temperature before application. Within 15 min of inoculation, the antibiotic discs were placed flat side down on the seeded agar surfaces using sterile forceps, followed by incubating agar plates for 24 h at 37^0C. The relative susceptibility of the polymicrobial growth to each antibiotic was shown by a clear zone of inhibition measured in mm. For control, polymicrobial growth-free agar plates were incubated with antibiotic discs.

Results and Discussion

Results: The susceptibility pattern of the dentinal caries microbes against 19 commercially used antimicrobial drugs in dentistry was assayed by Kirby-Bauer disc diffusion method. The polymicrobial growth showed susceptibility towards all the tested antibiotics except in few samples where Ampicillin/Cloxacillin, Cefixime/Clavulanic acid, Cephalexin, Cefixime and Ampicillin/Sulbactam showed no zone of inhibition; suggesting possible resistance to such antibiotics in these patients.

Among the fluoroquinolone group (tables 1 and 2) ; the susceptibility of the polymicrobial growth from the dentine samples were tested against both the first and second generation antibiotics, viz., Norfloxacin (Nx), Ofloxacin (OF), Ciprofloxacin (CIF), Gatifloxacin (GAF), Levofloxacin (LE) and Lomefloxacin (LOM). Nx exhibited largest inhibition zone and thus appears to be the most effective among the first generation fluoroquinolones, with zone of inhibition of 42 mm followed by OF (35 mm) and CIF (30 mm); while GAF ranked highest with 40 mm zone of inhibition followed by LOM (34 mm) and LE (30 mm). As a whole, the second generation fluoroquinolones were found to be more effective than the first generation antibiotics; since the additional fluoro and other substitutions in these drugs exhibited higher potency and had further extended their antimicrobial activity from Gram-positive cocci to anaerobes (figure 1).

Among the β-Lactam antibiotics group (tables 3 and 4); the susceptibility of the polymicrobial growth was tested against cephalosporins and extended spectrum penicillins. Cephalosporins tested included both the first and third generation antibiotics, viz., Cephalexin (CN), Cefixime (CFM) and Cefatrixone (CTR). CN exhibited greatest potency with zone of inhibition of 45 mm followed by CTR (33 mm) and CFM (25 mm). 5/30 samples of CN and 2/30 samples of CFM exhibited no clear zone of inhibition. The extended spectrum penicillin i.e., ampicillin (AMP) was also tested and showed an inhibition zone of 35 mm. Among the antimicrobial combinations used: the greatest potency was shown by Amoxycillin / Clavulanic acid (AMC) (40 mm) followed by Cefixime / Clavulanic acid (CMC) (39 mm), Ampicillin / Sulbactam (A/S) (39 mm) and Ampicillin / Cloxacillin (Ax) (38 mm). Combinations were used with an objective to broaden the spectrum of antimicrobial action. It is interesting to note that two samples each of Ax and CMC; and one sample of A/S exhibited no clear zone of inhibition against the polymicrobial growth (figure 2).

The other groups selected for the present study were aminoglycosides, macrolide and broad spectrum antibiotics (tables 5 and 6). Among the aminoglycosides, two antibiotics were selected, viz., Gentamicin (GEN) and Amikacin (AK); showing almost similar susceptibility pattern with maximum zone of inhibition of 32 mm and 31 mm respectively. From the macrolide antibiotics group, Erythromycin (E) was selected for the present study since the drug is known to be highly active against *S. pyogenes*. From the broad spectrum antibiotic group, Tetracycline (TE) and Doxycycline Hydrochloride (DO) were tested against the polymicrobial growth. TE (37 mm) was found to exhibit greater zone of inhibition than DO (32 mm) (figure 3).

