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Typeable ampicillin-resistant *Haemophilus influenzae* strains in Tunisian's children

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Abstract

Background: Here, we report the frequency of capsulated ampicillin-resistant *Haemophilus influenzae* strains isolated from children in Tunisia, particularly capsular serotype b, by polymerase chain reaction (PCR) to determine the molecular mechanisms underlying ampicillin resistance.

Methods: We considered 22 capsulated *H influenzae* strains selected from a series of 91 ampicillin-resistant *H influenzae* strains isolated from children between 2010 and 2011 in Tunisia. The capsular serotypes of these strains were identified by slide agglutination and PCR.

Results: By PCR, 19 (20.88%) serotype b, 1 (1.1%) serotype a, 2 (2.2%) serotypes d and f and 69 (75.82%) non-typeable strains were found among the 91 ampicillin-resistant *H influenzae* strains. 100% of the assumption between the consequences of antigenic examinations and PCR was found. The serotype b strains showed biotypes I, II, III, IV, VI, and VIII. The other capsulated strains showed biotypes IV and VIII. Thirteen of the serotype b strains created β-lactamase (14.28%). The 19 serotype b ampicillin-resistant *H influenzae* strains were subdivided into 3 bunches as indicated: The gathering of the β-lactamase positive, ampicillin-resistant where 11 strains (57.89%) were β-lactamase positive bla_{TEM-1} (+) and *fts*I (+). The second gathering of the β-lactamase negative, ampicillin-resistant strains, where 6 isolates (31.58%) were β-lactamase negative bla_{TEM-1} (–) and *fts*I (–), and lastly, the gathering of the β-lactamase positive, amoxicillin-clavulanate resistant where 2 isolates (10.52%) were β-lactamase positive bla_{TEM-1} (+) and *fts*I (–).

Conclusion: PCR should be used in our country because it may contribute to decreasing the probability of transmission of these strains, especially those showing the two mechanisms of resistance among children in Tunisia.

Abbreviations: AMC = amoxicillin/clavulanic acid, CEF = cefotaxime, BLNAR = β-lactamase negative, ampicillin-resistant, MIC = minimum inhibitory concentrations.

Keywords: antibiotics, disease, resistance

1. Introduction

Haemophilus influenzae is a standout among the most critical bacterial pathogens of pediatric diseases. This bacterium can cause clinical signs in children. *H influenzae* can be partitioned into 6 capsular serotypes (a–f) based on fundamentals and serologically unmistakable capsular polysaccharides. The enhancement of the *bexA* gene by polymerase chain reaction (PCR) is used to recognize capsular typeable strains from non-typeable strains. Strains having the *bexA* gene are assumed to be typeable, while strains without this gene are considered non-typeable.[1] The predominant bacterial pathogens of invasive diseases are capsulated *H influenzae* type b strains in children younger than 5 years old. In the USA, *H influenzae* serotype b invasive diseases are presently very uncommon; because of the widespread use of an *H influenzae* type b conjugate vaccination.[2] This sort of vaccine was presented in 2005 in Tunisia at the same time; it was halted for reasons of cost. The Hib vaccine was reintroduced in 2011. The rate of invasive illnesses caused by *H influenzae* type b strains (b-strains) with a capsule deficient mutant has been recorded.[1] This kind of *H influenzae* is especially important during the immunization period, as it is one of the reasons for vaccine failure.[3]

H influenzae serotypes were previously determined using a Slide Agglutination Test with 6 antisera (a–f), one for each of the capsular serotypes. This process is simple and produces a quick result, but it is temperamental. In comparison to the slide agglutination approach, PCR has recently been utilized for serotyping *H influenzae* strains and has shown great affectability and specificity. Hence, it is important to use PCR for serotyping *H influenzae* strains isolated from patients.

The point of the present investigation is to assess the recurrence of capsulated ampicillin-resistant *H influenzae* strains isolated from children in Tunisia, particularly capsular serotype

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b, using the PCR technique, and to depict their ampicillin resistance molecular mechanisms.

