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RESEARCH ARTICLE

Susceptibility of Porphyromonas Gingivalis Isolated from Patients Suffering from Periodontitis to Antibiotics

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Abstract

Twenty three of *P. gingivalis* isolated from ninety one patients complaining from periodontitis with different age from both gender in Babylon city. According to routine culture methods were done using differential media, anaerobic condition and morphological criteria such as microscopically and biochemically testsand percentage (25.3%). The activity of 27 antibiotics were tested towards the current isolates, and the results showed high degree of resistance for most antibiotics represented by: penicillin, ampicillin, amoxicillin ,carbenicillin, , tetracycline, doxycycline (100%), ticarcillin(92.6%) ,piperacillin (95.7%),erythromycin (85.2%) ,Co-trimoxazole and chloramphenicol (81.5%),cefepime (63.0%), cefixime ,ampiclox ,colistin(51.9%), ciprofloxacin and norfloxacin (56.5%), tobromycin (52.1%), ceftrixzone, aztreonam (47.8%), azithromycin, gentamicin and cefotaxime (44.4%) amoxiclave (37.1%),amikacin (33.3%),while The meropenem and impinem showed more effective against current isolates .The present results were consistent the what is known multidrugresistant *P.gingivalis*.The minimum inhibitory concentrations (MIC) of most present isolates were higher.

Keywords: *P.gingivalis, Periodontitis, Antibiotics resistance, Minimum inhibitory concentration.*

Introduction

P.gingivalis belongs to the family of Porphyromonadaceae and order, Bacteroidales, whose name is derived from the Greek word for purple and its first unsatisfactory strain, W83, by H. Werner in the 1950s from oral infections. In 2003, W83 became the first strain of *P. gingivalis* to be sequencedof this bacteria [1].In humans, these bacteria are often under the gums of the oral cavity, which is part of the biofilm that is in the dental plaque, causing the pain resulting from gingivitis [2], and finally lead to loss of tooth, if the inflammation of the gums not treated and finally destroyed the bacteria [3].

And located in uppergastrointestinal tract, respiratory tract, large intestine, *P.vaginalis* considered apportunistic pathogen ,also it has been isolated from adult females with bacterial vaginosis[4] , short rod-shapedor spherical globules in the liquid media and size 0.5 µm and 1-2 µm in size, while its colonies on the solid media were circles and smooth, shiny and high, and darkened on dark red blood because they take up the blood iron that oxidizes to hemin which aggregate on the surface of the cell, negative to gram stain, some of its strains are encapsulated, non-motile, non-forming of spores, having fimbriae, and anaerobic respiration, where metabolic stratification is similar to many bacterial species as it relies on peptide chains from host animal as a source of carbon and nitrogen secretion material called gingipain degrades peptides[5].

It is one of the most important causes of human disease in areas around the teeth causing acute and chronic injuries worldwide, including Iraq, as the sugars and starchy substances and food residue in the mouth and lack of care of cleanliness, especially in people who do not use the brush and medical toothpaste or huge people [6], which qualifies them to benefit from the growth of the mouth and the growth and reproduction due to fermentation of the mouth by doing, creating an environment suitable for survival and exercise activities that exceed the gums surrounding the teeth and traverses to reach the parts of the age of cannibalism and finally lead to inflammation, especially in

patients with diabetes [7]. dental disease is a group of factors leading to inflammation of the tissues surrounding the teeth also known as gum disease in the early stages called gingivitis and then become swollen gums, red and perhaps bleeding and more serious forms called periodontitis and gum pulls away from the teeth to reach the bone leading to loss or impairment or fall and may get bad breath, these infections occur in humans with periodontal infection affects 10-15% of adult male and female worldwide.

