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ORIGINAL

Antimicrobial resistance properties of *Helicobacter pylori* strains isolated from dental plaque and saliva samples

Propiedades de resistencia antimicrobiana de cepas de Helicobacter pylori aisladas de muestras de placa dental y saliva

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Abstract

Background: It is likely that oral cavity, particularly dental plaque and saliva samples, may represent an important reservoir of *H. pylori* infection for gastric infections. The present research was performed to assess the distribution and antimicrobial resistance of *H. pylori* strains in dental plaque and saliva samples.
Methods: A total of 80 *H. pylori* strains were isolated from dental plaque (n= 42) and saliva (n= 38) samples of patients referred to the Dental Clinic of the Armenia for routine check-ups. *H. pylori* was isolated using culture. Antimicrobial resistance was determined using disk diffusion.
Results: Twenty-two out of 80 (27.50%) examined specimens were positive for *H. pylori*. *H. pylori*ori prevalence amongst dental plaque and saliva samples was 33.33% and 21.05%, respectively (P <0.05). H. pylori strains harbored the high prevalence of resistance against ampicillin (77.27%), amoxicillin (72.22%), erythromycin (68.18%), and tetracycline (68.18%), Resistance rate toward metronidazole was lower than other antimicrobials (40.90%).
Conclusion: The role of dental plaque and saliva samples as H. pylori reservoirs was determined. Due to the high antimicrobial resistance of isolates against ampicillin, amoxicillin, erythromycin, tetracycline, and even metronidazole, there is a big demand for substitute antimicrobials for the oral colonized *H. pylori*.

Keywords: Helicobacter pylori, dentalplaque, saliva, prevalence, antimicrobial resistance.

Resumen

Antecedentes: Es probable que la cavidad oral, en particular la placa dental y las muestras de saliva, puedan representar un importante reservorio de *H. pylori* para las infecciones gástricas. La presente investigación se realizó para evaluar la distribución y la resistencia antimicrobiana de las cepas de *H. pylori* en la placa dental y las muestras de saliva.
Métodos: Se aislaron un total de 80 cepas de *H. pylori* de muestras de placa dental (n= 42) y saliva (n= 38) de pacientes remitidos a la Clínica Dental de Armenia para revisiones rutinarias. El *H. pylori* se aisló mediante cultivo. La resistencia a los antimicrobianos se determinó mediante difusión en disco.
Resultados: Veintidós de las 80 muestras examinadas (27,50%) fueron positivas para *H. pylori*. La prevalencia de *H. pylori* en las muestras de placa dental y saliva fue del 33,33% y el 21,05%, respectivamente (P <0,05). Las cepas de *H. pylori* presentaban una alta prevalencia de resistencia a la ampicilina (77,27%), la amoxicilina (72,22%), la eritromicina (68,18%) y la tetraciclina (68,18%).
Conclusión: Se determinó el papel de las muestras de placa dental y saliva como reservorios de *H. pylori*. Debido a la elevada resistencia antimicrobiana de los aislados frente a la ampicilina, la amoxicilina, la eritromicina, la tetraciclina e incluso el metronidazol, existe una gran demanda de antimicrobianos sustitutivos para el *H. pylori* colonizado por vía oral.

Palabras clave: Helicobacter pylori, placa dental, saliva, prevalencia, resistencia antimicrobiana.

Introduction

The oral cavity is the primary way for the entrance of foods into the body. As the oral cavity is the entry port and first component of the gastrointestinal system, researchers have been interested in the presence of some kinds of bacteria in this niche[1]. Both dental plaque and saliva samples can be infected with diverse kinds of bacteria responsible for digestive infections and disorders[2]. There are a few studies in the literature claiming to have isolated the *Helicobacter pylori* (*H. pylori*) from dental plaque and saliva samples[3].

Helicobacter pylori (*H. pylori*) is a microaerophilic and coccoid, and spiral bacterium recognized as a reason of gastric adenocarcinoma, peptic ulcer disease, duodenal ulcer, type B gastritis, and B-cell lymphoma[4-7]. Even though the human stomach is considered the main *H. pylori* reservoir[8], it was routinely identified in the oral cavity, particularly dental plaques, saliva, tongue, root canals, tonsil tissue, and oral mucosa[9]. It has been suggested that the *H. pylori* fermenting carbohydrates in food produce a low pH in the oral cavity, and this microaerophilic acidic environment with an average oral temperature of 35-37°C can be ideal for its growth and survival[10,11].

