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Antibiotic resistance of pathogenic bacteria from odontogenic infections in Taiwan

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The purpose of this study was to evaluate the susceptibility of facultative and anaerobic odontogenic infectious flora to various antibiotics. We assessed 178 bacterial strains isolated from 74 patients with odontogenic infections. The E-test was used to determine susceptibility. The microbial flora was predominantly facultative gram-positive organisms and anaerobic gram-negative bacilli. The results of antimicrobial susceptibility test showed that ampicillin resistance was found with a very high level of minimum inhibitory concentration (MIC) in approximately one third of *Fusobacterium nucleatum*, *Prevotella intermedia*, *Peptostreptococcus micros*, and *Eikenella corrodens* isolates (MIC ≥8 μg/mL). Greater activity was generally noted with amoxicillin than with ampicillin, but even β-lactamase inhibitor incorporated amoxicillin showed resistance in more than 10% of all groups except viridans group streptococci and *Porphyromonas gingivalis*. Tetracycline and erythromycin were considerably less active against the majority of the tested bacterial strains, while minocycline and doxycycline exerted strong antimicrobial activity and could inhibit strains grown at a very low concentration. Among all the tested antibiotics, travofloxacin appears to be a promising drug expressing the highest activities (MIC_{90} ≤1 μg/mL), and was regarded as a potent bactericidal drug in odontogenic infections.

Key words: E-test, odontogenic infection, antimicrobial susceptibility

The dentist commonly encounters infections of the oral and maxillofacial region which always offers a management problem. The essential features of infection management include drainage and debridement of the affected tissues. Unless the source of the infection is removed, all therapeutic methods will fail. In general, most bacteria in odontogenic or oral infections are anaerobic species similar to those found in subgingival plaque [1,2]. Because of the awareness that these infections are caused by particular bacteria, several microbiological studies have been performed over the past decade to establish the etiological bacteria [3-5]. The majority of the microbiota were noted to consist of both facultative and obligatory anaerobic microorganisms, which often occurred in more than 2 species [6]. For this reason, the use of antimicrobial chemotherapy has been considered in order to improve the therapeutic effect [4,5]. Unfortunately, although dentists might be successful in treating many oral infections without identification or knowledge of the specific etiologic agents, they usually neglect the susceptibility tests for the appropriate antibiotic agents (selection and dosage). This approach is not effective, allowing unpredictable resistance to possibly occur [7]. Therefore, it is necessary to determine the antimicrobial susceptibility of the bacteria involved and the appropriate antibiotic dosages to maintain therapeutic levels.

This study was initiated to isolate the predominant microorganisms in odontogenic abscesses and determine the species. The results were provided in 2 settings: (a) for the clinician to know which antibiotics are effective against the pathogens likely to be present and managing selected individual patients; (b) determining the susceptibility test patterns for new agents.

Materials and Methods

Patients, specimen, collection, and processing

Seventy-four patients, ages ranging from 9 to 72 years old, were randomly selected for this study. All patients suffered from pyogenic infections of odontogenic origin.

The lesion site to be sampled was irrigated with sterilized normal saline, then thoroughly dried and isolated using 2 cotton rolls. Pus or exudates were collected with 2 paper points by absorbing the lesion

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under aseptic conditions. After sampling, the specimen was immediately transferred into an anaerobic viable microbiostatic gelatin agar III in sterilized transport vials [8]. After appropriate dilution, the bacterial culture specimen was spread evenly on the entire plate using a sterilized glass rod. The processed specimen was then kept in an anaerobic chamber (Anaerobic workstation AW 200, Electrotek, West Yorks, UK) at 37°C, which provided information on the total viable bacterial counts.

Bacterial culture and identification

The medium used in this investigation for bacterial isolation was Brucella Agar (BBL Microbiology systems, Cockeysville, MD, US) enriched with 5% defibrinated horse blood, 0.5% hemolyzed blood, and 5 μg/mL of menadione (BBAP). The growing bacteria were inspected after 5 to 9 days incubation at 37°C. The preliminary bacterial species identification included colony morphology, diameter of colony, form, long-wavelength ultraviolet light fluorescence, pigmentation, agar pitting, spread and hemolytic types, Gram-stain, motility, flagella, spore, shape, arrangement, and cell branching [9]. For further biochemical identification of the bacterial species, all of the isolates were confirmed by RapID ANA II system (Innovative Diagnostic Systems Inc., GA, US).

E-test

The antimicrobial agents in this study included ampicillin, amoxicillin, amoxicillin/clavulanate, cephalexin, cefixime, clindamycin, doxycycline, erythromycin, metronidazole, minocycline, tetracycline, and trovafloxacin. The PDM E-test (AB Boidisk, Solna, Sweden) was used as described by the manufacturer for determination of minimum inhibitory concentration (MIC) [10]. The MIC values were determined using the elliptical intersection of the scale on the strip where complete inhibition zones were seen. Regular quality control practices were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) recommended method [11].

