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Review

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Odontogenic Infections, Opportunistic Prevotella and Metronidazole

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ABSTRACT: Treating odontogenic infections of the head, neck and oral cavity requires the understanding of surgical and pharmacotherapeutic interventions as they apply to single and multispace infections.   Three clinical cases are presented where *Prevotella* species were significant opportunistic pathogens. Since *Prevotella* can become virulent under conditions including post-extraction wound healing of an extraction site adjacent to an intact dentition, excessive plaque and calculus, nearby gingivitis or periodontitis, or an immunocompromised host, Metronidazole administration was deemed necessary in the cases described here. This intervention mitigated or resolved these odontogenic infections that were refractory to conservative surgical therapy that included source control. The involvement of *Prevotella* in odontogenic infections and pericoronitis, and the antibiotics used to treat common oral and maxillofacial infections with this bacterium are reviewed. *Prevotella* is susceptible to Metronidazole (Flagyl), and the antibiotic is effective in mitigating the disease process when basic principles of the treatment of odontogenic infections are employed.

Keywords: *Prevotella*; odontogenic infection; metronidazole (flagyl); pericoronitis

Introduction

Prevotella species are inhabitants of the periodontal sulcus, living in the crevicular fluid and in the dental biofilm attached to hard tissue surfaces (teeth) and mucosa. These obligate anaerobic Gram-negative rods are part of the normal oral microbiome and are not opportunistic microorganisms in the immunocompetent host [1].

Mixed infections comprising anaerobic, facultative anaerobic, and aerobic bacteria colonize perialveolar and fascial spaces, contributing to odontogenic infections of the head and neck that can progress to life-threatening circumstances, including bacteremia, sepsis, airway compromise and death [2, 3]. Pericoronitis, perialveolar infections (sometimes called subperiosteal infections), and multi-space odontogenic infections all show evidence of *Prevotella* species as isolates in the mixed oral flora.

Odontogenic infections are infections of tooth-related origins usually relating to endodontic or periodontal sources [4, 5]. Possible etiologies can include necrotic pulp from carious or fractured teeth, pericoronitis, or deep periodontal pockets where bacteria cause infections [6]. Anaerobic and aerobic bacteria are implicated in 50–60% of odontogenic infections, with the most commonly isolated facultative anaerobic organisms being Viridans streptococci, and the most commonly isolated obligate anaerobic bacteria being *Bacteroides spp, Prevotella spp, and Peptostretpococcus spp*. [4].

Pericoronitis is an infection of the soft tissue around the crown of a partially erupted tooth and is usually caused by normal microorganisms that inhabit the oral cavity [4]. Bacterial plaque can accumulate underneath the soft tissue which may lead to inflammation [7]. The bacteria that are found at the highest levels in symptomatic pericoronitis include *Actinomyces oris, Eikenella corrodens,*

Eubacterium nodatum, Fusobacterium nucleatum, Treponema denticola, Eubacterium saburreum, and various *Streptococcus* species [8]. With proper oral hygiene and strong host defenses, most patients can eliminate the bacteria that are trapped under the operculum without leading to further complications or infections. If the bacteria are not removed under the operculum, pericoronitis can lead to jaw inflammation and phlegmon [9]. Infection may spread, causing a multi-space infection of the head and neck regions.

Management of odontogenic infections in the oral cavity includes controlling the source of the infection by surgical removal, establishing drainage, and mobilizing the host defense system to fight the infection. The clinician must have fundamental knowledge of the anatomy of the head and neck region, specifically the fascial planes through which infections can spread. Odontogenic infections typically begin as vestibular space abscesses where it is localized and can be treated easily [4]. The infection, when localized, may present as an abscess, swelling, cellulitis, and trismus. If the infection persists and spreads, it will take the path of least resistance, potentially leading to life-threatening complications. Deep fascial space infections of the neck are most frequently odontogenic in origin and can be life-threatening if the abscess extends to the adjacent fascial space [10, 11].

Principles of source control include (i) decreasing the bacterial load by incision, opening the wound to introduce air, and changing the environment from anaerobic to aerobic, thus eradicating obligate anaerobes; (ii) drainage that comprises decompressing the swelling, releasing the purulent exudate, and promoting the blood supply; (iii) irrigation, thereby washing out bacteria, bacterial toxins and inflammatory by-products; and (iv) tooth extraction [12]. Empiric antibiotics targeted to opportunistic *Prevotella* species are used in immunocompromised hosts, severe infections, and infections refractory to surgery.

Methods

Three clinical cases are presented below where *Prevotella* species were significant opportunistic pathogens in odontogenic infections, and surgery combined with metronidazole was used to eradicate the infections. For the narrative review, the PubMed database was searched using the following terms: "*Prevotella* AND Odontogenic Infection AND (OR Amoxicillin OR Clindamycin OR Metronidazole OR Flagyl)." We identified 46 articles from 1996 to 2024 of which 40 were published in English. Twenty-five additional references were used to develop the section on *Prevotella* pathogenesis, and other publications were cited within the paper. A total of 68 references were reviewed and cited. Observational/retrospective studies, literature reviews, and randomized controlled trials were reviewed; foreign language papers were excluded. The three cases discussed were correlated with the literature.

Case Review

CASE 1: Perialveolar Infection

A 25-year-old female presented for extraction of teeth numbers 1, 16, 17, and 32 (Figure 1). Her medical history was unremarkable. Surgery was performed with removal of bone, sectioning of teeth and irrigation with dilute Chlorhexidine 0.12% mouthwash and sterile saline. The surgical sites were closed with 4.0 chromic gut sutures. She returned to the office for a post-operative visit one week later that revealed pink healthy healing mucosa in four quadrants. There was no erythema, edema or exudate. The sockets were irrigated with sterile saline alone, and there was no evidence of food impaction. The sutures were removed at this visit. She was told to return to the office if she exhibited any subjective signs of pain or swelling (Figure 2).

