MICs of 407 Oral Streptococci strains isolated from closed abscess of odontogenic infection

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The MICs of 407 strains of Oral Streptococci, collected and measured in clinical tests of new antimicrobial agents conducted during the period from September, 1989 to June, 1991, were studied. The following 9 antimicrobial agents were used: ampicillin (ABPC), cefteram (CFTM), cefuroxime (CXM), cefpodoxime (CPDX), cefdinir (CFDN), cefixime (CFIX), cefaclor (CCL), ofloxacin (OFLX) and levofloxacin (LVFX). We have come to the conclusion that ampicillin has a higher MICs than previously. On the other hand, ester-type cephems, cefteram pivoxil (cefteram), cefuroxime axetil (cefuroxime) and cefpodoxime proxetil (cefpodoxime), proved to have low and excellent MICs. Cefdinir (non-ester-type) also showed a low, and excellent, MICs.

Key words: Oral Streptococci, odontogenic infection, MIC

We have already submitted a study on measurement of the MICs of 1,024 strains of causative organisms from odontogenic infectious diseases for which oral administration of drugs is appropriate. This study focuses on measurement of the MICs of Oral Streptococci which constitute significant portion of the above mentioned causative organisms.

I. Material

1. Bacterial strains

The strains of causative organisms were identified from samples obtained from closed abscesses by the needle aspiration method. The strains studied here were isolated at the institutes which conducted clinical tests of new antimicrobial agents from September, 1989 to June, 1991 by the multi centric isolation method. A streptococcal identification kit, Api-STREP system® was used for biochemical analysis, with special reference to the carbohydrate metabolism of test strains. A total of 1024 strains of bacteria were isolated from closed abscesses. Oral Streptococci constituted 407 of these strains.

Table 1 shows the details of the 407 strains.

These strains were stored in 10% skim milk at −80°C until the measurement.

2. Antimicrobial agents

The following 9 antimicrobial agents were used:

1) ampicillin (ABPC, Meiji Seika)

For oral administration, lenampicillin, talampicillin and bacampicillin were used. The antimicrobial activity was measured in the same way as

Table 1. Details of Oral Streptotococci

	strains
Streptococcus anginosus	33
Streptococcus constellatus	84
Streptococcus intermedius	47
Streptococcus milleri	60
Streptococcus milleri II	1
Streptococcus mitis	42
Streptococcus salivarius	20
Streptococcus sanguis	97
Streptococcus sanguis II	19
Streptococcus mutans	4
Total	407

that of ampicillin.

2) cefteram (CFTM, Toyama Kagaku)

For oral administration, an ester-type cefteram-pivoxil was used. The antimicrobial activity was measured in the same way as that of cefteram.

3) cefuroxime (CXM, Glaxo)

For oral administration, an ester-type, cefuroxime axetil was used. The antimicrobial activity was measured in the same way as that of cefuroxime.

4) cefpodoxime (CPDX, Sankyo Seiyaku)

For oral administration, an ester-type, cefpodoxime proxetil was used. The antimicrobial activity was measured in the same way as that of cefpodoxime.

5) cefdinir (CFDN, Fuzisawa Seiyaku)

Because it does not change in internal organs, the antimicrobial activity was measured as was that of cefdinir.

6) cefixime (CFIX, Fuzisawa Seiyaku)

Because it does not change in internal organs, the antimicrobial activity was measured as was that of cefixime.

7) cefaclor (CCL, Shionogi Seiyaku)

Because it does not change in internal organs, the antimicrobial activity was measured as was that of cefaclor.

8) ofloxacin (OFLX, Daiichi Seiyaku)

This is a typical pyridonecarboxylic acid agent. The antimicrobial activity was measured in the same way as that of ofloxacin.

9) levofloxacin (LVFX, Daiichi Seiyaku)

This agent consists of lasemi bodies, a major component of ofloxacin. The antimicrobial activity was measured in the same way as that of levofloxacin.

II. Methods of susceptibility tests

The minimal inhibitory concentrations (MIC) of the test agents against organisms were determined in the following manner: A Mueller Hinton medium combined with 5% horse serum and a 2-fold serial dilution of each antimicrobial agent was inoculated with each strain, previously adjusted to 10^6 CFU/ml. After incubation at 35°C for 24 hours, the minimal concentration at which no bacterial growth was noted, was designated as the MIC of the agent[1].

The β-lactamase activity was determined by the acidometric and nitorocefin methods.