Results

The bacterial species and the number of strains isolated in each species from patients are shown in Table 1. Six major species of bacteria were isolated from 74 specimens in this study. According to the bacterial isolation rate, the viridans group streptococci were the most abundant. The isolation rate for this group was 54.1% (40/74 specimens). Other species with a high isolation rate included anaerobic gram-negative bacilli such as *Fusobacterium* and black-pigmented bacteroides. The isolation rates for *Fusobacterium nucleatum* and *Prevotella intermedia* were 47.3% (35/74) and 39.2% (29/74), respectively. *Peptostreptococcus micros* was found in 36.5% (27/74) of the isolates. Other odontogenic pathogens including *Eikenella corrodens* and *Porphyromonas gingivalis* were also identified, and their isolation rates were 32.4% (24/74) and 31.1% (23/74), respectively. These bacteria were used for the *in vitro* antibacterial activity test.

The susceptibility test results for all of the tested antimicrobial agents against these bacteria are presented in Table 2. *F. nucleatum* demonstrated a very high level of MIC to both ampicillin and amoxicillin. It was noted that about 11% of the *F. nucleatum* strains were resistant at concentrations above 256 μg/mL to ampicillin and the MIC_{90} was equal to 128 μg/mL to amoxicillin. The

Table 1. Microorganisms isolated from patients with odontogenic infection

	Microorganism (no. of isolates)	
	Anaerobic bacteria	Aerobic and facultative bacteria
Gram (+) cocci	*Peptostreptococcus micros* (27)	Viridans group streptococci (40)
		Enterococcus spp. (13)
		Staphylococcus epidermidis (1)
		Staphylococcus aureus (2)
Gram (+) rods	*Corynebacterium* spp. (2)	*Actinomyces* spp. (4)
	Eubacterium spp. (4)	*Lactobacillus* spp. (1)
Gram (-) cocci	*Veillonella* spp. (3)	
Gram (-) rods	*Prevotella intermedia* (29)	*Eikenella corrodens* (24)
	Fusobacterium nucleatum (35)	*Pseudomonas aeruginosa* (1)
	Porphyromonas gingivalis (23)	
	Bacteriodes melaninogenicus (4)	
	Bacteriodes gracilis (2)	
	Capnocytophaga ochracea (1)	

Table 2. *In vitro* susceptibilities of dominant microorganisms isolated from odontogenic infections

Microorganism, no. of isolates (%)	Antimicrobial agent	MIC (μg/mL)				Susceptible rates (%)
		Range	50%	70%	90%	92.5
Viridans group streptococci,	Ampicillin[b]	0.64-16	1	2	3	95.0
40 (54.1)	Amoxicillin[c]	0.125-16	0.5	1.0	4.0	100
	Amoxicillin/clavulanate[c]	0.125-8	1.5	4	8	100
	Cefixime[e]	0.19-24	1.5	8	16	62.5
	Cephalexin[e]	0.25-128	16	48	64	100
	Clindamycin[b]	0.016-2	0.75	1.5	2	97.5
	Doxycycline[c]	0.032-48	0.75	2	8	32.5
	Erythromycin[e]	0.25->256	6	32	192	45.0
	Metronidazole[d]	0.25->256	32	64	>256	100
	Minocycline[c]	0.125-8	1.5	2	4	45.7
	Tetracycline[c]	0.75-128	16	64	128	100
	Trovafloxacin[a]	0.064-1.0	0.25	0.75	1	74.3
Fusobacterium nucleatum,	Ampicillin	0.25->256	1	3	256	80.0
35 (47.3)	Amoxicillin	0.19-256	6	7	128	88.6
	Amoxicillin/clavulanate	0.19-24	0.75	4	12	91.4
	Cefixime	0.25-96	4	16	32	68.6
	Cephalexin	0.047-192	16	32	96	85.7
	Clindamycin	0.094->256	2	4	16	100
	Doxycycline	0.014-4	1	3	4	48.6
	Erythromycin	0.5->256	32	64	128	51.4
	Metronidazole	6->256	16	32	>256	100
	Minocycline	0.032-4	0.75	1	4	57.1
	Tetracycline	0.5->256	32	48	>256	100
	Trovafloxacin	0.125-1	0.25	1	1	74.3
Prevotella intermedia,	Ampicillin	0.64-128	1	8	12	69.0
29 (39.2)	Amoxicillin	0.025-96	2	8	16	86.2
	Amoxicillin/clavulanate	0.16-24	0.125	0.75	4	96.6
	Cefixime	0.016-64	1	4	8	96.6
	Cephalexin	0.5->256	4	16	64	79.3
	Clindamycin	0.016-1.5	<0.016	0.38	1.5	100
	Doxycycline	0.19-2	0.75	1.5	2	100
	Erythromycin	0.19->256	48	64	>256	44.8
	Metronidazole	0.5-16	0.25	0.75	8	100
	Minocycline	0.047-4	0.094	0.5	2	100
	Tetracycline	0.19-128	16	32	48	48.2
	Trovafloxacin	0.016-0.75	0.25	0.38	0.75	100
Peptostreptococcus micros,	Ampicillin	0.064->256	3	16	128	70.3
27 (36.5)	Amoxicillin	0.023-64	4	8	32	77.8
	Amoxicillin/clavulanate	0.016->256	1.5	4	>256	88.9
	Cefixime	0.5-24	2	4	12	100
	Cephalexin	0.38-128	32	32	64	74.1
	Clindamycin	0.016-96	0.75	1.0	1.5	100
	Doxycycline	0.25-128	1	2	4	85.7
	Erythromycin	0.25-96	24	32	64	44.4
	Metronidazole	0.25-128	0.094	2	16	92.6
	Minocycline	0.047-12	0.25	1	8	92.6
	Tetracycline	12->256	32	64	>256	50.0
	Trovafloxacin	0.016-1.5	0.75	1	1	100

