

Original article

Progress in laboratory-based *Haemophilus influenzae* surveillance of bacterial meningitis and pneumonia, by real-time PCR, in Sao Paulo State (2010-2019)

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Progress in laboratory-based *Haemophilus influenzae* surveillance of bacterial meningitis and pneumonia,
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ABSTRACT

Haemophilus influenzae (Hi) is an important pathogen causing bacterial Meningitis (BM) and bacterial pneumonia (BP), especially in countries where immunoprevention is poor or absent. Hi is differentiated into encapsulated (serotypes a, b, c, d, e, f), and unencapsulated (HiNt), according to the presence or lack of the polysaccharide capsule, respectively. The capsule is the main Hi virulence factor; the *bexA* gene, responsible for its expression, has been largely used for molecular detection and surveillance of BM and BP. In 2010, the Adolfo Lutz Institute (ALI) implemented real-time PCR (qPCR) using the *bexA* gene for detecting Hi; but reports on its failing to detect some encapsulated Hi and HiNt caused IAL to replace *bexA* with *hpd* as the target gene in the qPCR assay, extending Hi detection to both encapsulated and unencapsulated Hi. In this study, we assessed the impact of replacing the target gene on BM and BP surveillance, by analyzing the use of *bexA* target gene, within the period from 2010 to July 2012, compared with the use of *hpd*, from August 2012 to 2019. Adopting the *hpd* target gene in BM and BP surveillance improved the detection of non-vaccine Hi variants by 37% and 23%, respectively, predominantly Hia and HiNt; and it has contributed to improve laboratory surveillance of invasive Hi diseases.

KEYWORDS: *H. influenzae*, Bacterial Meningitis, Bacterial pneumonia, Real-time PCR, Diagnosis.

RESUMO

Haemophilus influenzae (Hi) é um importante patógeno causador de meningites (MB) e pneumonias bacterianas (PB), principalmente em países onde a imunoprevenção é precária ou inexistente. O Hi é classificado em tipáveis (sorotipos a, b, c, d, e, f) e não tipáveis (HiNt), de acordo com a presença ou ausência da cápsula polissacarídica, respectivamente. A cápsula é o principal fator de virulência dos Hi e o gene *bexA*, responsável pela sua expressão, é comumente empregado na detecção molecular e vigilância das MB e PB causadas por Hi. Em 2010, o Instituto Adolfo Lutz (IAL) implantou a PCR em tempo real (qPCR) empregando esse alvo genético para a detecção de Hi. Entretanto, relatos de falha na detecção de alguns Hi encapsulados e HiNt motivaram a substituição do gene alvo para essa bactéria. Desta forma, em agosto de 2012, o IAL fez a substituição do *bexA* pelo alvo genético *hpd* no ensaio de qPCR, permitindo a detecção de Hi tipáveis e não tipáveis. Neste estudo, avaliamos o impacto da substituição do alvo genético na vigilância das MB e PB analisando o emprego do alvo genético *bexA*, no período de 2010 a julho de 2012,

em comparação com o emprego do *hpd*, de agosto de 2012 a 2019. Esta substituição promoveu a melhoria na detecção de variantes não vacinais de Hi nas MB e PB em 37% e 23%, respectivamente, com predomínio de Hia e HiNt, contribuindo para o aprimoramento da vigilância laboratorial das doenças invasivas causadas por Hi.

PALAVRAS-CHAVE: *H. influenzae*, Meningites bacterianas, Pneumonias bacterianas, PCR em tempo real, Diagnóstico.

INTRODUCTION

The Gram-negative coccobacillus *Haemophilus influenzae* (Hi) is still a main pathogen causing bacterial Meningitis (BM) and bacterial pneumonia (BP), especially in countries where immunoprevention is poor or absent. The nasopharyngeal airway is the main entry route for that bacterium, which may or may not cause diseases; children and infants are the main reservoirs.¹

Transmission is person-to-person, through direct contact with infected droplets expelled by sufferers or carriers, which may reach the mucus in the respiratory tract and spread throughout the body, causing diseases in individuals of diverse age groups, preferably in susceptible children.² In newborns, transmission may occur by contact with amniotic fluid, or genital secretion.¹ Some factors favoring the spreading and susceptibility to the illness are dry weather, crowds, pollution, poor nutrition, and others.³