III. Results

Table 2 shows MICs against *Streptococcus anginosus*. Table 3 shows MICs against *Streptococcus constellatus*. Table 4 shows MICs against *Streptococcus intermedius*. Table 5 shows MICs against *Streptococcus milleri*. Table 6 shows MICs against *Streptococcus mitis*. Table 7 shows MICs against *Streptococcus sanguis*. Table 8 shows MICs of all the Oral

Table 2. MICs for *Streptococcus anginosus*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	13	10	1	1	1										≦0.05	0.1	0.2
CFTM	27	18	3	6											≦0.05	0.2	0.2
CXM	24	6	10	5	1	2									0.1	0.2	0.39
CPDX	14	8	2		3	1									≦0.05	0.39	0.39
CFDN	11	8	2		1										≦0.05	0.1	0.1
CFIX	3						1		2						6.25	6.25	6.25
CCL	18			1	1	3	10	2	1						1.56	1.56	3.13
OFLX	2				1	1									0.39	0.78	0.78
LVFX	4				2	2									0.39	0.78	0.78

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

Table 3. MICs for *Streptococcus constellatus*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	31	17	2	5	5	1	1								≦0.05	0.39	0.39
CFTM	48	30	7	8	2	1									≦0.05	0.2	0.2
CXM	39	17	10	6	3	2	1								0.1	0.2	0.39
CPDX	26	12	3	2	7	2									0.1	0.39	0.39
CFDN	16	12	1	2	1										≦0.05	0.1	0.2
CFIX	9					2		3	4						3.13	6.25	6.25
CCL	48		3		3	6	18	10	6		1	1			1.56	3.13	6.25
OFLX	15			1		3	10		1						1.56	1.56	1.56
LVFX	20					9	11								1.56	1.56	1.56

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

Table 4. MICs for *Streptococcus intermedius*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	16	6	4	4		2									0.1	0.2	0.78
CFTM	18	12	1	5											≦0.05	0.2	0.2
CXM	12	12													≦0.05	≦0.05	≦0.05
CPDX	22	5	1	4	11		1								0.39	0.39	0.39
CFDN	5	5													≦0.05	≦0.05	≦0.05
CFIX	6							5	1						3.13	3.13	6.25
CCL	35		1	2	6	9	6	8	2	1					0.78	3.13	3.13
OFLX	11					3	7	1							1.56	1.56	1.56
LVFX	7				2	5									0.78	0.78	0.78

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

Streptococci examined at this time. Ampicillin, as well as four kinds of cephem (cefteram, cefuroxime, cefpodoxime and cefdinir), proved to have low MICs.

β-lactamase-producing capacity was not in these Oral Streptococci.

IV. Discussion

In the United States, *S. anginosus, S. constellatus,* and *S. intermedius* (including *S. milleri*) were collectively called SMG[2] With recent advances in DNA hybridyzation techniques, however, efforts are being made to group them under the name of *S. anginosus*[3]. Researchers in the United Kingdom object to this classification[4]. In view of the diverse susceptibility of these organisms to antimicrobial agents, in Japan, we do not think it appropriate to group them under the single heading of *S. anginosus*[5].

When compared with the results of 1989 compilation, ampicillin resistance of Oral Streptococci

Table 5. MICs for *Streptococcus milleri*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	2	1		1													
CFTM	6	5		1											≦0.05	≦0.05	0.2
CXM	5	2	3												0.1	0.1	0.1
CPDX	36	3	3	6	22	2									0.39	0.39	0.39
CFDN	0																
CFIX	1							1									
CCL	43			2	3	12	9	14	3						1.56	3.13	3.13
OFLX	2					1	1										
LVFX	17						16	1							1.56	1.56	1.56

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

Table 6. MICs for *Streptococcus mitis*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	18	9	5		3			1							≦0.05	0.39	0.39
CFTM	20	13	4		2				1						≦0.05	0.1	0.39
CXM	15	6	4	3	2										0.1	0.2	0.39
CPDX	21	11	6			2	1	1							≦0.05	0.1	0.78
CFDN	7	5	1	1											≦0.05	0.1	0.2
CFIX	4			1		2			1						0.78	6.25	6.25
CCL	34		1		5	8	8	5	3	2		1	1		1.56	6.25	12.5
OFLX	11		1			1	6	3							1.56	3.13	3.13
LVFX	1						1										

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

appeared to have increased. Specifically, it was reported that the MIC_{80} and MIC_{100} of ampicillin were 0.025 and 0.10 μg/ml, respectively, in 225 strains of Oral Streptococci that were examined from 1987 to 1989[6]. While the current data reveal that Oral Streptococci have gained resistance to ampicillin (MIC_{80} and MIC_{100}, 0.20 and 3.13 μg/ml, respectively, Table 8).

Their resistance to cephems is unchanged.

The 1989 compilation yielded the following data: MIC_{80} and MIC_{100} of cefteram: 0.05 and 0.39 μg/ml; MIC_{80} and MIC_{100} of cefuroxim: 0.1 and 0.39 μg/ml; and MIC_{80} and MIC_{100} of cefaclor: 1.56 and 12.5 μg/ml.