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Table 2. *In vitro* susceptibilities of dominant microorganisms isolated from odontogenic infections (continued)

Microorganism, no. of isolates (%)	Antimicrobial agent	MIC (μg/mL)				Susceptible rates (%)
		Range	50%	70%	90%	92.5
Eikenella corrodens, 24 (32.4)	Ampicillin	0.25->256	3	32	256	66.7
	Amoxicillin	0.19-96	2	32	96	79.2
	Amoxicillin/clavulanate	0.19-24	0.75	4	32	91.6
	Cefixime	0.25-128	4	32	64	98.5
	Cephalexin	0.047-192	32	64	96	62.5
	Clindamycin	0.094-56	4	16	32	66.6
	Doxycycline	0.014-4	1	3	4	100
	Erythromycin	0.5-256	32	48	128	45.8
	Metronidazole	6-256	16	128	>256	20.8
	Minocycline	0.032-24	0.75	1	4	95.8
	Tetracycline	0.5->256	16	48	>256	41.6
	Trovafloxacin	0.125-1	0.25	1	1	100
Porphyromonas gingivalis, 23 (31.1)	Ampicillin	0.094-32	1	2	3	91.3
	Amoxicillin	0.016-24	0.75	2	4	95.7
	Amoxicillin/clavulanate	0.19-16	0.25	1.5	8	95.7
	Cefixime	0.25-32	0.5	8	32	91.3
	Cephalexin	0.047-128	4	32	64	52.2
	Clindamycin	0.016-1.5	<0.016	0.25	1	100
	Doxycycline	0.125-8	<0.016	0.75	2	95.7
	Erythromycin	0.19->256	12	48	128	56.6
	Metronidazole	0.75-8	0.19	1.25	6	100
	Minocycline	<0.016-4	<0.016	1	2	100
	Tetracycline	0.125-192	8	24	96	56.5
	Trovafloxacin	0.016-0.75	0.19	0.5	0.75	100

Abbreviation: MIC = minimum inhibitory concentration
MIC breakpoints for susceptible isolates [a]≤1 μg/mL; [b]≤4 μg/mL; [c]≤8 μg/mL; [d]≤16 μg/mL, [e]≤32 μg/mL.

susceptibility rates to ampicillin and amoxicillin were 74.3% and 80%, respectively. Approximately one-third of the *P. intermedia*, *P. micros*, and *E. corrodens* isolate strains exhibited resistance to ampicillin, although both ampicillin and amoxicillin exhibited good activity against viridans group streptococci isolates and *P. gingivalis* (MIC_{90} ≤4 μg/mL). Comparing the bactericidal activity, amoxicillin/clavulanate was more effective than amoxicillin alone in susceptibility against those species. Both the MIC_{50} and MIC_{90} of amoxicillin/clavulanate were 2 to 4 folds lower than those of amoxicillin and ampicillin, respectively, but there were some strains of *E. corrodens* (8.4%), *F. nucleatum* (11.4%), and *P. micros* (11.1%) that were resistant to amoxicillin/clavulanate.