Tables-1 and 2

Antibiotic susceptibility pattern of the polymicrobial growth samples (1 – 30) towards fluoroquinolones. The numbers in the column indicates zone of inhibition (mm)

Fluoroquinolones	Samples ↓														
Antibiotics ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Nx	30	35	21	12	19	19	25	11	21	20	19	20	35	34	09
OF	26	35	20	23	20	21	21	17	18	18	20	24	28	33	17
GAF	30	35	30	22	30	29	38	22	29	28	23	25	34	40	21
LOM	23	27	22	22	20	22	20	10	14	17	18	20	25	28	11
CIF	29	26	30	08	25	25	17	15	22	24	18.5	25	28	11	23
LE	26	27	29	13	26	25	30	18	20	22	21	19	14	25	16

Fluoroquinolones	Samples ↓														
Antibiotics ↓	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Nx	32	18	35	26	42	12	10	28	20	10	19	31	19	12	22
OF	33	18	25	29	34	23	17	25	20	18	21	24	19	21	25
GAF	39	26	32	34	44	22	24	30	29	22	27	33	23	25	30
LOM	30	18	15	23	34	22	11	25	22	16	20	28	12	26	27
CIF	09	21	11	22	14	10	25	26	11	28	23	24	17	23	18
LE	18	14	25	20	24	15	26	19	17	14	25	28	23	25	27

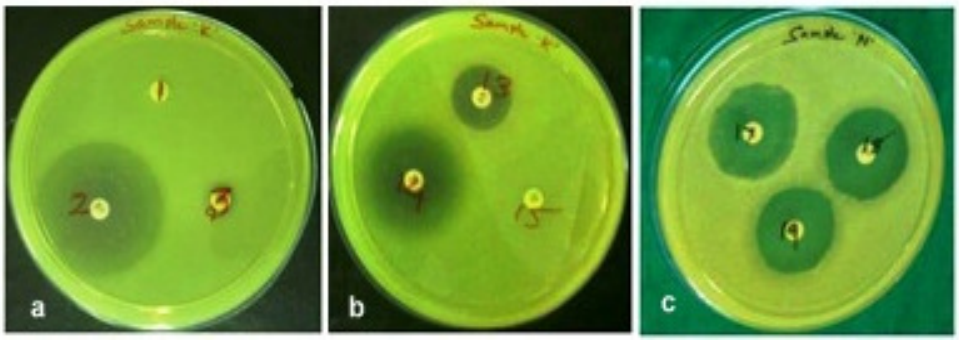

Figure-1

Zones of inhibition exhibited by Nx [a], LOM [b] and CIF [c] on Mueller Hinton agar media seeded with the polymicrobial growth from dental caries samples.

Tables-3 and 4

Antibiotic susceptibility pattern of the polymicrobial growth samples (1 – 30) towards β-Lactam Antibiotics. Column number indicates zone of inhibition (mm)

β-Lactam Group	Samples ↓														
Antibiotics ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ax	34	06	24	15	11	11	21	15	38	23	08	06	-	28	07
CMC	28	17	25	20	15	15	39	16	23	23	12	10	-	30	11
AMC	36	06	25	16	18	19	40	20	24	23	12	13	09	25	14
CN	29	-	33	16	18	19	12	12	25	23	18	06	-	34	-
CTR	26	12	30	22	28	27	30	17	21	20	15	13	22	33	18
CFM	20	06	08	20	25	25	10.5	12	15	31	-	11	13	15	13
A/S	36	07	33	18	20	20	39	20	30	29	12	12	12	30	15
AMP	35	06	15	17	14.5	12	21	14	15	13	20	16	23	18	22

β-Lactam Group		**Samples**													
Antibiotics	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Ax	29	12	35	26	-	15	18	08	25	17	13	16	23	20	10
CMC	29	19	19	25	-	20	16	09	22	13	15	09	10	18	14
AMC	28	18	15	28	07	16	20	11	23	16	19	10	09	25	10
CN	45	-	16	31	-	16	12	07	30	08	19	14	25	13	09
CTR	33	24	22	30	16	22	17	22	15	19	27	20	13	26	14
CFM	12	13	06	20	-	20	12	13	11.5	13	24	17	10	07	08
A/S	34	14	30	33	-	18	20	14	33	21	16	18	11	23	09
AMP	16	23	18	22	11	17	14	20	15	16	12	20	22	13	08

