Prevalence and Antimicrobial Susceptibility Pattern of Pathogens Isolated from Patients with Juvenile Periodontitis in Jamaica: A Prospective Multi-centre Study of 15 Cases over a 15-year Period

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ABSTRACT

Prevalence and antimicrobial susceptibility pattern of most frequent pathogens isolated from patients treated with juvenile periodontitis at three separate dental centres in Jamaica from 1989 to 2003 were studied. Swabs were taken from these patients periodontal pathologic pocket or root of most of their teeth with active disease processes. These swabs were processed at the microbiology department of the University Hospital of the West Indies Kingston, Jamaica and the Microbiology laboratory, School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago. The identification of the micro-organisms from positive cultures and their antimicrobial susceptibility profile were performed using standard microbiological procedures and dick diffusion (Kirby-Bauer) methods. Over 80% of the patients were females.

The most frequent micro-organisms isolated were Enterobacter (40.5%), followed by Klebsiella species (19%) and Acinetobacter species (10.8%). Actinobacillus actinomycetemcomitans, *a widely known key pathogen in juvenile periodontal diseases was encountered only in 5.4% (2/37) of the cases in this study. The most frequent organism isolated were still highly susceptibility to the commonly used and available antimicrobials such as amoxycillin/clavulanate,trimethoprim/sulphamethoxazole, chloramphenicol and aminoglycosides.*

The most frequent pathogens encountered in this study were totally different from what obtains in other places. There is the need to be aware of microbes in other countries during the microbiology investigations of juvenile periodontitis and that the antimicrobial chemotherapy should always be based on susceptibility test results. Surgical treatment for mechanical debridement of the site and bone grafting with guided tissue regeneration should be mandatory in conjunction with specific antimicrobial chemotherapy.

Keywords: Juvenile, pathogens, periodontitis

Prevalencia y Patrón de Susceptibilidad Antimicrobiana de Patógenos Aislados de Pacientes con Periodontitis Juvenil en Jamaica: Estudio Prospectivo Multicentro de 15 Casos por un Período de más de 15 Años

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RESUMEN

Se estudió la prevalencia y el patrón de susceptibilidad antimicrobiana de los patógenos más frecuentemente aislados de los pacientes tratados por periodontitis juvenil en tres diferentes centros

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odontológicos en Jamaica de 1989 a 2003. Se tomaron muestras de las bolsas patológicas periodontales de estos pacientes, o de la raíz de la mayor parte de sus dientes, en medio del proceso activo de la enfermedad. Las muestras fueron procesadas en el departamento de Microbiología del Hospital de la Universidad de West Indies, Kingston, Jamaica, y en el laboratorio de Microbiología, Escuela de Medicina Veterinaria, Facultad de Ciencias médicas, Universidad de West Indies, San Agustín, Trinidad y Tobago. La identificación de los microorganismos a partir de cultivos positivos y su perfil de susceptibilidad antimicrobiana, se realizaron mediante procedimientos microbiológicos estándares y métodos de difusión por disco (Kirby-Bauer). Más del 80% de los pacientes eran mujeres.
Los microorganismos más frecuentemente aislados fueron Enterobacter (40,5%), seguido por especies de Klebsiella (19%) y Acinetobacter (10,8%). Actinobacillus actinomycetemcomitans *– un patógeno clave ampliamente conocido en enfermedades periodontales juveniles – se encontró sólo en 5,4% (2/37) de los casos en este estudio. Los organismos más frecuentemente aislados mostraban todavía una alta susceptibilidad frente a los antimicrobianos comúnmente usados y disponibles, tales como amoxicilina/clavulanato, trimetoprima/sulfametoxazol, cloranfenicol y los aminoglicósidos. Los patógenos más frecuentemente encontrados en este estudio fueron totalmente diferentes de lo que se obtiene en otros lugares. Es necesario tomar conciencia de los microbios en otros países durante las investigaciones de microbiológicas de la periodontitis juvenil, y no perder vista que la quimioterapia antimicrobiana debe basarse siempre en las pruebas de susceptibilidad. El tratamiento quirúrgico para el desbridamiento mecánico del sitio, así como el injerto óseo con regeneración tisular guiada debería, deben ser obligatorios en conjunción con la quimioterapia antimicrobiana específica.*