Tetracycline exhibited poor activity against the oral pathogenic bacteria. Conversely, the new tetracycline derivatives, minocycline and doxycycline with a breakpoint of 8 μg/mL, expressed very pronounced antimicrobial activity and could inhibit more than 95% of the isolated species at a MIC_{90} ranging from 1.5 to 4 μg/mL. The highest inhibition concentration was observed in *E. corrodens* with a MIC_{90} of 4 μg/mL.

Metronidazole was relatively inhibitory (MICs ≤16 μg/mL) against a broad spectrum of the anaerobic species in this study. It had less effect on facultatives such as the viridans group streptococci (MIC_{50} = 32 μg/mL) and *E. corrodens* (MIC_{50} = 16 μg/mL).

Clindamycin was inhibitory (MICs ≤8 μg/mL) against most of the anaerobic and facultative organisms. However, about 30% of the *E. corrodens* isolates and 17% of the *F. nucleatum* strains were resistant to this antibiotic.

Of the cephalosporins tested, although some strain isolates were fairly susceptible to these agents, 20% to 50% of all tested species were resistant to cephalexin (breakpoint, 32 μg/mL) and exhibited a pronounced high MIC level (MIC_{50} ≥32 μg/mL). Another third-generation cephalosporin antibiotic, cefixime, was at least 2- to 4-fold more active *in vitro* than cephalexin, although about 30% of the *E. corrodens* and 20% of the *F. nucleatum* were resistant to this new derivative of cephalosporin (MIC ≥32 μg/mL).

Erythromycin was not particularly effective against oral-dental infectious organisms. Resistance to erythromycin was the most widespread among the

antibiotics tested and was noted in all bacterial species.

Trovafloxacin, the third-generation of fluoroquinolones, demonstrated the highest activity among the 12 antibiotics in this study against all tested species (MICs ≤1 μg/mL).

Discussion

The antimicrobial susceptibility of the oral anaerobes was difficult to forecast. Increased drug resistance of microorganisms has become a serious problem in Taiwan over the past 2 decades [12-14]. In general dental practice, the antibiotic doses administered were not always bactericidal, and the doses for successful treatment usually did not depend on the antibiotic effectiveness against all microorganisms present. In severely ill patients, such as immunocompromised patients, enough doses of appropriate antibiotics must be administered to achieve bactericidal levels [15]. Unfortunately, insufficient antibiotic doses usually induced drug-resistance during a treatment course. The elimination of sensitive strains and the dissemination of resistant microbes might lead to a situation that many pathogens became resistant to normal chemotherapy [16].

The present results showed that ampicillin-resistant organisms from odontogenic infections were very common and some of them were resistant to Augmentin. This suggests that the oral aminopenicillins should no longer be considered the drugs of choice for the management of odontogenic infections. Although cephalosporins including the third generation of cephalosporin, cefixime, has a broad spectrum, they were less active against some strains of bacteria in this study. Cephalosporin should therefore only be used as an alternative regimen based on the results of susceptibility tests.

Of the antimicrobial agents that have been tested against odontogenic infection microorganisms, erythromycin is relatively ineffective [17]. In this study, approximately 50% or more of the tested species were resistant to erythromycin.

The family of tetracycline antibiotics has been available since the mid-1950s. However, the intensive use of this antibiotic has led to the emergence of drug-resistant microorganisms. Minocycline and doxycycline inhibited a higher proportion of the strains in almost all groups than did tetracycline in this study. Clindamycin exhibited excellent activity against most anaerobic organisms isolated in odontogenic infections. Since clindamycin therapy has been proved to be associated with severe colitis, it should be reserved for severe infections when less toxic antimicrobial drugs were ineffective. However, when the microbiological susceptibility was clearly documented, clindamycin should be considered as a treatment modality for oral infection cases.

Metronidazole has an excellent activity against strict anaerobes [7,18]. But it had absolutely no effectiveness against facultative bacteria such as streptococci and *E. corrodens*. In addition, the therapeutic efficacy of metronidazole might be reduced when used alone. However, some microorganisms are difficult to isolate, and antibiotics are generally prescribed before sensitivity can be established. Putatively synergistic combinations based on metronidazole and β-lactam antibiotics, with a wide spectrum of activity would thus be required. Such combination may be value in the treatment of some selective infections and may delay the development of antimicrobial resistance [19].

In this study, trovafloxacin, the third generation of fluoroquinolones, demonstrated a notable *in vitro* activity against a variety of pathogenic bacteria commonly isolated from odontogenic infections. However, the antimicrobial activity of fluoroquinolones diminished quickly after the drug was clinically launched. Further clinical efficacy evaluations are necessary [20].

In conclusion, the activities of commonly used antibiotics against clinical odontogenic pathogen has decreased remarkably in Taiwan. The results could be used as a reference for choosing the appropriate antibiotic.

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