Figure 1. Impacted third molars with inadequate space for eruption.
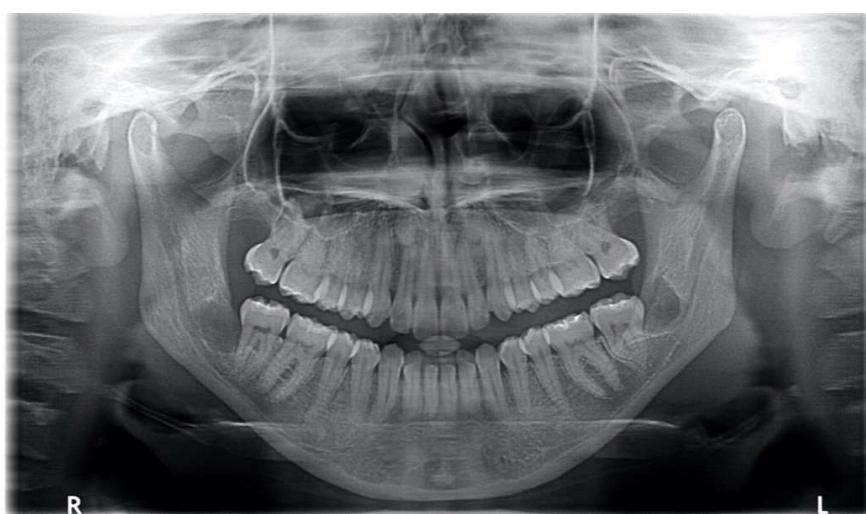
Figure 2. Bony extraction "crypts" with potential to harbor bacteria.

Twenty-one days after extraction surgery, she returned to the office with concern of yellow fluid in the socket of tooth #17, swelling and mild pain.   The floor of mouth and buccal space were not involved.   The socket was gently opened with cotton pliers and irrigated with 20 ml of dilute Chlorhexidine 0.12% mouthwash mixed with sterile saline. A prescription was given for Amoxicillin 500 mg, 15 tablets, take one by mouth three times a day until completed. An empiric antibiotic approach with conservative irrigation and drainage was performed as in many perialveolar infections previously treated in our practice.

Twenty-nine days after extraction surgery, she returned to the office with concern of yellow fluid in the socket of tooth #32, swelling and mild pain. The floor of mouth and buccal space were not involved. The socket was opened gently with cotton pliers and irrigated with 20 ml of dilute Chlorhexidine 0.12% mouthwash and sterile saline. A prescription was given for Chlorhexidine 0.12% mouthwash and the patient was instructed to irrigate the socket twice a day with the mouthwash alone for one week. A new prescription was given for Clindamycin 300 mg, 20 tablets, "take one by mouth four times a day until gone."

The patient returned thirty-six days after extraction surgery for follow-up and the #32 socket was irrigated with dilute Chlorhexidine 0.12% and sterile saline without evidence of purulent exudate.

Forty-five days after extraction surgery, the patient called the weekend on-call doctor and reported that there was exudate coming from the #32 socket again.   The doctor explained to the patient that effective tissue levels of the antibiotic may not have been achieved, and prescribed Amoxicillin 500 mg, 40 tablets, take one by mouth three times a day until gone.

On day forty-seven after the extraction surgery, we saw the patient, concerned that there was a resistant bacterium colonizing the #32 space. The #17 space was without evidence of erythema, edema or exudate at this time. Incision and drainage were performed at this site and there was no evidence of purulent exudate. Samples were taken at the #32 site for culture of anaerobic and aerobic bacteria, Gram staining and antibiotic sensitivity testing.   The socket of #32 revealed caseous material and shiny white collagen-like tissue. The area was irrigated with 60 ml of dilute Chlorhexidine 0.12% mouthwash and sterile saline.   The patient continued taking the Amoxicillin.

Fifty days after the extraction surgery, after completing the Amoxicillin prescription, the patient called and reported that the #17 site was draining purulent exudate.   The Gram-stain results for the #32 socket reported "No white blood cells. No organisms seen." The aerobic culture reported at fifty-one days after the extraction surgery revealed, "Moderate growth of normal oral flora from the #32 extraction wound." The anaerobic culture reported at day 53 after extraction surgery revealed, "*Prevotella buccae* isolated, with heavy growth" (Table 1).

The #17 site was opened with cotton pliers, irrigated with 20 ml dilute Chlorhexidine 0.12% and sterile saline, and the fluid was cultured again. Only one report was filed by the hospital microbiology department, "Moderate growth of mixed oropharyngeal flora. Final report."

Based on the anaerobic culture and sensitivity results from the day 50 surgery, Metronidazole 500 mg, 40 tablets, take one by mouth four times a day until gone, was prescribed. The patient continued to irrigate the #17 socket at home with dilute Chlorhexidine 0.12% and water.   The patient was followed weekly for improvement and there was no recurrence of the perialveolar infections at the #17 or #32 sockets.

We had never treated a refractory perialveolar infection like this in 23 years of clinical practice. Therefore, it is important to emphasize careful follow-up and repeated surgical interventions to mitigate these odontogenic infections.

Table 1. MBC for *Prevotella buccae* (anaerobic Gram-negative rod).

	Anaerobic Gram-negative rods MBC (µg/mL)	
Ampicillin + Sulbactam	1	Susceptible
Clindamycin	256	Resistant
Meropenem	0.25	Susceptible
Metronidazole	0.5	Susceptible

CASE 2: Multi-space Odontogenic Infection

A 23-year-old male patient presented from the hospital emergency department to the lead author's private office to be evaluated for an oral infection. His medical history was unremarkable. Six days prior, he was treated for a carious, impacted tooth #32 by a community dental provider and the tooth was removed. An Amoxicillin prescription was given upon discharge. He was given intravenous Clindamycin at the hospital.