Some strains of these bacteria have the ability to resist treatment, also a harmful microorganism when infection occurs.*P. gingivalis* has been associated with rheumatoid arthritis,and those affected by the disease, the most common people with periodontitis are diabetes, one of the most common toxins of LPS and Lipid A, Siderophore, and the enzymes (collaginase, peptidyl-deiminase, aminopeptidases, hemagglutinin, phospholipase C, prosterases, alkaline phosphatase, hemolysins) Arg-gingipain cysteine proteases were localized in the outer membrane vesicles in *P.gingivalis*,it was to invade the host's tissues, and its ability to form the thin tissue, which helps them to avoid treatment [8].

The importance of clinical bacteria as one of the main causes of infections of the gums and bokets and inflammation of the teeth and teeth loss and increase these cases in Iraq led us to isolate the current bacteria and identification of common laboratory tests, In addition to the main objective of the current study is to investigate resistance to antibiotics [9].

Materials and Methods

Collection of Samples

A total of 91samples of patients from both genders of different ages,who suffer from gingivitis and specifically from their pockets, are supervised by a gynecologist using paper paper inside the pockets of the gums and then left for1-2minutes.heart infusion broth orphysiological solution.Scientific methods were used in the treatment of samples, their transfer to the laboratory, their implantation, incubation and examination to investigate the bacteria under study and to test the drug sensitivity.

Bacterial Isolates

*P.gingivalis*isolates were isolated from gingivitis patients after culturing of the samples on the blood agar base,the MacConkey agar (Himedia),and in brain heart infusion broth containing5µg /ml of VitamineK and(1ug/ml)Cysteine, under anaerobic conditions. Identified based on phenotypic traits (culture and microscopic) and biochemical tests [10].

Antibiotic Susceptibility Test

Twenty-seven antibiotics equipped with Bioanalyse-Turky-ready tablets were used, and the lowest inhibitory concentration of antibiotics was determined by macro-dilution method [11].

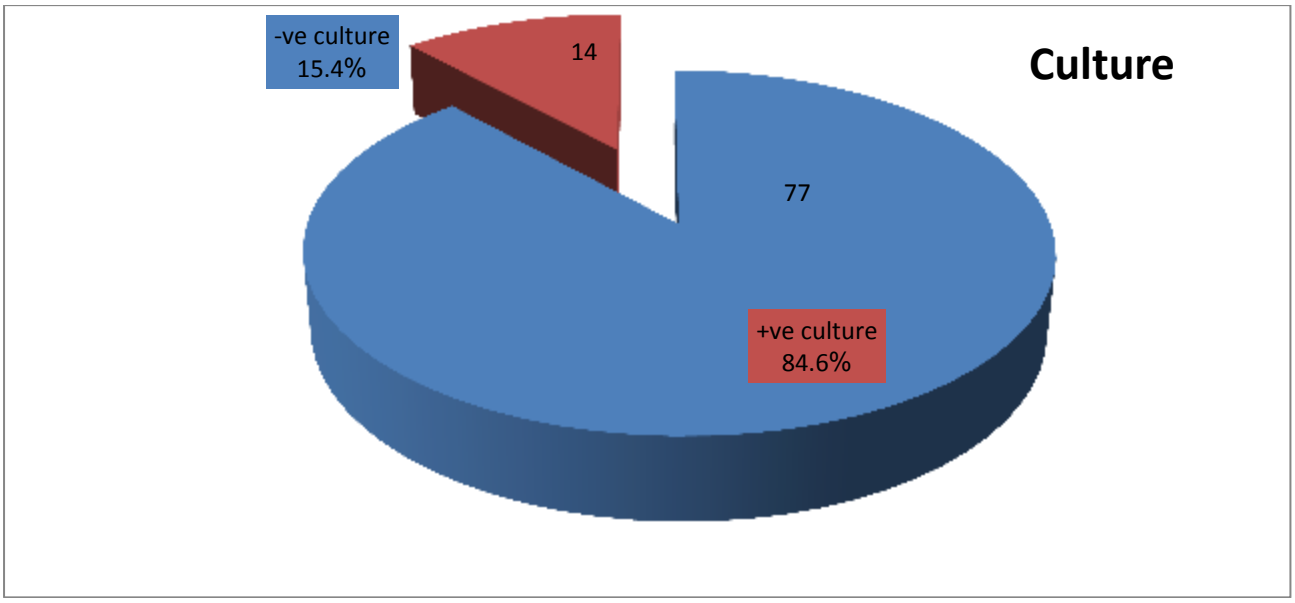
Results and Discussion

23 isolates were isolated from periodontitis patients, its numbers and percentage were distributed as shown in Table 1.Depending on the characteristics of the cultural and microscobiclfor bacteria and the biochemical tests.