Palabras claves: Juvenil, patógenos, periodontitis

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INTRODUCTION

Periodontitis is a chronic inflammation of supporting tissues of the teeth with progressively destructive changes that lead to loss of the supporting bone and periodontal ligament. Three types of periodontitis acute, chronic and juvenile are described in the literature. These entities, particularly juvenile periodontitis, can be mistaken for rapidly progressive periodontitis seen in AIDS and the varying degrees of periodontal bone loss associated with systemic conditions such as diabetes mellitus and syndromes such as Papillon-Lefevre syndrome (1).

Following the recent reclassification of periodontal diseases, aggressive periodontitis is the term now given to those conditions previously known as juvenile periodontitis and rapidly progressive periodontitis (2). Juvenile periodontitis is a localized or generalized (rapidly progressive) degenerative disease of the periodontium. It is a relatively uncommon disease with a predilection for females and may have a higher prevalence in developing countries and among black patients (3, 4). It can be differentiated from acute or chronic periodontitis because of its specific clinical and radiological presentation.

The understanding of the aetiology of periodontal disease has improved markedly over the last three decades. Several studies have evaluated the micro-flora typically associated with periodontitis (including juvenile periodontitis) and available data strongly implicate such organisms as relevant aetiological pathogens including, *Actinobacillus actinomycetemcomitans, Tannerela forsythenesis (*formerly *Bacteroides forsythus), Campylobacter rectus, Eikenella corrodens, Eubacterium species, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Micromonas micros (*formerly *Peptostreptococcus micros), Treponema denticola* as well as viruses *(*5–7).

Factors such as genetic and environmental have also been known to be strongly associated with different patterns of colonization by periodontal pathogens (8–10). *Pseudomonas aeruginosa, E coli* and several other pathogenic microbes have achieved sophistication in the type of characteristic secretory systems they have to produce highly specialized translocation machinery that is able to promote processes that enable their adherence and/or internalization (11–12).

The purpose of this present study is to highlight the prevalence of some other common bacterial pathogens that could be associated with juvenile periodontitis and their antimicrobial susceptibility patterns for an understanding by healthcare providers of the varying aetiology, epidemiology and geographical agents of the disease.

SUBJECTS AND METHODS

Clinical evaluation

This was an observational prospective study that involved 26 swabs taken from appropriate sites in the oral cavity of 15 patients with clinical and radiological features of juvenile periodontitis seen at three dental centres in Jamaica between 1989 and 2003. The dental centres were Chapelton Community Hospital located in the central region; "Fish" Medical and Dental Clinics in the southeast region and Cornwall

Dental Centre located in the northwest region of the country, respectively.

Specimen collection and processing

The swabs on each occasion were taken from the periodontal pathologic pocket or root of most of the affected teeth with active disease processes. Swabs were taken using Ames and transported at room temperature to the laboratory within 24 hours for processing. The specimens were processed at the Microbiology department at the University Hospital of the West Indies, Kingston, Jamaica and the Microbiology laboratory, School of veterinary medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago at different times during the study period.

Gram-staining was performed on each clinical specimen and this was followed by culture using appropriate solid media such as blood agar, chocolate agar, sabourauds agar etc and were incubated at 35–37ºC overnight under both aerobic and anaerobic conditions.

Isolates identification and susceptibility tests

Specimens that yielded positive growth in the culture were further subjected to biochemical tests such as catalase, coagulase, oxidase, several sugar tests and so on to identify the organism growing from the swab.

The disk diffusion (Kirby-Bauer) methods were used to determine the antimicrobial susceptibility profiles of the microbial isolates from the swabs following Clinical Laboratory and Standards Institute, CLSI recommendations (13).