Upon examination, his temperature was 99.4° F and he was trismatic to 1 cm of mouth opening (Figure 3, left image). The perialveolar, buccal and sublingual spaces exhibited moderate edema. There was a purulent exudate expressed from the socket of tooth #32 (Figure 3, right image) Nitrous oxide-Oxygen sedation was used to facilitate oral opening to approximately 3 cm.   Incision and drainage surgery was performed with full thickness mucoperiosteal flap, and 5 ml of purulent exudate was removed, and the space was irrigated with dilute Chlorhexidine 0.12% mouthwash and sterile saline. The surgical site was left open for continued drainage. Cultures of the extraction socket of #32 were taken for Gram-stain, aerobic bacteria, and anaerobic bacteria and sent to the hospital. The patient returned to the hospital for a blood test and observation.

Figure 3. Right buccal swelling and trismus (left image); #32 area serosanguinous fluid and pus (right image).

A Complete Blood Count with differential analysis was ordered and the results revealed "White blood cells 12.4K, 86% Neutrophils." The Gram-stain revealed "Moderate white blood cells, few Gram-positive cocci, few Gram-negative rods, and rare Gram-positive rods." The aerobic culture revealed "Light growth *Eikenella corrodens*, light growth normal oral flora, rare growth *Streptococcus anginosus (S. milleri* group)."

We recommended the hospitalist send him home with Metronidazole 500 mg, 40 tablets, "take one tablet every six hours until gone."

The patient was followed in the private office the next day with improvement of trismus, swelling and pain. He was instructed to rinse with Chlorhexidine 0.12% diluted with water at home for one week. At day 3 post incision and drainage, the anaerobic culture was returned and revealed, "Mixed varieties of *Prevotella* species isolated. Light growth of mixed anaerobic flora including *Prevotella buccae*." CLSI standards lead the microbiology laboratory to not do antibiotic sensitivity testing as no bacteria revealed itself in high quantities outside the expected mixed odontogenic normal flora.

The patient was seen again in the lead author's office at post operative day 8 with resolution of his infection.

CASE 3: Severe Pericoronitis

A 42-year-old male presented to the office with acute pericoronitis, trismus and pain. The patient had an unremarkable medical history. His vital signs were BP 160/110 with a HR of 90. His BMI was 33. Upon oral evaluation, he exhibited a severe pericoronitis associated with tooth #17 that had extended into the left buccal space (Figure 4). There was no floor of mouth raise or purulent exudate present. He was trismatic to approximately 2 cm of oral opening. After he was examined in the private office, he was sent to the hospital for impending surgery and intravenous antibiotics.
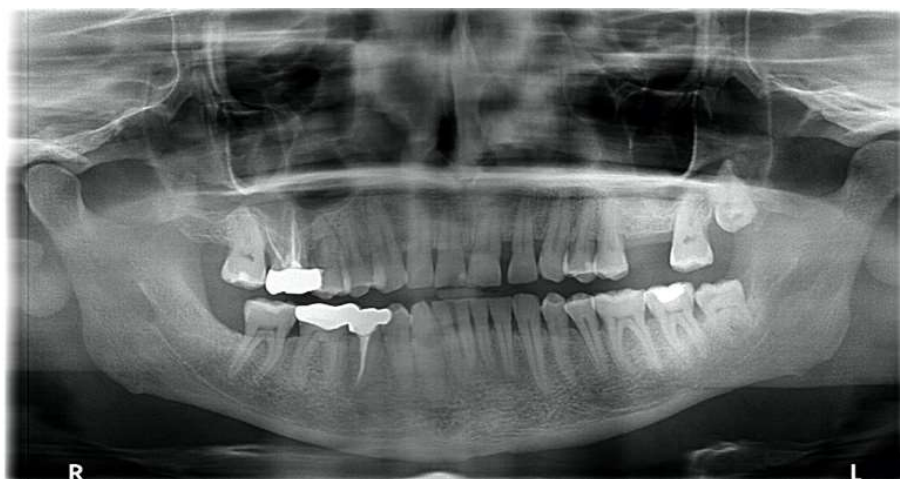
Figure 4. Impacted #17 with caries, mesial bone loss and distal cupping resorption.

He was advised to have nothing to eat or drink (NPO) until surgery in the operating room under general anesthesia. He was prescribed Unasyn (ampicillin-sulbactam) 3 g intravenously upon arrival at the hospital, due to trismus and the suspicion of an advancing polymicrobial infection.

Surgery was performed in the operating room under oral-endotracheal general anesthesia. A full thickness mucoperiosteal was elevated and tooth #17 was extracted with removal of alveolar bone, and the space was irrigated with Chlorhexidine 0.12% mouthwash diluted with sterile saline, approximately 60 ml. There was serosanguinous fluid in the buccal space without the presence of purulence indicative of cellulitis. The wound was left open to promote drainage. Cultures were taken for aerobic and anaerobic bacteria, and a Gram-stain was requested from the laboratory. The Gram-stain revealed, "Many white blood cells, moderate Gram-positive cocci, and few Gram-negative rods." The aerobic culture revealed, "Moderate growth of mixed skin flora, light growth of *Klebsiella oxytoca*, light growth of *Streptococcus anginosus* (*S. milleri* group)." Three days later, the anaerobic culture revealed, "Heavy *Fusobacterium*, moderate growth *Parvimonas micra* (*Peptostreptococcus micros*), and moderate growth of mixed varieties of *Prevotella* species including *Prevotella intermedia*" (Figure 5).

