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The use of rapid antigen testing and matrix-assisted laser desorption/ionization-time of flight mass spectrometry in the diagnosis of group A beta-hemolytic streptococci in throat swab samples

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Background/aim: We aimed to evaluate the efficacy of a rapid antigen test in detecting group A beta-hemolytic streptococci (GAS) in throat samples in comparison with the culture method and to compare the efficiency of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and traditional methods in identifying GAS in cultures.

Materials and methods: A total of 3668 throat samples from patients with a prediagnosis of tonsillopharyngitis were assessed by the QuickVue+Strep A antigen test and culture. For GAS identification from cultures, bacitracin sensitivity, PYR, and latex agglutination tests and MALDI-TOF MS were used.

Results: A total of 567 (15.5%) and 536 (14.6%) of the samples were positive for GAS culture and rapid antigen testing, respectively. The sensitivity and specificity of the rapid antigen test compared to culture was 89.07% and 99%, respectively, while positive and negative predictive values were 94.22% and 98.02%. Traditional methods were in full concordance with MALDI-TOF-MS for all 567 isolates. In all densities of growth in culture, the time to diagnosis with MALDI-TOF MS was significantly lower than with traditional identification tests.

Conclusion: This study shows that both the rapid antigen testing of samples and bacterial identification with MALDI-TOF MS contribute much to the rapid diagnosis of GAS tonsillopharyngitis.

Key words: MALDI-TOF MS, rapid antigen test, *Streptococcus pyogenes*, tonsillopharyngitis

1. Introduction

Group A beta-hemolytic streptococci (GAS) are important pathogens that cause tonsillopharyngitis and more severe infections such as septicemia, pneumonia, or meningitis (1). Due to the possibility of the occurrence of complications such as acute poststreptococcal glomerulonephritis and acute rheumatic fever after streptococcal infections, rapid diagnosis and treatment of GAS gains importance (2,3).

Throat culture is the gold standard for diagnosis of GAS tonsillopharyngitis, but it requires at least one day for detection. Therefore, rapid antigen tests were developed. Reported sensitivity and specificity rates of rapid tests with regard to culture are 66%–99% and 95%, respectively (4–6). Using a highly sensitive rapid test makes a significant contribution to early diagnosis and appropriate therapy. Rapid antigen tests used to diagnose acute tonsillopharyngitis have resulted in significant reductions in antibiotic prescriptions over the past 10 years (7).

Bacitracin sensitivity, the pyrrolidonyl arylamidase test (PYR), and latex agglutination tests are used traditionally in order to identify GAS in culture. With those tests, culture results are usually available in 1–3 days (8). By using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), which is a novel rapid and automated identification system in clinical microbiology laboratories, bacteria can be identified in minutes at the genus and species level based on unique proteomic fingerprints of microorganisms (9).

The aim of this study is to assess the effectiveness of the QuickVue+Strep A Test (Quidel Corporation, USA) in determining GAS in throat swab samples in terms of rapid diagnosis and to evaluate the effectiveness of the MALDI-TOF MS system compared to traditional methods in the identification of GAS growth when culture is done.

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2. Materials and methods

2.1. Patients

This research was performed with the ethical approval of the Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee (Decision Number 1000). In this study, 3668 throat swab samples from 3668 patients sent routinely with the prediagnosis of pharyngitis to the clinical microbiology laboratory between November 2014 and May 2015 were assessed. The demographic data of the patients were noted. The throat swab samples were taken using BD BBL CultureSwab EZ II combined double swabs (Le Pont de Claix, France) and were transported to the laboratory in one hour. After arrival of the samples to the laboratory, one of the swabs was used for rapid antigen testing while the other one was used for throat culture.

2.2. Rapid antigen test

A lateral immunoassay kit, the QuickVue+Strep A Test (Quidel Corporation, USA), was used according to the manufacturer's recommendations in order to detect GAS antigens from throat swab samples.

2.3. Throat culture and GAS identification

The samples were cultured on 5% sheep blood agar and incubated at 37 °C in a 5%–10% CO_2 incubator for up to 48 h. The plates were assessed after 24 and 48 h of incubation for the existence of beta-hemolytic colonies. Gram-positive and catalase-negative colonies were identified traditionally by using the PYR test (BBL DrySlide PYR Kit, Becton, Dickinson and Company, USA), the latex agglutination Streptococci Grouping Test Kit (Plasmatec, UK), and sensitivity to 0.04 U bacitracin discs (Oxoid, UK). The Microflex LT (Bruker Daltonics, Germany) platform was used for MALDI-TOF MS identification of any suspected beta-hemolytic colony on the primary diagnostic plate. Direct transfer of the colony to the target and direct transfer-formic acid methods were used for MALDI-TOF MS evaluation. Data processing was performed using MALDI Biotyper 3.1 software (Bruker Daltonics, Germany). Identification results by traditional methods (GAS) and by MALDI-TOF MS (*Streptococcus pyogenes*) were compared. The amount of GAS growth seen in the agar plates (enumerated from 1 to 4, i.e. growth in first to fourth quadrant of the agar plate) (10) and time to diagnosis for each test, where arrival of the sample to the laboratory was considered as time zero, were recorded.