Table2: The Numbers and percentages of P.gingivalis isolates isolated from periodontitis

Age(year)	No. samples		No.Total Isolates	(%) For isolates
	-ve	+ ve		
Male(16-70Years)	9	53	18	33.9
Female(16-70Years)	5	24	5	20.8
Total	14	77	23	29.9
	91			

The current results showed that percentage of *P.gingivalis* isolates were 25.3 from total samples and 29.9% were from positive culture for both males and females and from different age. The depth of the pocket and the type of infection was determined to be acute or chronic, of chronic cases were higher than acute cases and the depth of the pockets were 4-10 mm.



Figur 1: Positive & negative culture of sample taking from periodontitis patients

While [12] showed that (28.9%) of isolation were from subgingivival plaque biofilm from patients with periodontal health samples ,(20.4%) from gingivitis ,(13.3%) of generalized aggressive and 36.3% of chronic periodontitis, and this because the possession of these bacteria factors of virulence and in particular antibiotic repel enable it to exercise its vital activities of the growth and multiplication of beneficiaries of whatever the food and environment mouth unhealthy, leading to the intricacies of oral accompaniment to hit specific to the gums and teeth may reach the bone, causing its inflammation, [13] were enable of isolated this bacteria under study and causing the inflammation of periodontal chronic causing the problems of the teeth and gums and showed , it was very important from the clinical side.

Table2: the Numbers and percentages of *P.gingivalis* isolates according to Age

Age(year)	No.Totalinfections			
	Males		Females	
	No.	%	No.	%
Less of 20Years	1	5.5	0.0	0.0
(20-30Years)	4	22.3	2	40
(31-40Years)	7	38.9	1	20
(41-50Years)	3	16.7	1	20
(51-60Years)	2	11.1	1	20
(61-70Years)	1	5.5	0	0.0
Total	18	100%	5	100%

The resistance to Merppenem, Impenem, was 0.0%, the lack of resistance of *P. gingivalis*was due to the resistance of many antibiotics due to the modernity of these bacteria for antibiotics and itsnot continuous use as well as its cost expensive.

The presence of bacteria in the environment container of antimicrobial antibiotics, such as hospitals, which are continuously exposed, increases the selective pressureand increases the resistance of bacteria, the inaccuracy of the identification of bacteria and the treatment of infections caused by inappropriate treatments and indiscriminate use without consultation of these antibiotics. The results showed that the resistance of *P.gingivalis* to amikacin and amoxiclave were (34.8%) ampiclox, colistin (60.8%), ciprofloxacin and norfloxacin (56.5%),tobromycin (52.1%), while azthromycin (39.1) [14]. Refers tothe resistance of *P. gingivalis* isolates to azithromycin,amoxicillin andclavunilic acid were 100%, and its resistance may be were to the production of bacterial isolates for virulence factors, containing broken-down enzymes such as beta lactamaseand cephalosporinase, which encoded by chromosome or plasmid,and a fracturedor inhibitor for antimicrobial cephalosporines, thebeta-lactame antibioticswhich inhibit cellular wall construction by interfering with peptidoglycan synthesis,as well as the others mechanics of its resistance, such as the

narrow channels through which the antibiotic penetration , as some bacteria have many channels are very narrow or sometimes closed, making it more resistant than other bacteria, in addition to the characteristic permeability of the plasma membrane in bacteria and the amount of lipid whichthe presence of it more than what is present in the sensitive bacteria, as well as the absence or decrease of the receptors of penicillin antibiotics to the bacterial cell wall or changes in the permeability of antibiotics or prevent passage of the antibiotics through the holes scattered in the outer membrane. as well as mucus secreted by bacteria and it was formation of biofilm that protect bacteria from external influences such as hating ,disinfectant,sterilization and antibiotics, relative to free bacteria,which sensitive to it,and this was indicatedby[15] in his study of resistance antibiotics antibiotics by *P. gingivalis* were isolated from the biofilms formed on the teeth.