The patient was discharged that day with a prescription for Metronidazole 500 mg, 28 tablets, take one tablet four times a day until gone; Augmentin 875 mg, 14 tablets, take one tablet twice a day until gone; Chlorhexidine 0.12% mouthwash, rinse ½ oz. twice a day and spit out, for one week (Table 2). We also prescribed an opioid-containing analgesic as needed for pain.

The patient was followed in the office the next day for improvement and his symptoms had subsided by the one-week post-operative examination.
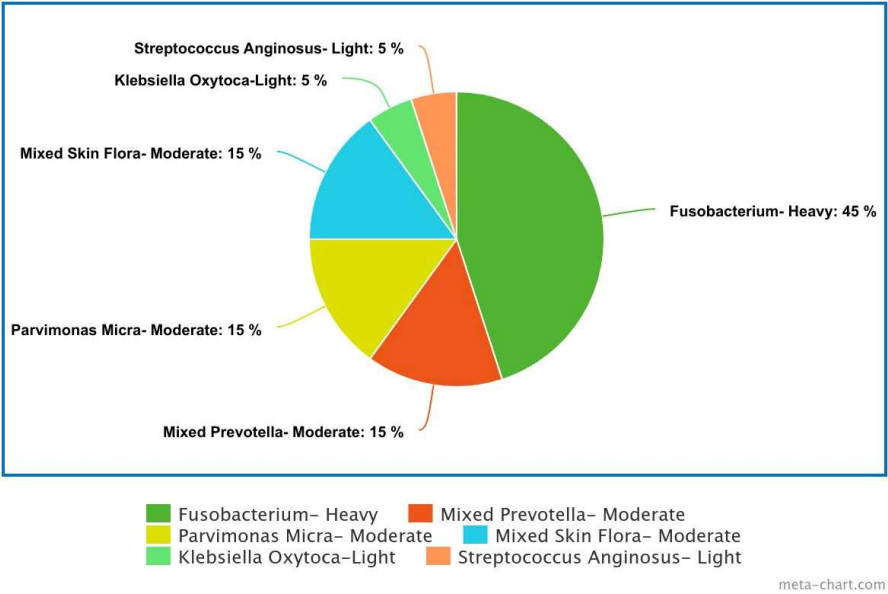
Figure 5. Anaerobic Organisms: *Fusobacterium*, Mixed *Prevotella*, *P. micra* (75%).

Aerobic Organisms: Mixed Skin Flora, *K. oxytoca, S. anginosus* (25%)

Table 2. Susceptibility Testing for *Prevotella intermedia* and *Fusobacterium* species.

Susceptibility		
Prevotella intermedia		
Antibiotic	*Interpretation*	*Value Comment* (µg/mL)
Ampicillin + Sulbactam	Susceptible	<=0.03
Clindamycin	Susceptible	<=0.03
Meropenem	Susceptible	0.06
Metronidazole	Susceptible	0.12
Fusobacterium Species		
Ampicillin + Sulbactam	Susceptible	<=0.03
Clindamycin	Susceptible	0.06
Meropenem	Susceptible	0.03
Metronidazole	Susceptible	<=0.03

Oral Microflora and Odontogenic Infections

The microbiome of the oral cavity is one of the most varied flora in the human body.   The variety of bacteria in the oral cavity is a result of the many microenvironments, generated by the various surfaces of teeth, the gingival sulcus, buccal mucosa, and tongue [13]. Each organism has a unique set of conditions that allows the bacteria to survive in that region. Each location in the oral cavity has appropriate nutrients and oxygen tension or physical protection from unfavorable conditions [13]. Plum et al. have proposed that *Streptococcus salivarius* and *Veillonella spp.* often colonize the tongue and buccal surfaces; *Actinomycetes* and *Streptococcus spp.* often colonize the enamel; and *Fusobacterium, Prevotella, Porphyromonas,* and *Spirochetes* colonize gingival surfaces [14]. *Streptoccus spp., Fusobacteria,* and *Prevotella* are bacteria identified in the cases we have discussed above.   When managing odontogenic infections, it is important to understand the nature of the flora and the environment – specifically analyzing the organism's oxygen requirement, which will be crucial when identifying the possible bacteria and how to treat the infection. The most common

causes of odontogenic infections include caries (65%), pericoronitis (36%) and periodontitis (21%) [15, 16].

Prevotella Pathogenesis

The *Prevotella* species, *P. buccae, P. intermedia, P. loescheii, P. melaninogenica, P. nigrescens* are pleomorphic, strict anaerobes, and form black-pigmented colonies. They can convert glucose into acetic acid and succinic acid, and require vitamin K and hemin for growth. *P. intermedia* is [normally] associated with chronic periodontitis and endodontic infections. Its virulence factors include phospholipase A, IgA/IgG proteases, mercaptans, and hydrogen sulfide [17]. *P. intermedia* is one of the microorganisms in the "orange complex," as decribed by Socransky et al. [18]. Increasing the colonization of periodontal plaque with microorganisms in this group, which also includes *Fusobacterium nucleatum* and *Peptostreptococcus micros*, leads to more sites being colonized by *Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola*, which are, in turn, characterized as the "red complex" [17, 18]. *P. buccae* was identified in Case 1 and Case 2. Case 3 identified *P. intermedia* and "other *Prevotella spp.*" that were not specified in the culture results returned from the hospital.

In 1990, the moderately saccharolytic and predominantly oral species, *Bacteroides oralis* and *B. melaninogenicus*, were reclassified in the genus *Prevotella*, [19] and the genus name *Bacteroides* was restricted to *Bacteroides fragilis* and closely related species, including *B. thetaiotaomicron*.