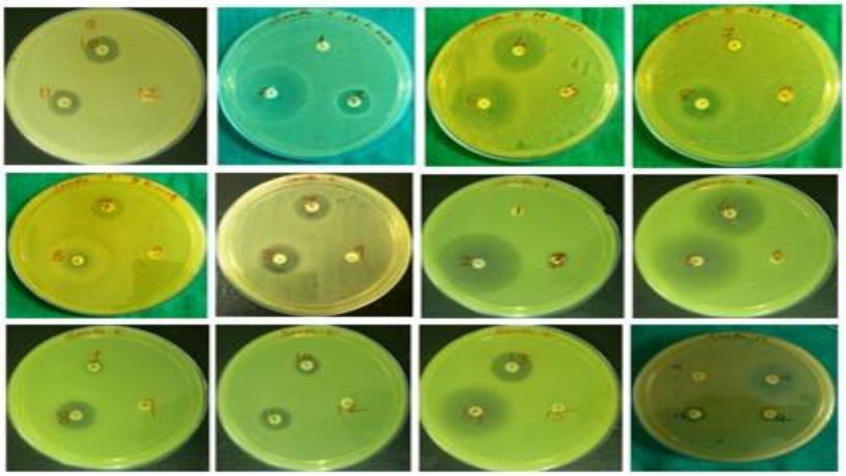

Figure-2
Samples exhibiting no clear zone of inhibition on Mueller Hinton agar media seeded with the polymicrobial growth from dental caries samples

Tables-5 and 6
Antibiotic susceptibility pattern of the polymicrobial growth samples (1 – 30) towards other antibiotics selected. The numbers in the column indicates zone of inhibition (mm).

Other Group		**Samples**													
Antibiotics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
DO	32	27	29	16	19	19	32	25	29	28	19.5	17	12	20	22
GEN	23	32	26	15	19	20	24	19	19	20	18	19	16	26	20
TE	29	23	32	23	23	24	29	25	28	31	15	22	19	27	20
AK	24	31	26	18	20	20	25	25	23	24	18	20	20	30	19
E	23	27	30	11	21	18	30	24	24	14	19	23	26	20	19

Other Group		**Samples**													
Antibiotics	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
DO	29	20	22	32	12	16	25	14	17	17	19	11	15.5	16	24
GEN	30	18	21	21	20	16	19	16	26	23	20	18	16	24	23
TE	29	23	37	31	15	23	25	19	18	20	24	27	21	27	25
AK	31	23	26	25	21	19	25	20	25	19	21	22	20	21	13
E	23	26	21	19	20	19	22	24	20	19	19	21	23	22	21

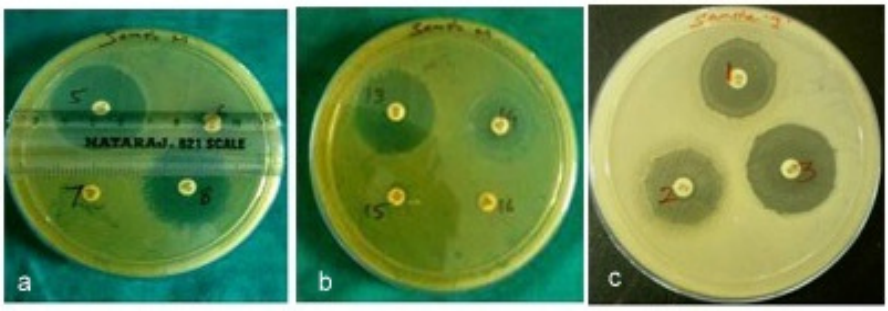

Figure-3
Zones of inhibition exhibited by GEN [a], AK [b] and DO [c] on Mueller Hinton agar media seeded with the polymicrobial growth from dental caries samples

Discussion: Dental caries is a worldwide disease known to affect every population throughout life. Due to its prevalence and high cost of treatment; it has emerged as key health predicament in developed countries. Furthermore, determining the bacterial species implicated directly in the disease or recognizing the mere by-standers, makes the etiology of dental caries challenging[3,12].