The antibiotics tested included ampicillin, amoxycillin-clavulanate, chloramphenicol, trimethoprim-sulphamethoxazole, gentamicin and amikacin. All the patients had surgery and were treated with the appropriate antibiotic regime for the isolated pathogens. Post "appropriate" antimicrobial chemotherapy, culture and susceptibility test were done for only one patient, particularly because she requested advanced fixed restoration for her missing teeth that was lost because of juvenile periodontitis.

Each patient also had radiological examinations of the site of the periodontitis. All the patients gave their permission to be included in the study when consented for treatment at the different dental centres.

RESULTS

All the patients included in this study presented with clinical and radiological features consistent with a diagnosis of juvenile periodontitis. A photograph of one of the affected teeth as well as a radiological picture of one of the cases with juvenile periodontitis is shown in Figs. 1 and 2a–b. Also, a summary of the distribution of the sites of swab collection and the micro-organisms isolated are depicted in Table 1. Of the 26 swab specimens collected from these 15 patients (13 females and 2 males, ages between 11 and 28 years, mean

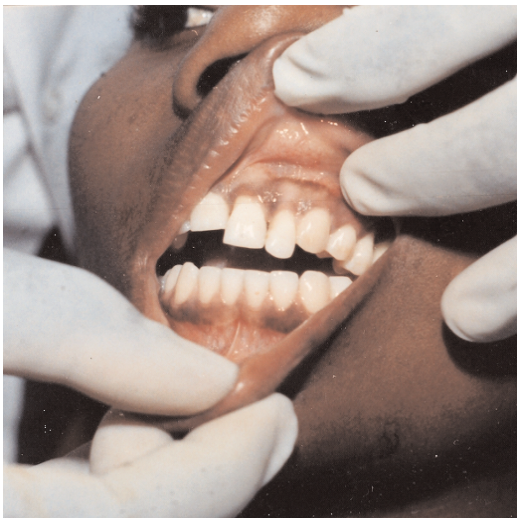

Fig. 1: A photograph of one of the patients seen in Jamaica with severe pathological migration of the upper anterior with clinical grade 3 mobility.

Table 1: Distribution of specimen collection site and micro-organisms isolated from 15 patients with juvenile periodontitis from 1989 – 2003, in Jamaica

Patient	Site of swab	Micro-organism isolated
1	Gingival crevice	Enterobacter spp
	PPP of 32	*Candida albicans*, Enterobacter spp
2	PPP mesial side of 17	*K. oxytoca* & Enterobacter spp
3	Gingival crevice of 16	Enterobacter spp, Acinetobacter spp;
	PPP of 14	Alcaligenes spp
4.	Apex of 46 post extraction	*Staph aureus*, Klebsiella spp
	Apex of 15 post extraction	Microaerophilic Strep, Enterobacter
5	Gingival crevice 35	Enterobacter spp, Acinetobacter spp
	Gingival crevice 36	*P. aeruginosa*, *K. oxytoca* Enterobacter spp
6	PPP of 11	Acinetobacter spp
	PPP of 16	Enterobacter spp
7	PPP of 21	Microaerophilic Strep, Klebsiella spp
	Gingival crevice of 21	Enterobacter spp & Klebsiella spp
8	PPP of 41	Enterobacter spp
	Gingival recession of 41	Acinetobacter spp
9	PPP of 26	Enterobacter spp, Klebsiella spp
	Gingival recession of 26	Enterobacter spp
10	PPP 18	No organism isolated
11	Gingival recession 11	*A. actinomycetemcomitans*
	Gingival recession 31	Enterobacter spp
	Gingival recession 41	Microaerophilic Strep spp
12	No swab taken	–
13	PPP of 24	No organism isolated
	Gingival crevice of 22	*A. actinomycetemcomitans*
14	PPP of 45	*P. aeruginosa*
	PPP of 46	*S. viridans*, Enterobacter spp
15	PPP of upper anterior teeth	Klebsiella spp, Enterobacter spp