2.4. Statistical evaluation

IBM SPSS Statistics 22 (IBM SPSS, Turkey) was used for statistical analyses. Goodness of fit for normal distribution was determined by using the Shapiro–Wilks test, as well as using descriptive statistical methods (median, standard deviation, frequency). The Freidman test was used for mean comparison and the Wilcoxon sign test was used in post hoc evaluations. The chi square test was used in the dependency analysis for contingency tables and Spearman's rho correlation analysis was used in examining correlations between parameters. Screening programs were used in calculation of sensitivity and specificity. Significance was evaluated at the level of $P < 0.05$.

3. Results

A total of 3668 patients were included in the study, of which 1615 were female (44%) and 2053 were male (56%). Ages of patients ranged from 0 and 72 years and the mean average age was 7.9 ± 7.14 years. A total of 3467 (94.5%) samples were sent from pediatrics clinics, 3060 (83.4%) being from emergency pediatrics, and 201 (5.5%) from adult clinics. The GAS antigen was detected by rapid tests in 536 cases (14.6%). GAS was identified in throat culture in 567 cases (15.5%). General characteristics of patients and culture results are shown in Table 1. The sensitivity and specificity of rapid antigen tests compared to the gold standard of culture for the 3668 throat swab samples was 89.07% and 99%, respectively, while positive and negative predictive values were 94.22% and 98.02%, respectively. In 31 cases (0.8%) GAS did not grow in culture while the rapid antigen test was positive. The distributions of rapid antigen test and culture results are shown in Table 2. The accuracy of the test was 97.46%. The result of throat culture was positive in 15.1% of female patients and 15.7% of male patients; there was no significant difference between distribution of culture results according to sex ($P > 0.05$). Rapid antigen tests yielded positive results in 130 (83.3%) of 156 cases with low growth concentration (+1, +2) and in 376 (91.5%) of 411 cases with high growth concentration (+3, +4), presenting a statistically significant increase in higher growth concentrations ($P = 0{,}001$; $P < 0.01$).

All isolates that were grouped as GAS on latex were PYR-positive and bacitracin-susceptible. MALDI-TOF MS results were concordant with the latex agglutination test for all 567 isolates. With MALDI-TOF MS, 89% (502/567) of isolates were identified by direct transfer method from the colony on a target, while 11% (65/567) were identified when the direct transfer-formic acid method was used (score value of ≥2.0).

At all growth densities, the time to diagnosis with the MALDI-TOF MS method was significantly shorter than that of PYR, latex agglutination, and bacitracin sensitivity tests (Friedman test, $P < 0.01$) (Table 3).

4. Discussion

The prevalence of GAS in throat swabs from children presenting with sore throat usually varies at a rate of 20%–40%, while it is seen at a rate of 5%–15% in adults (4). Although there may be some distinctive symptoms such as cough, conjunctivitis, and nasal flow in viral tonsillopharyngitis, patients usually exhibit a clinical

Table 1. General characteristics and culture results of patients.

		n	%
Sex	Female	1615	44
	Male	2053	56
Rapid antigen test result	Positive	536	14.6
	Negative	3132	85.4
Culture results (GAS)	Positive	567	15.5
	Negative	3101	84.5
Distribution of culture results	RF	3059	83.4
	GCS	15	0.4
	GGS	27	0.7
	GAS	567	15.5
GAS growth in throat culture (5–15 years)	Positive	501	20.5
	Negative	1945	79.5
Distribution of culture results between ages 5 and 15	RF	1913	78.2
	GCS	13	0.5
	GGS	19	0.8
	GAS	501	20.5

RF: Respiratory flora, GCS: group C streptococci, GGS: group G streptococci, GAS: group A streptococci.

Table 2. Comparison of results of the rapid antigen test with culture for 3668 throat swab samples.