Infections caused by *H. pylori* are mainly treated with antimicrobial therapies[12]. However, recent surveys have shown the high *H. pylori* resistance rate toward commonly prescribed antimicrobial agents[13]. In this regard, the highest resistance rate was observed against specific antimicrobials and choices of the oral and gastrointestinal infections, particularly ampicillin, amoxicillin, metronidazole and clarithromycin[14,15]. Changes in the antimicrobial administration and prescription caused severe changes in the *H. pylori's* resistance pattern during the time[16]. Thus, it is essential to assess the antimicrobial resistance of *H. pylori* strains to determine the exact bacterial manner and antimicrobial choices.

Rendering to the uncertain role of dental plaque and saliva samples as a reservoir of antibiotic-resistant H. pylori strains, the present survey was aimed to assess the antimicrobial resistance of *H. pylori* strains isolated from dental plaque and saliva samples of patients referred to the Armenia dental clinics for the routine check-up.

Materials and methods

Samples

From January 2020 to March 2021, a total of 80 H. pylori strains were isolated from dental plaque (n=42) and saliva (n=38) samples of patients referred to the Dental Clinic of the Armenia for routine check-ups.

H. pylori isolation and identification

The dental plaque and saliva specimens from each patient was cultured into a sterile tube containing 5% sheep blood agar, chocolate agar, and a selective medium and transported to the microbiology laboratory to be incubated microaerophically (5% oxygen, 85% nitrogen, and 10% CO_2) using the MART system (MART system, Lichtenvoorde, The Netherland) at a temperature of 37°C for seven days. Culture media were supplemented with 5% of horse serum (Sigma, St. Louis, MO, USA), nalidixic acid (30 mg/L), vancomycin (10 mg/L), cycloheximide (100 mg/L), and trimethoprim (30 mg/L) (Sigma, St. Louis, MO, USA). Suspected colonies were then identified using Gram stain, motility, colony morphology, and biochemical tests such as urease, oxidase, and catalase tests. For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed[17].

Antimicrobial resistance

Mueller–Hinton agar (Merck, Germany) assessed antibiotic resistance patterns using the simple disk diffusion technique. Antibiotic resistance profile of *H. pylori* bacteria was researched toward different antibiotic against (Oxoid, UK) using the guidelines of previous research[18] and also those of Performance Standards for Antimicrobial Susceptibility Testing- Clinical and Laboratory Standards Institute - NCCLS, 2007[19]. Bacterial suspensions were adjusted to the 0.5 McFarland standard (equivalent to 1–2 × 10^8 CFU/mL) and inoculating Muller Hinton agar plates (Merck, Germany). The resistance of bacteria was examined toward metronidazole (5 µg), ampicillin (10 µg), tetracycline (30 µg), clarithromycin (2 µg), erythromycin (5 µg), and amoxicillin (10 µg) (Oxoid, UK). Antibiotic disks were placed on media contained the bacteria, and the plates were incubated under microaerophilic conditions at 35°C for 16-18 h. The zones of growth inhibition produced by each antibiotic were measured and interpreted by standard procedure.

Data analysis

Data were subjected to Microsoft Office Excel (version 15; Microsoft Corp., Redmond, WA, USA). The statistical analysis was performed employing the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were applied to measure any significant relationship. *P*-value <0.05 was considered as a significant numerical level[20-25].

Results

H. pylori distribution

Table I shows the H. pylori distribution amongst the studied population. Twenty-two out of 80 (27.50%) examined specimens were positive for *H. pylori*. *H. pylori* distribution amongst dental plaque and saliva samples was 33.33% and 21.05%, respectively. A statistically significant difference was obtained for the *H. pylori* distribution between dental plaque and saliva specimens (P <0.05).

Table I: *H. pylori* distribution amongst the studied population.

Specimens	N. specimens collected	N. specimens positive for *H. pylori* (%)
Dental plaque	42	14 (33.33)
Saliva	38	8 (21.05)
Total	80	22 (27.50)

H. pylori antimicrobial resistance

Table II shows the antimicrobial resistance of *H. pylori* strains isolated from dental plaque and saliva specimens. *H. pylori* strains isolated from examined specimens harbored the high prevalence of resistance against ampicillin (77.27%), amoxicillin (72.22%), erythromycin (68.18%), and tetracycline (68.18%), antimicrobials. Resistance rate toward metronidazole was lower than other antimicrobials (40.90%). *H. pylori* isolates of dental plaque samples had a higher prevalence of resistance toward all examined antimicrobial agents (P <0.05).