2. Material and Methods

2.1. Bacterial strains

A total of 22 capsulated *H influenzae* strains were isolated from a series of 91 ampicillin-resistant *H influenzae* strains isolated from kids between 2010 and 2011 in Tunisia.

The *H influenzae* strains were confined to cerebrospinal fluid (LCR), blood culture, and pneumonic examples, and different examples were recognized based on lab standard philosophy. A gathering of 4 American Type Culture Collection (ATCC) *H influenzae* strains was used as positive controls: *H influenzae* ATCC 49247 (Ampicillin-resistant, β-lactamase negative), *H influenzae* C425 (bla_{TEM-1} positive), *H influenzae* C322 (bla_{ROB-1} positive), and *H influenzae* ATCC 10211 (capsular type b). One strain resistant to nontypeable *H influenzae* (ATCC 49766) was used as a negative control.

2.2. Biotyping

The effects of three biochemical tests (ODC, urea, and indole) permitted us to decide eight biotypes. Biotyping was performed using the Api 10 S (bioMerieux, France).

2.3. Serotyping by slide agglutination tests

The 24-hour subcultures of our *H influenzae* strains were tried by a business slide agglutination kit (Difco) to agglutinate the strains into three groups: serotype b, non-serotype b, and non-typeable. From there on, serotypes of the capsular non-serotype b strains were resolved with the antisera of a, c, d, e, and f serotypes, respectively (Difco).

2.4. PCR

Two to three colonies of *H influenzae* strains were obtained and suspended in refined water after being subcultured for 24 hours on Chocolate Agar supplemented with polyvitex (bioMérieux). After boiling for 5 minutes at 100°C, the mixture was centrifuged for 20 minutes at 25,857 g. Finally, the supernatant was collected and stored at –20°C for future use. Non-typeable *H influenzae* strains were separated from typeable strains using a pair of primers (HI-1 and HI-2), which are specific for the bexA gene. As a result, for the majority of the strains, PCR tests with six sets of primers, including a1, a2; b1, b2; c1, c2; d1, d2; e1, e2; f1, f2, which are specific for capsular kinds a through f, were done to determine serotypes exactly.[1] In subsequent testing, nontypeable strains were submitted to PCR analysis using two primers, b1 and b2, that are specific for capsular type b, to identify type b-strain. When a strain yielded a negative PCR result with primers HI-1 and HI-2 but a positive result with primers b1 and b2, it was assumed to be type b-. The reaction contained 50 M of each oligonucleotide primer, 10 Taq Buffer, 50 M of MgCl2, 5 M of dNTP mix, 1 L of DNA, 0.5 U of Taq polymerase, and distilled water in a 10 L reaction volume. A PCR thermal cycler was used for PCR cycling (Applied Biosystems, United States). 2 percent agarose gel electrophoresis and BET staining were used to look for PCR products.

2.5. Testing for susceptibility

Antibiotic susceptibility of *H influenzae* strains was determined by determining the antibiotic drugs' minimum inhibitory concentrations (MIC): ampicillin, amoxicillin/clavulanic acid (AMC), and cefotaxime (CEF) (CTX). MIC was estimated by *E* Test as indicated by the strategies depicted by CA-SFM.[4] The MIC was characterized as the most reduced centralization of antibiotics, which repressed noticeable development of the inoculum. Plates were analyzed following 24 hours of incubation at 5% CO2 at 37°C.

2.6. Beta-lactam resistance genes

The bla_{TEM} and bla_{ROB} genes determine the beta-lactamase type; the ftsI gene encodes the PBP 3 transpeptidase area.[5]