		Culture		
		Positive	Negative	Total
		n (%)	n (%)	n (%)
Rapid antigen test	Positive	505 (13.8)	31 (0.8)	536 (14.6)
	Negative	62 (1.7)	3070 (83.7)	3132 (85.4)
	Total	567 (15.5)	3101 (84.5)	3668 (100)

appearance similar to the symptoms of GAS pharyngitis (11). Appropriate treatment of GAS tonsillopharyngitis prevents suppurative and nonsuppurative complications and decreases the duration of clinical symptoms and risk of transmission. Therefore, the determination of GAS in patients with tonsillopharyngitis has significant clinical importance in patient management (12).

A study that reported data from eight different studies performed in five different countries stated that the GAS prevalence was 21%–48% in children with pharyngitis (2). In two studies from Turkey involving patients with pharyngitis aged 0–18, GAS prevalence was 25% (13,14). In the study of Küçük et al. (15), prevalence was observed as 19.4% in the 0–6 age group and was almost doubled in the 7–17 age group (35.9%). In our study, while the rate of GAS tonsillopharyngitis was 15.5% in the total patient group, it was determined as 20.5% in children aged 5–15 years, consistent with other studies.

Although throat culture is the gold standard method for the diagnosis of GAS tonsillopharyngitis, it requires

Table 3. Time to diagnosis for each test according to growth densities.

	Growth density			
	1	2	3	4
Test (days)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)
MALDI-TOF MS	1.56 ± 0.63 (1)	1.19 ± 0.48 (1)	1.08 ± 0.33 (1)	1.01 ± 0.09 (1)
PYR	2.13 ± 0.34 (2)	1.99 ± 0.33 (2)	1.53 ± 0.54 (2)	1,09 ± 0.3 (1)
Latex agglutination	2.15 ± 0.36 (2)	1.99 ± 0.33 (2)	1.53 ± 0.54 (2)	1.09 ± 0.3 (1)
Bacitracin sensitivity	2.15 ± 0.4 (2)	2.05 ± 0.26 (2)	2.02 ± 0.13 (2)	2 ± 0.05 (2)
P-value	0.001**	0.001**	0.001**	0.001**

MALDI-TOF MS: Matrix-assisted laser desorption/ionization-time of flight mass spectrometry; PYR: pyrrolidonyl arylamidase, SD: standard deviation. Friedman test, **P < 0.01.

at least 24–48 h, which may cause delays in treatment or unnecessary use of antibiotics (16). Turkey was recently reported to be the country with the most frequent use of antibiotics in a European-wide study including 17 countries (17). Today, new-generation rapid antigen tests based on lateral migration immunoassay and rapid immunochromatographic methods are widely used to guide treatment of GAS pharyngitis (18). Cohen et al. (2) reviewed data from studies evaluating rapid antigen tests and stated that the sensitivity and specificity of the tests was 66%–94% and 40%–86%, respectively. In more extensive research, 116 different rapid antigen tests in 98 publications were examined and the average sensitivity of rapid antigen tests was reported as 86% while the specificity was 95% (4). In our study, the sensitivity of the rapid antigen test was determined as 89.07%, specificity as 99%, positive predictive value as 94.2%, negative predictive value as 98.02%, and accuracy of the test as 97.46%, demonstrating that the sensitivity and specificity of the rapid test used in the present study is considerably high .

There are various factors affecting the sensitivity and specificity of rapid antigen tests, including the presence of signs and symptoms supporting the GAS pharyngitis, the test kit, and the amount of inoculum (2,4,19). In a study by DiMatteo et al. (20) the sensitivity of rapid antigen tests in patients with Centors score of 0, 1, 2, 3, and 4 was found to be 61%, 76%, 90%, and 97%, respectively. In an experimental study investigating the effect of amount of inoculum, sensitivity of five different tests was investigated in various dilutions of *S. pyogenes* and it was shown that the increase in the amount of bacteria in the sample increased the sensitivity of the test (21). In our study, we found a correlation between growth density and positive results of rapid antigen tests. While the rapid antigen test positivity rate was 91.5% in the group with intensive growth, the positivity rate was 83.3% in the group with low growth density.

Culture-negative antigen-positive test results have been reported in the literature, although at a low rate (13–15). It has been suggested that the reasons for this include the use of antibiotics, competition between several bacteria in the flora, inhibition of growth by secreted bacteriolytic agents, and carriage of common group A streptococcal carbohydrate antigen in several streptococci in the flora (19,22,23). In our study low rates of antigen test positivity in culture-negative samples were identified.