PPP = Periodontal pathologic pocket

age 20.3 years), 92.3% (24/26) had positive culture yielding 37 micro-organisms; the rest had no growth. The results show that 50% (13/26) of the swab specimens were taken from periodontal pathologic pockets followed by gingival crevices 23% (6/26) and gingival recession 19.2% (5/26). From the same patient, there were multiple swabs taken and more than one micro-organism was isolated from 46.1%

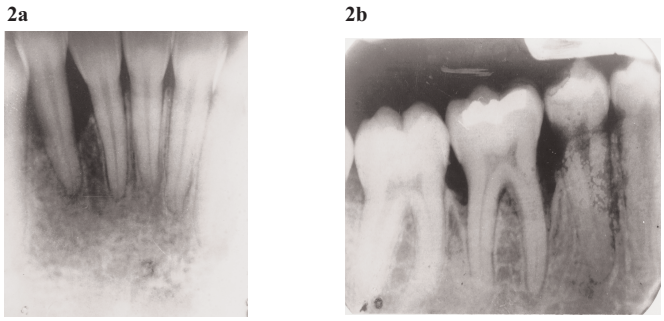

Fig. 2a: Radiograph of the periapical teeth of one of the patients showing evidence of vertical and horizontal bone loss of the lower anterior teeth; and Figure 2b – periapical radiograph of another patient showing surcerized bone loss of the lower first molar with evidence of furcation involvement.

(12/26) of such specimens. As shown in Fig. 3, 97.3% (36/37) of the micro-organisms were bacteria. Gram-negative bacilli organisms accounted for the majority 83.8% (31/37), 13.5% (5/37) were gram-positive cocci while 2.7%

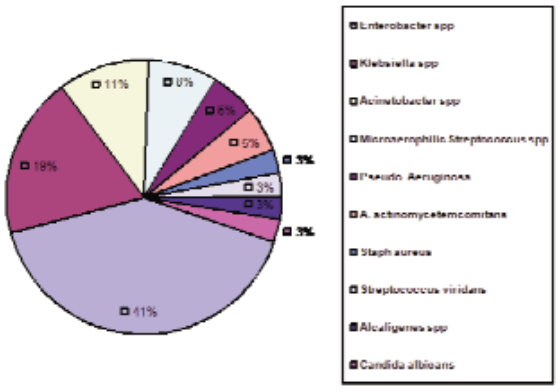

Fig. 3: Frequency distribution of 37 micro-organisms isolated from 26 oral cavity swabs of 15 juvenile periodontitis cases seen at three dental centres over a 15-year period in Jamaica.

(1/37) were fungi. Previously unmentioned aetiologic micro-organisms in the pathogenicity of juvenile periodontitis were found in these patients. The most frequently isolated micro-organism was Enterobacter species 40.5% (15/37) followed by Klebsiella species 19.0% (7/37) and Acinetobacter species 10.8% (4/37). *Actinobacillus actinomycetemcomitans,* a widely known key pathogen in juvenile peridontal diseases was encountered only in 5.4% (2/37) of the cases in this study.

The antibiotic susceptibility pattern of the three most frequently isolated bacterial organisms (Enterobacter spp, Klebsiella spp and Acinetobacter spp) are depicted in Fig. 4.

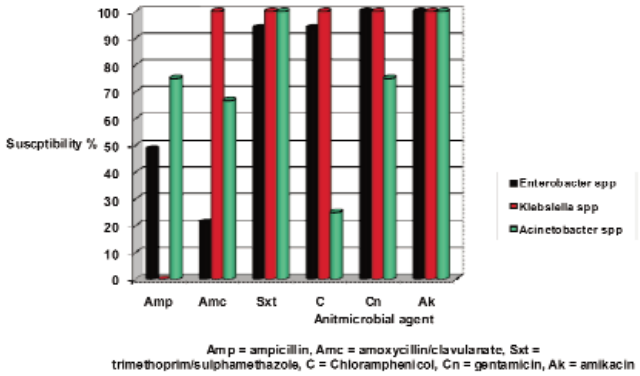

Fig. 4: Antimicrobial susceptibility patterns of most frequent isolates from the oral cavity; swabs taken from 15 juvenile periodontitis patients in Jamaica.