3. Results

An aggregate of 22 capsulated *H. influenzae* strains were chosen from a series of 91 ampicillin-resistant *H influenzae* strains isolated from children between 2010 and 2011 in Tunisia. The 22 capsulated ampicillin-resistant *H influenzae* strains were confined to LCR (36.36%), blood culture (13.6%), pneumonic examples (45.5%), and different examples (4.5%). Among the 22 capsulated strains, 19 were of serotype b. These type b strains were separated from LCR (8; 42.1%), blood culture (2; 10.53%), pneumonic examples (8; 42.1%) and other samples (1; 5.26%). By using the slide agglutination technique, 19 serotype b, 3 capsular non-type b strains, and 69 non-capsulated strains were found. Based on serotyping with 6 different antisera, the non-type b strain just gave a positive result with a, d, and f antisera. A *bexA* gene was amplified in the majority of the strains using PCR using capsular-specific primers HI-1 and HI-2. Those strains were then submitted to PCR using six pairs of type-specific primers. The results revealed that 19 strains delivered specific DNA sequences amplified with primers b1 and b2, 2 strains were of type d and f, and 1 strain displayed a specific DNA sequence amplified with primers a1 and a2. For the remaining 69 non-typeable strains, PCR using primers b1 and b2 was used to identify type b-strains. Despite this, none of the 69 non-typeable strains tested positive for type b-strain characteristics. In this investigation, 100% of the assumptions were found between the aftereffects of antigenic examinations and PCR strategy. The serotype b strains showed biotypes I, II, III, VI, and VIII. The other capsulated strains showed biotypes IV and VIII.

The 19 capsulated type b ampicillin-resistant *H influenzae* strains showed MIC estimations of amoxicillin extending from 1 to >256 mg/L and AMC MICs going from 1 to 6 mg/L. The majority of the type b strains were susceptible to CEF. The 19 type b ampicillin-resistant *H influenzae* strains tried were subdivided as follows as indicated by their ampicillin-resistance genes bla_{TEM}, $bla_{ROB,}$ and *fts*I: Six strains (31.58%) had changes in the *fts*I gene (β-lactamase negative, ampicillin-resistant, BLNAR); 11 (57.89%) created β-lactamase TEM-1 write (bla_{TEM-1} (+)) and had no *fts*I changes (β-lactamase positive, ampicillin-resistant); no strain created the bla_{ROB-1} gene. Two strains (10.52%) delivered β-lactamase (bla_{TEM-1} (+)) and, furthermore, had changes in the *fts*I gene (β-lactamase positive, amoxicillin-clavulanate resistant).

4. Discussion

Slide agglutination has been used for serotyping *H influenzae* strains that cause diseases in kids. Be that as it may, this system is inconsistent. The inconsistency of this procedure may be due to serological cross-responses, specialized practice, as well as antiserum quality. In contrast to antigenic exams, the PCR approach for ensuring *H influenzae* serotypes has shown excellent affectability and specificity. To distinguish typeable strains from nontypeable strains, PCR using capsular-specific primers was used for all strains. Capsular typeable isolates, which gave positive results with the *bexA* gene, were inspected with 6 sets of type-specific primers to precisely distinguish the serotypes. Furthermore, the strains that gave negative outcomes with the

bexA gene were tried with type b particular primers to recognize b-strain.

Serotype b-strains were not identified in this report nor in past investigations in Tunisia.[6] The b-strain of *H influenzae* is thought to be a capsular-lacking mutant of serotype b strains. As a result, the segregation rate of that form of *H influenzae* is said to be extremely low. The frequency of type b-strain was previously noted in a report.[1] This investigation demonstrated that no inconsistency between the after-effects of slide agglutination and PCR was found among our isolates. In this report, the proportion of ampicillin-resistant serotype b *H influenzae* strains is 20.87%. This rate varies from one country to another. In Bangladesh, 15% of isolates were serotype b (Hib), with the remainder being non-Hib.[7] As indicated by Bae et al,[8] in Korea, over 90% of the respiratory strains were non-typeable, and the predominance of capsulated *H influenzae* has been just 7.4%. 2 isolates were of type b, 16 were of type a, 2 were of type c, eight were of type d, six were of type e, and six were of type f. According to William and Geddes,[9] Hib carriage was present in 5% of Nepalese households.

The 19 capsulated type b ampicillin-resistant *H. influenzae* strains exhibited MIC estimations of amoxicillin running from 1 to >256 mg/L and AMC MICs extended from 1 to 6 mg/L. The sort b strains were susceptible to CEF.