Traditional methods such as bacitracin sensitivity, PYR test, and latex agglutination test are used for GAS identification in culture. While overnight incubation is needed for bacitracin sensitivity, the PYR test and latex agglutination test yield results earlier. The existence of bacitracin-resistant, PYR-negative *S. pyogenes* isolates and PYR-positive streptococcal species can cause confusion in identification, leading to the requirement to use all tests simultaneously (8). Fast and easily applied identification methods with high accuracy are needed in clinical microbiology laboratories. Automated identification systems have been developed and MALDI-TOF MS systems based on the mass spectrometry method have become available recently for rapid identification (24,25). Schulthess et al. (26) stated that the MALDI-TOF MS method and traditional methods gave consistent results at a rate of 95.6% and *S. pyogenes* isolates were identified at an accuracy rate of 94.7% (18/19) at the species level. In a study with a greater number of similar isolates, 66 of 97 *S. pyogenes* isolates were identified accurately with the direct transfer method; the unidentified 31 isolates were tested again by ethanol formic acid extraction method and 25 more isolates were identified accurately (27). Other studies reported accurate identification of *S.*

pyogenes with MALDI-TOF MS systems (27–30). In our study, 567 GAS isolates were identified with the MALDI-TOF MS system in full concordance with the traditional methods. The strength of the present study is that it includes much higher numbers of isolates compared to other studies conducted. However, the study isolates were not sequenced and MALDI-TOF MS results could not be compared to sequencing results, which can be considered as a limitation of the study.

In our study, when the MALDI-TOF MS method and the traditional methods were compared in terms of time to diagnosis in growth densities numbered from 1 to 4, time to diagnosis with MALDI-TOF MS was significantly lower in all growth densities. While diagnosis can be made with a single colony by using the MALDI-TOF MS method, more colonies are needed for identification with other methods. The results can be obtained in the same day with PYR and latex agglutination methods in the presence of sufficient colonies; however, an additional overnight incubation period is required for bacitracin sensitivity.

In conclusion, the QuickVue+Strep A antigen test used in this study contributed significantly to the diagnosis of GAS tonsillopharyngitis with high sensitivity and specificity. The confirmation of negative rapid antigen test results by culture is important to avoid misdiagnosis and insufficient treatment. For throat culture evaluation, MALDI-TOF MS gives earlier results than the traditional methods and can lead to earlier introduction of appropriate therapy. The widespread use of MALDI-TOF MS in routine laboratories would bring a new perspective to microbiological diagnosis.

References

1. Bisno Al, Stevens DL. *Streptococcus pyogenes*. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, PA, USA: Churchill Livingston; 2010. pp. 2593-610.
2. Cohen JF, Cohen R, Levy C, Thollot F, Benani M, Bidet P, Chalumeau M. Selective testing strategies for diagnosing group A streptococcal infection in children with pharyngitis: a systematic review and prospective multicentre external validation study. Can Med Assoc J 2015; 187: 23-32.
3. Spinks A, Glasziou PP, Del Mar CB. Antibiotics for sore throat. Cochrane Database Syst Rev 2013; 11: CD000023.
4. Cohen JF, Bertille N, Cohen R, Chalumeau M. Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database Syst Rev 2016; 7: CD010502.
5. Van Limbergen J, Kalima P, Taheri S, Beattie TF. Streptococcus A in paediatric accident and emergency: are rapid streptococcal tests and clinical examination of any help? Emerg Med J 2006; 23:32–4.
6. Harbeck RJ, Teague J, Crossen GR, MaulDM, Childers PL. Novel, rapid optical immunoassay technique for detection of group A streptococci from pharyngeal specimens: comparison with standard culture methods. J Clin Microbiol 1993; 31: 839-844.
7. Gazzano V, Berger A, Benito Y, Freydiere AM, Tristan A, Boisset S, Carricajo A, Poyart C, Vandenesch F, Descours G. Reassessment of the role of rapid antigen detection tests in diagnosis of invasive group A streptococcal infections. J Clin Microbiol 2016; 54: 994-9.
8. Spellerberg B, Brandt C. *Streptococcus.* In: Versalovic J, Carroll KC, Jorgensen JH, Funku G, Landry ML, Warnock DW, editors. Manual of Clinical Microbiology. Vol. 1. 10th ed. Washington, DC, USA: ASM Press; 2011. pp. 331-349.
9. Angeletti S. Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) in clinical microbiology. J Microbiol Methods 2017; 138: 20-29.
10. Church DL. Aerobic bacteriology. In: Garcia LS, Isenberg HD, editors. Clinical Microbiology Procedures Handbook. Vol. 1 3rd ed., Washington, DC, USA: ASM Press; 2010. Section 3.3.2.4.
11. Van Brusselen D, Vlieghe E, Schelstraete P, De Meulder F, Vandeputte C, Garmyn K, Laffut W, Van de Voorde P. Streptococcal pharyngitis in children: to treat or not to treat? Eur J Pediatr 2014; 173: 1275-83.
12. Llor C, Madurell J, Balagué-Corbella M, Gómez M, Cots JM. Impact on antibiotic prescription of rapid antigen detection testing in acute pharyngitis in adults: a randomised clinical trial. Br J Gen Pract 2011; 61: e244-51.
13. Gözüküçük R, Göçmen İ, Nas Y, Yılmazer F, Ünüvar E. The effectiveness of rapid Strep A Test in the diagnosis of Streptococcal tonsillopharyngitis. J Child 2011; 11:157-159.
14. Altun HU, Meral T, Aribas ET. The specificity and sensitivity results of the rapid antigen test used in the diagnosis of group A beta hemoliytic Streptococcal tonsillopharyngitis. Acta Medica Mediterr 2015; 31: 287.
15. Küçük O, Biçer S, Giray T, Cöl D, Erdağ GC, Gürol Y, Kaspar CE, Vitrinel A. Validity of rapid antigen detection testing in group A beta-hemolytic Streptococcal tonsillopharyngitis. Indian J Pediatr 2014; 81: 138-142.
16. Vaz LE, Kleinman KP, Raebel MA, Nordin JD, Lakoma MD, Dutta-Linn MM, Finkelstein JA. Recent trends in outpatient antibiotic use in children. Pediatrics 2014; 133: 375-85.
17. Versporten A, Bolokhovets G, Ghazaryan L, Abilova V, Pyshnik G, Spasojevic T, Korinteli I, Raka L, Kambaralieva B, Cizmovic L, et al. Antibiotic use in eastern Europe: a cross-national database study in coordination with the WHO Regional Office for Europe. Lancet Infect Dis 2014; 14: 381-387.