In the present study, the MIC_{80} of cefteram, cefuroxime, and cefaclor were 0.10, 0.39 and 3.13 μg/ml, respectively.

There is a report that some highly ampicillin-resistant strains are also resistant to other, including cephem, antimicrobial agents[7]. Specifically, 3 strains of *S. sanguis* showed ampicillin susceptibilities of 12.5 μg/ml and 6 showed susceptibilities of

Table 7. MICs for *Streptococcus sanguis*

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	48	36	4	3	3	2									≦0.05	0.1	0.39
CFTM	54	49	4	1											≦0.05	≦0.05	≦0.05
CXM	45	35	7	1	2										≦0.05	0.1	0.1
CPDX	56	34	9	10	1	2									≦0.05	0.2	0.2
CFDN	31	28	1	2											≦0.05	≦0.05	≦0.05
CFIX	9		1	1		4	2	1							0.78	1.56	3.13
CCL	56		2	9	6	13	14	9	3						0.78	3.13	3.13
OFLX	17					1	6	10							3.13	3.13	3.13
LVFX	10				1	1	6	2							1.56	1.56	3.13

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

Table 8. MICs for Oral Streptococci

	strains	≦0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	MIC_{50}	MIC_{80}	MIC_{90}
ABPC	136	86	16	14	12	5	2	1							≦0.05	0.2	0.39
CFTM	207	146	23	27	6	1	1		3						≦0.05	0.1	0.2
CXM	170	90	38	22	12	4	1	3							≦0.05	0.2	0.39
CPDX	188	82	25	24	44	9	2	1	1						0.1	0.39	0.39
CFDN	75	62	5	5	2		1								≦0.05	≦0.05	0.2
CFIX	36		1	2	2	9	4	10	8						1.56	6.25	6.25
CCL	270	1	11	18	27	58	75	51	22	3	1	2	1		1.56	3.13	6.25
OFLX	61		1	1	1	10	30	17	1						1.56	3.13	3.13
LVFX	62				5	19	35	3							1.56	1.56	1.56

ABPC: ampicillin, CFTM: cefteram, CXM: cefuroxime, CPDX: cefpodoxime, CFDN: cefdinir, CFIX: cefixime, CCL: cefaclor, OFLX: ofloxacin, LVFX: levofloxacin.

25 μg/ml.

The MICs of cephalothin, against the same strains were 12.5 and 25 μg/ml. The MICs of cefaclor, for the same strains were 50 and 100 μg/ml. These resistant strains also showed notable resistance to macrolides. It has been reported that changes in the affinity of penicillin-binding protein (PBP) are have been considered in the mechanism of drug resistance development in streptococci[8~10] Indeed, PBP was found to be underlic the drug resistance mechanism of the aforementioned multi-drug-resistant *S. sanguis*[11]. It was thus concluded that the ampicillin-resistant strains showed different susceptibilities to each cephem.

Conclusion

After studying the MICs of 407 strains of Oral Streptococci, collected and measured at clinical tests of new antibacterial agents conducted during the period from September 1989, to June, 1991, we have come to the conclusion that ampicillin has a higher MICs than previously. We noted that the MIC of *S. constellatus* was 1.56 μg/ml and that of *S.*

intermedius was as high as 0.78 μg/ml. On the other hand, ester-type cephems, cefteram pivoxil (cefteram), cefuroxime axetil (cefuroxime) and cefpodoxime proxetil (cefpodoxime) proved to have low and excellent, MICs. Cefdinir (non-ester -type) also had a low, and excellent, MICs.

Assignment for future

We have already submitted a report on the pharmacokinetics of the antimicrobial agents examined here. Therefore, we intend to conduct further research on Post Antibiotic Effect (PAE) with which we expect to be able to clarify the break point.

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歯性感染症の閉塞膿瘍から検出されたOral Streptococci 407株の薬剤感受性

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1989年9月から1991年6月まで，新しい抗菌剤の臨床試験を行い，407菌株のOral Streptococci の薬剤感受性の検討を行った。抗菌剤はampicillin (ABPC), cefteram (CFTM), cefuroxime (CXM), cefpodoxime (CPDX), Cefdinir (CFDN), cefixime (CFIX), cefaclor (CCL), ofloxacin (OFLX) および levofloxacin (LVFX) の9薬剤について検討を行った。近年，ampicillinの耐性化傾向が認められるがエステル型セフェムのcefteram pivoxil (cefteram), cefuroxime axetil (cefuroxime) およびcefpodoxime proxetil (cefpodoxime) 非エステル型セフェムのefdinirの感受性は良好であった。

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