Table 3: The antibioticssusceptibility of *P.gingivalis* isolates isolated from periodontitis

P.gingivalis	Cifixime	Tobramycin	Amoxiclave	Meropenem	Impenem	Ampiclox	Cifipiem	Ticarcillin	Amoxcillin	Aztronam	Cloramphen	colstin	Gentamycin	Sifitrixon	Ciprofloxaci	Tetracyclin	Amikacin	Cefotaxime	carbencillin	Norfloxacin	Ampicillin	Pencillin	Doxycyclin	Pipracillin	Azithromyci	Erthromyci	C-	No.ofisolate
1	R	R	S	S	S	S	R	R	R	S	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	18
2	R	R	S	S	S	S	R	R	R	S	R	R	S	S	I	R	S	R	R	R	R	R	R	R	S	R	R	7
3	R	R	R	S	S	R	R	R	R	S	R	S	S	R	I	R	R	R	R	R	R	R	R	R	R	R	R	21
4	R	R	R	S	S	R	R	R	R	R	S	R	S	R	S	R	R	S	R	S	R	R	R	R	R	R	R	20
5	R	R	R	S	S	R	R	R	R	R	R	R	R	I	S	R	S	S	R	R	R	R	R	S	R	S	R	19
6	R	R	R	S	S	R	R	R	R	S	R	R	S	S	S	R	S	R	R	S	R	R	R	R	S	R	R	18
7	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	I	R	I	R	18
8	S	R	I	S	S	R	R	R	R	S	R	R	R	S	R	R	I	S	R	S	R	R	R	R	S	R	R	16
9	R	R	S	S	S	R	R	R	R	S	R	R	R	R	S	R	S	S	R	S	R	R	R	R	S	R	R	18
10	R	S	S	S	S	S	S	R	R	S	R	S	R	I	S	R	S	R	R	S	R	R	R	R	R	R	R	15
11	R	S	I	S	S	S	S	R	R	S	R	R	S	S	S	R	R	S	R	R	R	R	R	R	S	S	R	14
12	R	S	S	S	S	R	S	R	R	R	R	R	S	S	S	R	S	R	R	S	R	R	R	R	S	R	R	16
13	R	R	S	S	S	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	20
14	S	S	S	S	S	S	S	R	R	S	R	I	S	S	S	R	S	S	R	S	R	R	R	R	R	R	R	12
15	R	R	S	S	S	S	S	R	R	S	R	S	S	R	S	R	S	S	R	S	R	R	R	R	S	R	R	13
16	S	S	S	S	S	R	S	R	R	R	R	S	R	R	S	R	R	R	R	S	R	R	R	R	S	R	S	16
17	R	S	R	S	S	S	S	R	R	S	R	S	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	17
18	S	S	R	S	S	R	S	R	R	S	R	S	R	S	S	R	S	S	R	S	R	R	R	R	S	R	R	14
19	S	R	S	S	S	R	R	R	R	S	R	S	R	S	I	R	R	R	R	R	R	R	R	R	S	R	R	16
20	R	R	R	S	S	R	S	R	R	S	R	R	R	S	S	R	R	R	R	S	R	R	R	R	S	R	R	19
21	S	S	R	S	S	R	R	R	R	S	R	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	19
22	S	S	S	S	S	S	S	S	R	S	R	S	S	S	S	R	R	S	R	S	R	R	R	R	R	S	R	11
23	S	S	R	S	S	R	R	S	R	S	R	R	R	S	R	R	S	R	R	R	R	R	R	R	R	R	R	19