18. Procop GW, Church DL Hall GS, Janda WM, Koneman EW, Schreckenberger PC, Woods GL. Koneman's Color Atlas and Text Book of Diagnostic Microbiology. 7th ed. Philadelphia, PA, USA: Wolters Kluwer Health; 2017.

19. Cohen JF, Chalumeau M, Levy C, Bidet P, Benani M, Koskas M, Bingen E, Cohen R.. Effect of clinical spectrum, inoculum size and physician characteristics on sensitivity of a rapid antigen detection test for group A streptococcal pharyngitis. Eur J Clin Microbiol Infect Dis 2013; 32: 787-793.

20. DiMatteo LA, Lowenstein SR, Brimhall B, Reiquam W, Gonzales R. The relationship between the clinical features of pharyngitis and the sensitivity of a rapid antigen test: evidence of spectrum bias. Ann Emerg Med 2001; 38: 48-52.

21. Lasseter GM, McNulty CA, Richard Hobbs FD, Mant D, Little P; PRISM Investigators. In vitro evaluation of five rapid antigen detection tests for group A beta haemolytic streptococcal sore throat infections. Fam Pract 2009; 26: 437-444.

22. Coban B, Kaplan H, Topal B, Ulku N. The sensitivity and the specificity of rapid antigen test in group A streptococcal tonsillopharyngitis. J Pediatr Inf 2013; 7: 143-146.

23. Johnson DR, Kaplan EL. False-positive rapid antigen detection test results: reduced specificity in the absence of group A streptococci in the upper respiratory tract. J Infect Dis 2001; 183: 1135-1137.

24. Van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010; 48: 900-907.

25. Fenselau C, Demirev PA. Characterization of intact microorganisms by MALDI mass spectrometry. Mass Spectrom Rev 2001; 20: 157-171.

26. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol 2013; 51: 1834-1840.

27. Jensen CS, Dam-Nielsen C, Arpi M. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry identification of large colony beta-hemolytic streptococci containing Lancefield groups A, C, and G. Infect Dis (Lond) 2015; 47: 575-579.

28. Cherkaoui A, Emonet S, Fernandez J, Schorderet D, Schrenzel J. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of beta-hemolytic streptococci. J Clin Microbiol 2011; 49: 3004-3005.

29. Wang J, Xhou N, Xu B, Hao H, Kang L, Zheng Y, Jiang Y, Jiang H. Identification and cluster analysis of *Streptococcus pyogenes* by MALDI-TOF mass spectrometry. PLoS One 2012; 7: e47152.

30. Moon HW, Lee SH, Chung HS, Lee M, Lee K. Performance of the Vitek MS matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories. J Med Microbiol 2013; 62: 1301-1306.