The Enterobacter species demonstrated poor susceptibility to oral antibiotics including ampicillin (48.8%) and amoxycillin-clavulanate (21.4%); and high susceptibility to trimethoprim-sulphamethoxazole (93.8%), chloramphenicol (93.8%) and excellent (100%) susceptibility pattern to the aminoglycosides – gentamicin and amikacin. Klebsiella species susceptibility to amoxycillin-clavulanate, trimethoprim-sulphamethoxazole and chloramphenicol were perfect. Acinetobacter species also had an excellent (100%) susceptibility to trimethoprim-sulphamethazole and amikacin. Only one of the cases had a post antimicrobial treatment culture and susceptibility tests done and these revealed complete eradication of the pathogenic organisms. Post treatment culture was not done for the rest of the patients because they responded very well to the appropriate antibiotic treatment in conjunction with surgical manipulation.

DISCUSSION

The microbiological findings of this series clearly show a variation from what has been previously documented by other authors throughout the world as indicator micro-organisms or key pathogens that include *A actinomycetemcomitans, T forsythenesis, Eubacterium species, Porphyromonas and Prevotella species* that are implicated in the initiation and progression of Juvenile periodontal diseases (14–16).

Enterobacter, Klebsiella and Acinetobacter species were the most encountered micro-organisms in this study, and to the best of our knowledge, none of these microbes has been described in literature as associated with any form of Juvenile periodontal disease. These organisms are widespread throughout the environment or vegetal sources (17).

The Enterobacter and Klebsiella species are opportunistic pathogens belonging to the enterobacteriaceae family that includes several other microbes. They are facultative gram-negative rods and are described as glucose and lactose

fermenters, oxidase negative, capable of reducing nitrates to nitrites. While Enterobacter species are motile, Klebsiella species are not. Their virulence or pathogenicity lies in the fact that both organisms can establish infection through several factors such as their fimbriae, prominent capsule, cell wall containing lipopolysaccharide that act as endotoxin. These factors provide these organisms with an increased resistance to phagocytosis and the action of complement and antimicrobial resistance mechanisms (17).

Actinobacillus actinomycetemcomitans that is regarded as a key pathogen or most notorious bacterium linked to periodontitis, has been demonstrated to evade host defenses by different mechanisms such as production of leukotoxins, collagenase, polymorphonuclear (PMN) chemotaxis-inhibiting flora factors after its initial colonization (18–22).

A minimal number of the micro-organisms that were isolated in this study were gram-positive cocci. This is in contrast to reports from other researchers that observed a high relative proportion of gram-positive facultative cocci such as *S aureus* and Streptococcus species (15).

The three most frequently isolated micro-organisms in this study (Enterobacter spp. Klebsiella spp and Acinetobacter spp) had good susceptibility to many common and easily available antimicrobial agents in our locality. Since they were not the regularly isolated pathogens in previous reports by other researchers, report on their susceptibility is tardy. Although only a few classes of antimicrobial agents had their susceptibility tests performed for Enterobacter, Klebsiella and Acinetobacter, these organisms still had very high susceptibility rates to the commonly used and available antimicrobials in the country.

All the cases encountered in this present study were placed on the appropriate antimicrobial agent based on the culture and susceptibility test results.

In summary, the most predominant organism in cases with juvenile periodontitis from Jamaica is the Enterobacter species. The antimicrobial chemotherapy was predominantly based on the outcome of culture and susceptibility testing. We propose that further studies in other countries on the microbiology of juvenile periodontitis should always look out for these other organisms that were encountered in the present study from Jamaica and that antimicrobial treatment should always be based on susceptibility test results. Surgical treatment of juvenile periodontitis should also be mandatory.