Based on PCR, the 19 type b ampicillin-resistant *H influenzae* strains tried were subdivided as follows as per their ampicillin resistance genes bla_{TEM}, bla_{ROB} and *fts*I: Six strains had transformations in the *fts*I gene (BLNAR); 11 created β-lactamase TEM-1 type (bla_{TEM-1} (+)) and had no *fts*I changes (β-lactamase positive, ampicillin-resistant); No strain delivered the blaROB-1 gene. Two strains created β-lactamase (bla_{TEM-1} (+)) and furthermore had transformations in the *fts*I gene (β-lactamase positive, amoxicillin-clavulanate-resistant). As indicated by Hascelik and Kittana,[10] among the BLNAR *H influenzae* isolates, serotype b 110 (88%) was the most prevalent.

Haemophilus influenzae type b can cause a large number of instances of genuine illness worldwide and a huge number of deaths every year among young children.[11] The primary clinical indications of invasive Hib contamination include meningitis, which can prompt passing. Hib vaccines can avert numerous instances of Hib diseases. That is the reason they are prescribed in all routine infant vaccination programs. In Tunisia, Hib immunization was introduced in 2002 and ceased for reasons of cost. It was reintroduced in the vaccination plan for the public sector in January 2011.[12]

5. Conclusion

PCR should be used in our country because it may contribute to decreasing the probability of transmission of these strains, especially those showing the two mechanisms of resistance among children in Tunisia. Continued surveillance is expected to screen for serotype distribution of *H influenzae* diseases in Tunisia.

Author contributions

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References

[1] Falla TJ, Crook DW, Brophy LN, Maskell D, Kroll JS, Moxon ER. PCR for capsular typing of Haemophilus influenza. J Clinical Microb. 1994;32:2382–6.
[2] Jackson C, Mann A, Mangtani P, Fine P. Effectiveness of Haemophilus influenzae type b vaccines administered according to various schedules: systematic review and meta-analysis of observational data. Ped Infec Dis J. 2013;32:1261–9.
[3] World Health Organization. Haemophilus influenzae type b (Hib) vaccination position paper - July 2013: introduction = Note de synthèse: position de l'OMS sur la vaccination anti-Haemophilus influenzae type b (Hib) — Juillet 2013: introduction. Weekly Epid Rec. 2013;88:413–426.
[4] CA-SFM. 2020. Available at: https://www.sfm-microbiologie.org/2020/04/07/casfm-eucast-v1-0-avril-2020.
[5] Dabernat H, Delmas C, Seguy M, Pelissier R, Faucon G, Bennamani S, Pasquier C. Diversity of β-Lactam resistance-conferring amino acid substitutions in penicillin-binding protein 3 of Haemophilus influenzae. Antimicrob Agents Chem. 2002;46:2208–18.
[6] Oueslati S, Smaoui H, Joubert G, Dabernat H, Kechrid A. Étude de la résistance aux β-lactamines et des marqueurs moléculaires chez 157 souches d'Haemophilus influenzae isolées chez l'enfant à Tunis. Canad J Microb. 2009;55:5.
[7] Rashid H, Shoma S, Rahman M. Prevalence of betalactamase positive ampicillin resistant H. influenzae from children of Bangladesh. J Infect Dis Epidemiol. 2016;2:10.
[8] Bae S, Jaehoon L, Lee J, Kim E, Lee S, Yu J, Kang Y. Antimicrobial resistance in Haemophilus influenzae respiratory tract isolates in Korea: results of a nationwide acute respiratory infections surveillance. Antimicrob Agents Chemo. 2010;54:65–71.
[9] Williams JD, Geddes AM. Penicillins and Cephalosporins. Softcover reprint of the original 1st ed. 1976 edition. Springer. 2012; 357.
[10] Hascelik G, Kittana A. Investigation of serotype distribution and antibiotic susceptibility in betalactamase negative ampicillin resistant Haemophilus influenzae strains from adult patients. Inter J Infec Dis. 2018;73:130.
[11] WHO. Couverture vaccinale. Available at: https://www.who.int/fr/news-room/fact-sheets/detail/immunization-coverage. 2020.
[12] Mzilem S, Ksiaa S, Smaoui H, Kechrid A. Haemophilus influenzae strains in children: increasing resistance to Beta-lactam antibiotics. Inter J Microb Imm Re. 2015;3:084–9.