Several strains of *P. intermedia* were found to inhibit the mitogen-or antigen-induced proliferation of B-cells and T-cells, suggesting that immunosupression mediated by periodontal bacteria may contribute to the pathogenesis of periodontal disease [20].   *P. intermedia* can bind to the basement membrane protein, laminin, which is abundant in the periodontal pocket. It ferments glucose and sucrose, hydrolyzes starch, and produces various acids as metabolic end products, including acetic and succinic acids [21]. *P. intermedia* was identified in Case 3 reviewed above.

P. intermedia expresses a 65 kD molecule that acts as a receptor for the Fc region of human IgG, thereby shielding the bacterium from the humoral response of the host [22]. A 38 kD cysteine protease of *P. intermedia* completely degrades IgG, IgA and IgM within 24 h [23]. *P. intermedia* has hemolytic activity that enables it to acquire iron for its metabolism [24]. It also has hemoglobin receptors that can bind hemoglobin with a K_{diss} of 2.5×10^{-8} M [25], and has hemagglutination activity associated with its fimbriae [25].

Another virulence factor of *P. intermedia* is interpain A, which degrades the C3 component of complement, synergistically with gingipains [26]. Interpain A can also activate the C1 complex in serum, which results in the deposition of C1q on inert and bacterial surfaces. Endodontic pathogens, particularly *P. intermedia*, can kill infiltrating neutrophils, which may be a mechanism by which endodontic infections get established [27]. *P. intermedia* has the highest DNA degradation ability among periodontopathogenic bacteria, which may explain its ability to escape neutrophil extracellular traps [28].

P. intermedia lipopolysaccharide (LPS) can induce IL-8 gene expression in human dental pulp fibroblasts. This ability appears to be specific to *P. intermedia* LPS, since synthetic LPS and *Salmonella* LPS do not increase IL-8 mRNA levels in the host cells [29]. *P. intermedia* LPS stimulates the production of tumor necrosis factor-alpha (TNF-a) in monocyte-derived macrophages by activating the three types of mitogen-activated protein kinases (MAPKs), ERK1/2, JNK1/2 and p38 [30]. The adhesion of *P. intermedia* to HEp-2, KB and HeLa cells and fibroblasts is inhibited by lactoferrin in a dose-dependent manner [31].

In patients with advanced periodontal disease, systemic administration of Metronidazole plus Amoxicillin inhibits the growth of *Aggregatibacter actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*, and causes a reduction in the inflammatory lesion [32]. The MIC for Metronidazole against *P. intermedia* was found to be about 1 μg/mL [33].

Among other bacteria, *P. buccae* was found in peri-implant bone defects [34], periodontitis [35, 36], root canal infections, [37] and radicular cysts [38].

Prevotella and Odontogenic Infections

In line with recent studies addressing the treatment of odontogenic abscesses, the most prevalent anaerobic bacteria were: *Prevotella spp. Peptostreptococcus (Micromonas micros)*, *Bacteroides*, and different species of *Actinomyces, F. nucleatum,* comprising 53% of the anaerobes [13, 15, 39-43]. Along with *Prevotella spp.*, mixed oropharyngeal flora (which generally includes *Peptostreptococcus spp.)* was identified in Case 1. The aerobic and facultative anaerobic bacteria found in high count include: Viridans streptococci, alpha-hemolytic streptococci*, S. aureus, and E. coli [13, 15, 40, 44, 45].* Fungi have also been identified [41]. When addressing larger odontogenic infections, the bacteria involved are more pathogenic than those predominantly isolated from smaller odontogenic infections [46].

In the lead author's clinical practice, *Prevotella* is often resistant to β-lactam antibiotics. Refractory infection is generally observed when Amoxicillin is prescribed to treat odontogenic infections, and these patients continue to be reinfected even after Cleocin is also given. Amoxicillin appears to have become popular in the prophylaxis of Viridans group streptococcal infective endocarditis for dental procedures because of its minimal side effects and its improved absorption from the gastrointestinal tract, providing higher and more sustained serum concentrations. Viridans group streptococci are part of the oral microbiome and have been shown to be antagonistic to periodontal pathogens such as *Prevotella* and other obligate anaerobes [47]. Khalil, et. al discussed opportunistic bacterial pathogens exhibiting resistance after just days of antibiotic administration used for both prophylaxis against surgical wound infections or for the prophylaxis against Viridans group streptococcal infective endocarditis, and a single dose of Amoxicillin induced a significant selection of resistant strains and caused ecological disturbance in the microflora of the oral cavity [48].

Infections by *Prevotella, Eikenella, Fusobacterium* and other anaerobic Gram-negative organisms—that frequently include Viridans streptococci, *Neisseria, Klebsiella* and other facultative anaerobes—are mitigated by surgery and common orally administered antibiotics used to treat odontogenetic infections, including Amoxicillin, Penicillin, Augmentin, and Clindamycin (Table 3). These organisms live as part of the normal oral microbiome and biofilm—with facultative anaerobic and aerobic organisms—but they can become virulent given the opportunity in odontogenic infections. Although any antibiotic can contribute to pseudomembranous colitis, the use of Clindamycin continues to decrease due to its contribution to this disease.   It is important to emphasize here that Clindamycin is not always effective against all *Prevotella spp.*

Table 3. Anaerobic Organisms Other Than *Bacteroides spp.* and *Parabacteroides spp.* and their sensitivity to antibiotics. Reprinted with permission from Clinical and Laboratory Standards Institute from: CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.*30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