REFERENCES

1. Burgett F. Periodontal disease. In: Oral Pathology: Clinical-Pathologic Correlations. Regezi JA, Sciubba JJ, Ed. Philadelphia: Saunders; 1989: pp 503–19.
2. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; **4:** 1–6.
3. Melvin WL, Sandiff JBB, Gray JL. The prevalence and sex ratio of juvenile periodontitis in a young racially mixed population. J Periodontol 1991; **62:** 330–4.
4. Eisenmann AC, Eisenmann R, Sousa D, Slots J. Microbiological study of localized juvenile periodontitis in Panama. J Periodontol 1983; **54:** 712–13.
5. Kamma JJ, Nakou M, Persson RG. Association of early onset periodontitis microbiota with aspartate aminotransferase activity in gingival crevicular fluid. J Clin Periodontol 2001; **28:** 1096–1105.
6. Mullally BH, Dace B, Shelburne CE, Wolf LF, Coulter WA. Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. J Periodontol Res 2000; **35:** 232–41.
7. Kamma JJ, Contreras A, Slots J. Herpes viruses and peiodontopathic bacteria in early-onset periodontitis. J Clin Periodontol 2001; **28:** 879–85.
8. Umeada M, Chen C, Bakker I, Contreras A, Morrison JL, Slots J. Risk indicators for harboring periodontal pathogens. J Periodontal 1998; **69:** 1111–8.
9. Spektor DM, Vandesteen GE, Page RC. Clinical studies of one family manifesting rapidly progressive, juvenile and prepubertal periodontitis. J Periodontol 1985; **56:** 93–101.
10. Okada M, Awane S, Suzuki J, Hino T, Takemoto T, Kurihara H, Miura K. Microbiological, immunological and genetic factors in family members with periodontitis as a manifestation of systemic disease, associated with hematological disorders. J Periodontol Res 2002; **37:** 307–15.
11. Anderson DM, Schneewind O. Type III machines of gram-negative pathogens: injecting virulence factors into host cells and more. Curr Opin Microbiol 1999; **2:** 18–24.
12. Zambon JJ. Periodontal diseases: microbial factors. Ann Periodontol 1996; **1:** 879–925.
13. Wikler MA, Cockerill FR, Craig WA, Dudley MN, Eliopoulos GM. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Document M100-S14. Wayne, PA: NCCLS; 2004 p 30–5.
14. Nonnenmacher C, Mutters R, de Jacoby LF. Microbiological characteristics of subgingival microbiota in adult periodontitis localized juvenile periodontitis and rapidly progressive periodontitis subjects. Clin Microbiol Infect 2001; **7:** 213–7
15. Darby I, Curtis M. Microbiology of periodontal diseases in children and young adults. Periodontology 2000. 2001; **26:** 33–53.
16. Lee JW, Choi BK, Yoo YJ, Choi SH, Cho KS, Chai JK, Kim CK. Distribution of periodontal pathogens in Korean aggressive periodontitis. J Periodontol 2003; **74:** 1329–35
17. Abbott S. Klebsiella, Enterobacter, Citrobacter, and Serratia. In PR Murray, E J Baron M A Pfaller FC, Tenover, R H. Yolken (ed), Manual of clinical microbiology, 7th ed: Washington, DC: ASM Press; 1999: pp 475–80.
18. Gillelf R, Johnson NW. Bacterial invasion of the Periodontium in a case of Juvenile Periodontitis. J Clin Periodontol 1969; **40:** 40.
19. Carranza FA, Saglie R, Newman MG. Scanning and transmission election microscopy study of tissue invading microorganism in localized juvenile periodontitis. J Periodontol 1983. **54:** 598.
20. Zambon JJ, Christerssons EA, Slots J. Actinobacillus actinomycetemcomitans in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. J Periodontal 1983; **54:** 707.
21. Hillman JD, Socransky SS. Bacterial interference in oral ecology of Actinobacillus actinomyceteuscomitans and its relationship to human periodontosis. Arch oral Biol 1982; **27:** 75.
22. Page RC, Baab DA. A new look at the etiology and pathogenesis of early onset periodontitis. Cementopathia revisited J Periodontal 1985; **56:** 748.