Utilization of antimicrobials in treating dental infections has gained much attention worldwide. But the widespread concern about the increasing problem of antimicrobial resistance has emphasized the need for rationalization of antibiotic use. Therefore, in context to lowering down the antimicrobial resistance rate; it is imperative to consider routine susceptibility testing. Such tests could be useful in improving the treatment efficacy[13].

Susceptibility testing is the determination of the bacterial pattern of resistance to a number of antibiotics. Antimicrobial susceptibility testing can be done by several different methods. Kirby-Bauer disc diffusion method was utilized in the present study, since the method employed is easy to perform and reliable. The antibiogram of the isolated polymicrobial growth, in the present study indicated a wide range of susceptibility to different antibiotics and therefore, seems that the use of these antimicrobial drugs may reduce the incidence and severity of the disease per se and its sequelae.

Fluoroquinolones are quinolone antimicrobials having one or more fluorine substitutions; that further expand its antimicrobial activity, potency and /or confer metabolic stability. These are also active against many β-lactam resistant bacteria. β-lactams such as cephalosporins and penicillins are bactericidal drugs that act by inhibiting the bacterial cell wall synthesis. β-lactams are non-toxic and have minimum concentration-dependent adverse effects; but allergic reaction mainly with penicillins is the most significant undesirable effect[14].

Amoxycillin/clavulanic acid, combination of a β-lactam antibiotic (amoxicillin trihydrate) and a β-lactamase inhibitor (potassium clavulanate) has broad antimicrobial spectrum and effective against amoxicillin-resistant bacteria that produce β-lactamase[15]. Such antimicrobial agent may prove valuable for managing dental infections.

The tetracyclines are broad spectrum antibiotics that act by inhibiting bacterial protein synthesis. They are relatively inexpensive drugs and have been extensively used in the prophylactic and therapeutic regimens. These antibiotics have selective affinity for deposition in tooth substance, possibly through formation of a complex with calcium ions of hydroxyapatite crystals; leading to tooth discolouration[16]. Therefore, pertaining to dental infections, such antibiotics must be used with caution.

Selection of an antibiotic regimen should be based on knowledge of the efficacy of an antibiotic for the bacteria, most often associated with severe infections. It should also be remembered that dental caries represent an ecosystem of bacteria in which by-products of one species of bacteria may be nutrients for other species of bacteria. Thus, if an antibiotic is effective against some species of bacteria in a polymicrobial infection, it may indirectly affect other bacteria in that ecosystem.

Dental caries, though a disease of great antiquity, is not a disease of the past since it is reappearing in many countries as a public health crisis. It is therefore, important that the public health be taught on prevention, early recognition and reporting to dental clinics for proper prognosis. This underscores the need for laboratory diagnosis, confirmation of dental caries and the antibiograms of incriminating micro-organisms for proper management of patients and to reduce the development of resistant strains or multiple resistances to antibiotics. Since antibiotic sensitivity tests aids in determining the targets for therapeutic intervention; further studies using larger samples are necessary to assess the level of protection against dental caries offered by the use of these antibiotics.

Conclusion

The comprehensive data obtained from the present study may support the polymicrobial etiology of the disease. Continue surveillance in the form of routine susceptibility tests is an

excellent source of information on the prevalence of resistant pathogens within the dental caries. Further, since the results from the present investigation reveals that patients differ in their antibiotic sensitivities; therefore such studies may prove an invaluable aid to form a sound policy on antibiotics usage in the clinical practice.

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