Anaerobic Organisms	Ampicillin-Sulbactam			Pipercillin-tazobactam			Imipenem			Meropenem		
	# of strains	%S	%R	# of strains	%S	%R	# of strains	%S	%R	# of strains	%S	%R
Prevotella spp.	29	97	3	63	100	0	29	100	0	92	98	0
Fusobacterium spp	20	100	0	55	96	2	75	95	4	20	100	0
Anaerobic gram positive cocci	-	-	-	1853	99	1	134	99	0	1647	100	0
Clostridium perfringens	15	100	0	410	100	0	23	100	0	417	100	0
Clostridioides	76	99	0	542	93	0	480	69	4	609	99	0
Clostridium spp.	-			439	94	1	71	99	0	390	100	0
Anaerobic Organisms	Penicillin			Clindamycin			Moxifloxacin			Metronidazole		
	# of strains	%S	%R	# of strains	%S	%R	# of strains	%S	%R	# of strains	%S	%R
Prevotella spp.	63	100	0	29	69	28	92	66	25	92	99	0

Fusobacterium spp	-	-	-	75	77	21	75	68	23	75	95	5
Anaerobic gram positive cocci	1647	100	0	1826	97	3	300	72	21	0	100	0
Clostridium perfringens	402	90	4	425	83	12	23	83	9	425	100	0
Clostridioides	533	6	37	1013	32	38	480	74	25	1343	100	0
Clostridium spp.	390	69	13	461	67	25	71	62	35	461	100	119

We are surprised that Penicillin has been reported as 100% effective in eradicating *Prevotella* according to the CLSI (Tables 3 and 4). Penicillin or the aminopenicillin Amoxicillin will not eradicate resistant *Prevotella spp.,* as demonstrated in all three cases above. We suspect that we did not see *Prevotella* becoming an opportunistic pathogen, because of the old-fashioned "Penicillin plus Flagyl" regimens for odontogenic infections, as the Flagyl (Metronidazole) component was bactericidal for *Prevotella*. Metronidazole certainly fell out of fashion because of the Disulfiram-like reaction and its poor tolerance due to gastrointestinal upset. It is of interest to note that many antibiotics in the table are intravenous (IV) only. Community providers need a cost-effective and available oral (PO) medication.

The old "Penicillin plus Flagyl" regimen might be the first empiric line, if *Prevotella* is the suspected actor in a mixed flora multispace odontogentic infection or cellulitis. Surgery and Metronidazole alone seem adequate in treating mild to moderate odontogenic infections in the immunocompetent host.

The aerobic and facultative anaerobes found in high count include: Viridans streptococci, alpha-hemolytic streptococci, *S. aureus, and E.* coli [13, 15, 40, 44, 45]. Fungi have also been collected in small cultures [41]. When addressing larger odontogenic infections, the bacteria involved are more pathogenic than those predominantly isolated from smaller odontogenic infections [46]. These mixed flora odontogenic infections refractory to surgery alone require combination antibiotic therapy (Beta-lactams, or antibiotics covering facultative organisms in addition to Metronidazole) as Metronidazole only eradicates anaerobic bacteria as demonstrated in Case 3 above.

Metronidazole

Metronidazole is a commonly used antibiotic and antiprotozoal medication, given both orally and intravenously, belonging to the nitroimidazole class. It is synthetically derived from azomycin which was originally detected in *Streptomyces spp*. in the 1950s [49]. In 1962, it was used for the treatment of vaginitis due to *Trichomonas vaginalis* and has also been effective in treating patients with gingivitis. Metronidazole is the first choice in the treatment of *Clostridium difficile* [49].

Metronidazole exerts its antimicrobial effects through a stepwise mechanism. It is first taken into the microorganism by diffusion across the cell membranes of anaerobic pathogens [50]. It exerts its antimicrobial effects after the reduction of its nitro group through the transfer of an electron, activating the prodrug and creating reactive intermediates and radicals that are toxic to the microorganisms, leading to the inhibition of growth or outright killing by the breaking of DNA strands [49].

Metronidazole is absorbed rapidly by the gastrointestinal tract often bypassing the enterohepatic circulation, as observed in studies with mice and rats [49]. The liver breaks down Metronidazole into 5 metabolites. The hydroxy metabolite has a longer half-life than its parent compound. The majority of metronidazole and the metabolites are excreted in the subject's urine and feces, with approximately 12% being excreted unchanged [51]. Metronidazole proved to be effective in contributing to the eradication of the odontogenic infections in all three cases presented above.

Metronidazole and The Disulfiram-Like Reaction

There is a commonly known association of a disulfiram-like reaction with the use of Metronidazole and alcohol. Disulfiram is a medication commonly used to treat alcohol dependence. Its mechanism of action is to inhibit the enzyme aldehyde dehydrogenase thus leading to the

accumulation of acetaldehyde [52]. Metronidazole has a similar mechanism. While it does not inhibit aldehyde dehydrogenase, it does inhibit alcohol dehydrogenase. When alcohol is mixed with disulfiram or Metronidazole there are a range of unpleasant symptoms that accompany the build-up of acetaldehyde. Common symptoms include nausea, vomiting, abdominal pain, flushing of the face, tachycardia, throbbing headaches, and syncope. In more severe cases, the symptoms may include respiratory depression, cardiovascular collapse, arrhythmias, convulsions, and death [53].

While *Prevotella* has been shown to be highly susceptible to Metronidazole, the symptoms associated with the disulfiram-like reactions are a contributing factor for health care providers not wanting to prescribe this antibiotic.

Careful administration of Metronidazole with close patient follow-up, as presented in the cases above, proved to avoid the untoward outcome of a disulfiram-like reaction.