Discussion

Notwithstanding an enormous expansions in medicine, varied complicated infectious diseases faced with the human[26-30]. In this regard, *H. pylori* have become a developed public health issue in the last century[31].

H. pylori prevalence among the dental plaque and saliva specimens was 33.33% and 21.05%, respectively. Higher H. pylori prevalence in saliva than dental plaque specimens was also reported from Japan[32], Mexico (33), Malaysia[34], Iran[35], and Peru[36]. Yang et al. (2015) (37) showed that 76% of dental plaque samples collected in China were positive for *H. pylori*. Rasmussen et al. (2010)[38] stated that the *H. pylori* prevalence amongst the saliva and dental plaque samples was 42.30% and 47.40%, respectively. However, these is no strict data about the exact source of *H. pylori* in dental plaque and saliva samples. Maybe foods have the main role in transmission of *H. pylori* into the oral cavity and then stomach.

H. pylori isolates of dental plaque and saliva samples harbored the high resistance rate toward ampicillin, tetracycline, amoxicillin, erythromycin, and clarithromycin. These antimicrobials are the major therapeutic options used for *H. pylori* eradication, particularly in the mouth, among Iranian practitioners. High and illegal antibiotic prescriptions in medical and dental clinics and excessive use of disinfectants and mouthwashes solutions may cause the high antimicrobial resistance observed in the present survey. Our findings showed the higher antimicrobial resistance of H. pylori strains isolated from dental plaque than saliva samples. This finding may be due to the biofilm formation of H. pylori strains in the dental plaque samples[39]. Hanafiah et al. (2019)[40] reported that resistance rates of *H. pylori* strain to metronidazole and clarithromycin antimicrobial agents were 59.30%, and 35.6%, respectively. Mashak et al. (2020)[41] reported that the resistance rates of *H. pylori* strains against ampicillin, clarithromycin, erythromycin, metronidazole, levofloxacin, tetracycline, amoxicillin, rifampin, trimethoprim, cefsulodin, streptomycin, furazolidone, and spiramycin antimicrobial agents were 59.61%, 61.53%, 80.76%, 51.92%, 63.46%, 82.69%, 63.46%, 40.38%, 65.38%, 38.46%, 59.61%, 25.00%, and 21.15%, respectively.

Totally, this survey is the first report of identification of antimicrobial resistance of *H. pylori* strains isolated from dental plaque and saliva samples in Armenia. Findings are limited to the low number of isolated bacteria, lack of demographical characters of the studied population and also absence of the determination of the history of gastrointestinal disorders among patients.

Conclusion

To sum it up, diverse antimicrobial resistance was found in the *H. pylori* strains isolated from dental plaque and saliva samples. Ampicillin, amoxicillin, clarithromycin, tetracycline, erythromycin and even metronidazole antimicrobials were not effective against isolates. *H. pylori* isolates of dental plaque samples harbored the higher antimicrobial resistance. This finding may show the predominant role of dental plaque samples as a reservoir of antimicrobial-resistant *H. pylori*. Additionally, findings may suggest that the oral cavity, particularly dental plaque and saliva samples, may be a *H. pylori* reservoir and potentially a source of transmission or reinfection.

Interests conflict

The researchers declare that they have no conflict of interest.

Table II: Antimicrobial resistance of *H. pylori* strains isolated from dental plaque and saliva specimens.

Specimens	N. H. pylori isolates harbored resistance against each antimicrobial agents (%)					
(N. positive)	Clr*	Ert	Amx	Amp	Tet	Met
Dental plaque (14)	10 (71.42)	10 (71.42)	11 (78.57)	12 (58.71)	10 (71.42)	7 (50.00)
Saliva (8)	4 (50.00)	5 (62.50)	5 (62.50)	5 (62.50)	5 (62.50)	2 (25.00)
Total (22)	14 (63.63)	15 (68.18)	16 (72.72)	17 (77.27)	15 (68.18)	9 (40.90)

*Clr: clarithromycin (2 µg), Ert: erythromycin (5 µg), Amx: amoxicillin (10 µg), Amp: ampicillin (10 µg), Tet: tetracycline (30 µg), Met: metronidazole (5 µg).

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