Abbreviations: R: Resistance, S: Sensitive, I: Intermediate resistance. It may be that the excessive use of antibiotics is a major cause of resistance to bacteria and non-affected by its therapeutic effectiveness as almost all bacteria are more powerful and less responsive to treatment with antibiotics and antimicrobial materials, and this leads us to

find new types of antibiotics in conjunction with the rationalization of its current use, leading to complications and side effects that may lead to the death of the patient and confirmed some studies that antibiotics were the fourth cause of death for the high share of the side effects and toxicity of the liver and kidney regarding the lifting of enzymes, especially the rest of the other organs of the body antibiotics have an important role in the treatment of many infectious diseases of humans and animals in the case if the best use is used as a prescription and medical advice for each patient type appropriate to the condition and the duration and dosage specific, and this is what many people are prepared to deal with in front of a variety of delicious foods and sweets withoutscientific and cultural awareness of the side effects and toxicity of drugs due to misuse that may lead to the patient's life.

And has caused the weakness of the body's immunity and influence on balance and hearing and consideration, especially showing the cumulative effect of abuse for a long time and the emergence of other strains of hostility and strong in the prevention of antibiotics and non-response to it, and thus put us in front of infection and diseases and bacterial infections severe clinical symptoms and difficult to treat. Is manifested in children, many of them receive unnecessary antibiotics at the discretion of some recent studies which is in the department of the negative effects of the drug, and also extends to the fetus, so prevent pregnant, especially in the first three months of the use of any kind of antibiotics consult a doctor (9).

P.gingivalis resistance to gentamicin and tobromycin is higher than amikacin due to antibiotic-inhibition bacterial enzymes. Gentamicin and amikacin belong to the aminooglycosides group of antibiotics, which inhibits protein synthesis by interfering with protein-making sites in bacteria.

The current results revealed that all current isolates were resistant to tetracycline while for erythromycin were 85.8%, and were contrary to [13] that found in all their bacterial isolates of*P.gingivalis* was sensitive to it. May be this were The transfer of plasmids carrying antibiotic resistance genes (R-plasmid or a piece of DNA or transposon from strains resistant to other sensitive treatments leads to medical problems related to bacterial resistance or tolerance to many antibiotics, especially in hospitals which rich in bacteria, treatments and antimicrobial agents such as disinfectants and sterile dilators that help and stimulate bacterial resistance.

The minimum inhibitory concentration of 12antibiotics selected according to medical use in Iraq were identified to treat chronic gingivitis characterized by the presence of purulent and blood cells as well as the acute causes of *P. gingivalis*, especially Augmentin, ceftriaxone, cefotaxim and azithromycin. Based on the method of estimating the growth rate of bacteria in the brain heart infusion broth by the naked eye in determining the presence or absence of growth, as well as re-culturing (Sub culture) for fear of doubtful growth.

Table6: The minimum inhibitory concentrations of antibiotics against *P.gingivalis* isolated fromperiodontitis

Antibiotics	% of resistance for *P. gingivalis*	MICs (µg/ml) for *P.gingivalis*
Amoxiclave	37.1	1 -32
Ampicillin	100	64 ->128
Azithromycin	44.4	0.5 -32
Cefotaxime	44.4	2 ->128
Meropenem	0.0	0.5 -2
Amoxicillin	100	32 ->128
Amikacin	33.3	0.5 -32
Cloramphenicol	81.5	16 ->128
Gentamicin	44.4	1 -64
Ciprofloxacin	56.5	32 ->128
Erythromycin	85.2	16 ->128
Cefetriaxone	47.8	1 -16

Conclusion

P.gingivalis isolates associated with periodontitis showed multidrugresistance but it was highly sensitive to meropenem and imipenem. The result in this studydeclared that there are effects of gender and age on

isolation percentageand the current results showed that percentage of *P.gingivalis* isolates werehigh in 20-30 years and isolation of it inmales were more than females.

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