Odontogenic Infections and Antibiotic Susceptibility

Mixed flora bacterial isolates were sent to the laboratory for culture and sensitivity evaluation in all three cases presented above. Each case grew bacteria that was resistant to empiric antibiotic therapy. With the clinical laboratory evaluation and guidance, either Metronidazole or a combination antibiotic therapeutic approach was used to choose antibiotics mitigating or eradicating opportunistic organisms susceptible to drugs given at the appropriate MBC. Of the cases studied by Chunduri et. al., one-third of the patients were successfully treated solely with incision and drainage. Penicillin has been the antibiotic of choice for uncomplicated dental infections because of its activity against many facultative and anaerobic organisms recovered from these mixed infections [42]. Only in severe abscesses is penicillin prescribed following surgical incision and drainage [44]. The administration of the penicillin drug class is considered to be useful for the empirical management of odontogenic infections, as described by other studies published in Spain [41]. The advantages of such treatment are based upon the bacterial susceptibility to the antibiotic as well as the low cost [41, 54-56]. However, some authors have found that penicillin-resistant organisms are increasingly isolated from abscesses of odontogenic origin because of the production of b-lactamase, which restricts the effectiveness of the penicillin [9, 15, 45, 56-58]. The ability of species to synthesize b-lactamase should be determined before treating with b-lactam antibiotics to avoid potential antibiotic resistance [59]. Patel et al. found *Prevotella* to be the most prevalent in producing b-lactamase, followed by *Capnocytophaga, Veillonella* and *Bacteroides [60].* The b-lactamase produced protects the bacteria and other surrounding bacteria in the polymicrobial niches against antibiotic activity [61]. There are several mechanisms through which microorganisms can gain resistance to b-lactam antibiotics. These include alteration in penicillin-binding proteins, barriers to target sites, and the ability to inactivate the antibiotic through production of b-lactamase. *Prevotella*'s resistance to b-lactam antibiotics results from the expression of b-lactamase genes. Kuriyama et al. have found *Prevotella* to have many b-lactamase-positive strains [56]. When considering penicillin use, it is important to ascertain whether the patient has allergies to this or other antibiotics.

Other antibiotics that have proven to be effective include penicillinase-resistant penicillin, Clindamycin, or a combination of Metronidazole plus Amoxicillin or a macrolide [42]. In Cases 1 and 2, *P. buccae* was isolated, and Clindamycin was deemed ineffective and not used as a second-line therapy. Metronidazole has been studied extensively and has been seen to have excellent activity (100%) against all the anaerobic isolates, but possesses little to no activity against aerobes [15, 62]. Chan et al have found there to be a possible decrease in therapeutic effectiveness when Metronidazole is used alone [63]. Ciprofloxacin, Amoxicillin with a beta-lactam inhibitor like clavulanic acid, and Clindamycin were also effective [15, 64]. Maestre et al. found that *Prevotella* isolates were susceptible to Amoxicillin-clavulanic acid, while Amoxicillin alone exhibited resistance rates ranging from 17.1% in *P. buccae* to 26.3% in *P. denticola [65].* In all *Prevotella* species tested, resistance rates to Metronidazole was below 6%, and Clindamycin resistance ranged from 0% to 21.1% [65]. When testing *P. buccae, P. denticola,* and *P. intermedia,* resistance rates were found to range from 9.1% to 21% for Clindamycin, 0% to 5.9% for Metronidazole, and 5.9% to 36.4% for Tetracycline [65].

A study by Shakya et al. [15] examined the antibiotic susceptibility of *Prevotella* species and found that 0/8 were susceptible to penicillin, 6/8 to Clindamycin, 8/8 to Amoxicillin + clavulanic acid, and 8/8 to Metronidazole. For patients with penicillin allergies, Clindamycin, erythromycin, tetracyclines, and levofloxacin were found to be adequate alternative regimens [45]. The family of tetracycline antibiotics has been available and commonly prescribed since the mid-1950s [63]. Metronidazole was used in all of the cases presented above as it is a narrow-spectrum antibiotic targeting anaerobic organisms. Our objective was to avoid broader spectrum agents with either a narrow therapeutic index or more drug side effects.   Because of its widespread use and emergence of drug-resistant microorganisms, a culture and sensitivity test should be performed prior to prescribing tetracycline or its derivatives to ensure its effectiveness.

There has been some discussion of the use of combination therapy. Alou et al. found eradication of b-lactamase producing strains to be achieved quicker by the use of combination therapy of Amoxicillin + clavulanic acid + tinidazole and by Clindamycin + tinidazole than by a b-lactam antibiotic alone [63]. We concluded that combination therapy was essential in Case 3 as "moderate growth of mixed varieties of *Prevotella"* was identified. Although *P. intermedi*a was identified, we were concerned about the unidentified *Prevotella spp.* that could have been resistant to empiric therapy.

Based on literature researched and the clinical cases, when a specimen contains a single organism considered to be an opportunistic pathogen, or if there is a clear predominance of growth in a mixed culture, then susceptibility testing is performed on that organism. Occasionally, however, when known empiric therapies are found to be effective, such antibiotics will be prescribed without susceptibility testing. For example, a culture with *Eikenella sp.* and *S. anginosus* found in small quantities among other indigenous flora, no susceptibility testing would be performed. For *Eikenella*, oral therapy with Amoxicillin/clavulanate (Augmentin) is a well-accepted treatment. This would also cover much of the indigenous flora. (Dr. Steven Schapiro, Director of Microbiology, Kaiser Permanente-Northern California, personal communication)

Even for anaerobic infections that are often polymicrobial and do not require susceptibility testing, empiric therapy including surgery is usually curative [66]. If susceptibility testing is needed for anaerobic organisms such as *Prevotella*, Metronidazole is reliable as the first line of empiric treatment, as resistance in these organisms to Metronidazole is uncommon [67]. Meropenem is an intravenous broad-spectrum single use Carbapenem antibiotic reserved for the treatment of complicated infections.   It is effective against anaerobic organisms such as *P. buccae* and would be appropriate if organisms resistant to empiric treatment were identified. (Table 4)

To aid in the antimicrobial effectiveness, determination of the breakpoint values is important in analyzing susceptibility data for different species of organisms. The breakpoint value is used to categorize bacteria as susceptible, intermediate, or resistant to that specific antibiotic. In the present study, the susceptibility breakpoints were determined by the NCCLS (National Committee for Clinical Laboratory Standards) criteria. Breakpoints are determined based on data concerning the clinical outcome, the pharmacology of the agents, which includes the tissue and serum concentrations, the degree of protein-binding, and the susceptibility of bacteria to antimicrobial agents. When antibiotics are administered, concentrations of antibiotics in oral and maxillofacial regions are much smaller than those found in serum samples. This could be a result of the various factors of pharmacokinetics including absorption, distribution, metabolism and excretion [68]. Additionally, in specific sites there may be barriers that limit the ability for the antibiotic to penetrate through membranes of tissues, resulting in lower concentrations than serum levels.

The Clinical and Laboratory Standards Institute (CLSI) publishes standards for susceptibility testing and an antibiogram for anaerobic organisms (Table 3). The table shows that 99% of *Prevotella* samples tested were susceptible to Metronidazole. Other than Clindamycin and Moxifloxacin, most oral and IV therapies have excellent *Prevotella* coverage.   The table also reports that approximately 30% of *Prevotella spp*. are resistant to Clindamycin, and we were able to identify resistant organisms in Case 1 and 2.

Table 4. The number of resistant isolates and the percentage of resistance against different antibiotics and the prevalence of the corresponding resistance genes in *Prevotella* isolates. A.C.M. Veloo, W.H. Baas, F.J. Haan, J. Coco, J.W. Rossen. Prevalence of antimicrobial resistance genes in Bacteroides spp. and Prevotella spp. Dutch clinical isolates, Clinical Microbiology and Infection, Volume 25, Issue 9, 2019, Pages 1156.e9–1156.e13, ISSN 1198-743X, https://doi.org/10.1016/j.cmi.2019.02.017. (With permission).

	Resistant strains (n [%])			
	Amoxicillin	**Meropenem**	**Clindamycin**	**Metronidazole**
Species				
Breakpoint (mg/L)	**R>2**	**R>8**	**R>4**	**R>4**
P. baroniae (n=2)	1 (50.0)	0	0	0
P. bergensis (n=3)	2 (66.7)	0	2 (66.7)	0
P. bivia (n=17)	9 (52.9)	0	2 (11.8)	1 (5.9)
P. buccae (n=13)	5 (38.5)	0	0	0
P. buccalis (n=3)	0	0	0	0
P. copri (n=2)	1 (50.0)	0	1 (50.0)	0
P. denticola (n=7)	4 (57.1)	0	0	0
P. disiens (n=4)	1 (25.0)	0	2 (50.0)	0
P. histicola (n=2)	1 (50.0)	0	0	0
P. intermedia (n=4)	1 (25.0)	0	0	0
P. jejuni (n=2)	2 (100)	0	0	0
P. melaninogenica (n=21)	14 (66.7)	0	1 (4.8)	1 (4.8)
P. nigrescens (n=4)	3 (75.0)	0	1 (25.0)	0
P. oris (n=2)	2 (100)	0	0	0
P. timonensis (n=6)	1 (16.7)	0	1 (16.7)	0
Prevotella spp. (n=7)	1 (14.3)	0	0	0
Total, n (%)	48 (48.5)	0	10 (10.1)	2 (2.0)

Conclusions

The principles of surgery including incision, drainage (removing the source of the infection, often including tooth extraction, exposing the area to air which eradicates exposed obligate anaerobes, and decompressing the swelling), and irrigation (decreasing the bacterial load and removing bacterial inflammatory byproducts) will often cure an odontogenic infection in the immunocompetent host.

Infections refractory to surgery and empiric antibiotics should be cultured to identify opportunistic organisms. CLSI guidelines should be followed, except in light of recurrent infections where *Prevotella* isolates have been identified. *Prevotella buccae* is resistant to both beta lactam antibiotics and Clindamycin. *Prevotella buccae* and other empiric antibiotic-resistant *Prevotella* species remain part of the oral microbiome and must be ruled out as an opportunistic pathogen.

Metronidazole is bactericidal at the appropriate MBC to treat all *Prevotella* species. Multispace odontogenic infections with mixed flora isolates that include obligate anaerobic organisms can be very dangerous. Early surgery is necessary prior to infections spreading through fascial spaces and causing trismus and airway compromise, as these infections can result in morbidity and mortality.

Metronidazole seems to be the only oral antibiotic able to effectively eradicate *Prevotella buccae* and other antibiotic resistant *Prevotella* species, as well as a majority of obligate anaerobes [6]. Tolerance to the disulfiram-like reaction and stomach upset remain side effects of Metronidazole, limiting its use in some patients. Access to intravenous agents that can eradicate *Prevotella* species resistant to oral antibiotics often require hospitalization for administration and access to the drugs.

If treatment of these infections is late in the course of the disease, radiographs including CT scans and infectious disease specialist consultation may be indicated. A polypharmaceutical approach to serious odontogentic infections of the head and neck may be needed to supplement appropriate

surgery of the infected areas. Culture and sensitivity testing can guide antibiotic susceptibility choices.

Perioperative irrigation of dilute Chlorhexidine 0.12% mouthwash during dentoalveolar surgery seems to prevent both perialveolar infections and alveolar osteitis. Repeated use of Chlorhexidine in the presence of mixed flora odontogenic infections and pericoronitis may lead to disruption of a protective microbiome—resulting in the selection of resistant microorganisms—giving an opportunity for *Prevotella* to become virulent. Its use is meant to be adjunctive to surgery and systemic antibiotic administration, eradicating opportunistic bacteria during surgical procedures.

We look forward to a study of isolates from infections with a larger sample size that identifies resistant *Prevotella* species and directs the clinician to targeted effective narrow spectrum antibiotic therapy. Antibiotics available for oral administration with a wide therapeutic index and minimal side effects would be ideal.

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