Hi strains are differentiated into encapsulated (serotypes a, b, c, d, e, f), and unencapsulated (HiNt), according to the presence or lack of the polysaccharide capsule, respectively.⁴ The capsule is the main Hi virulence factor, offering resistance to the bactericidal activity in the host's complement system, thereby favoring the onset of an infection.⁵

The introduction of a vaccine against Hi, serotype b (Hib), in the 1980s-1990s⁶, had great impact on the Hi epidemiology worldwide. Before the vaccine, Hib was the main BM and BP pathogen infecting children from developing countries, being responsible for 20%-60% and 30% of those illnesses, respectively⁷. It is currently limited to sporadic cases, in closed or local communities which has not implemented vaccination against Hib. On the other hand, HiNt, which, previous to the vaccine, was commonly found in mucosa inflammatory infections—such as sinusitis, otitis, and conjunctivitis—, and in aggravations of the chronic obstructive pulmonary disease, now has been often found in invasive infections, especially in children and elders.^{1,8,9}

As Hib prevalence has been reduced, there has been reports on increased diseases associated with the other non-b serotypes, and with HiNts.^{7,9,11} According to the Annual Epidemiological Report for 2018 on Communicable Diseases in Europe (https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2018_haemophilus_influenzae.pdf), HiNt was responsible for 78% of the invasive () Hi diseases among the samples submitted to typing.

The vaccine against Hib was introduced in Brazil in 1999, resulting in a substantial reduction in BM cases, by approximately 90%,^{2,10} and in BP cases, by about 31%,¹² as well as in BM and BP mortality rates, among children under 5 years and infants under 18 months old.² According to the the Brazilian Reportable Diseases Surveillance System (SINAN), the state of Sao Paulo had a decreased incidence coefficient of the BM caused by Hib, from 1.18/100,000 inhabitants in 1998 to 0.13, in 2019.

Considering the possibility of transmission, invasive Hi diseases must have prompt notification, according to the National List of Compulsory Notification of Ailments, Diseases Aggravations, and Public Health Events, and to the Brazilian Ministry Department of Health Decree N. 264, of February 17, 2020. The clinical diagnosis of the infection considers signs and symptoms, which may be nonspecific, and is followed by laboratory confirmation. Treatment for those illnesses comprehends antibiotic administration after clinical suspicion, especially for meningitis, given its great severity, and high mortality.

Along the history of BM in Brazil, diagnoses have been made through many different methods: bacterial culture; latex agglutination test; counterimmunoelectrophoresis (CIE); cerebrospinal fluid (CSF) chemocytology; and polymerase chain reaction (PCR).^{2,3} Culture is the gold standard method for laboratory diagnosis of bacterial infections, but antibiotic therapy prior to biological sample collecting may harm the sensitivity of that test.¹³

CIE is an immunological technique largely used in Brazil for diagnosing *Neisseria meningitidis* (Men) and Hi infections. The method was adopted in the 1970s, and was used because it is fast, simple, and low-cost, comprehending bacterial antigens detection for the BM diagnosis (Men serogroups A, B and C, and Hib)¹⁴ However, that diagnosis test was gradually discontinued in the country, due to reports on cross-reactivity between *S. pneumoniae* (Spn) and Hib,¹⁵ attested by the Adolfo Lutz Institut (ALI), which have detected 57% false-positive results for Hib.¹⁶

Another widely adopted technique, especially in hospitals, is the latex agglutination test, whose particles, sensitized with specific antibodies to the serogroups/serotypes, form a visible precipitate in the presence of soluble antigens released by bacteria in

biological samples. Such method allows the detection of Men (some serogroups), Spn, and Hib, with 80%, 94%, and 90% sensitivity, respectively, and 97% specificity. However, in 2018, one of the commercial kits most commonly used in Brazil had reports on false-positive results for Spn, revealing positive reactivity in saline samples (Letter to the client- FSCA 10-18 IDD-BioRad AC_003/2018). Once the issue was settled, the current presentation of the latex kit for BM has limited use to CSF and blood culture supernatant samples, excluding serum samples (Kit package insert – Pastorex™ Meningitis; Marnes-la-Coquette, France: Bio-Rad, 2017).

The real-time polymerase chain reaction (qPCR) is a molecular technique implemented in 2010 by Sacchi *et al.* in the ALI diagnostic routine,¹³ and it has been contributing for reliable diagnosis of BM cases in the city of Sao Paulo. From its implementation until 2015, there was a 50% reduction in the nuBMer of indeterminate BM cases.¹⁷

The qPCR assay simultaneously detects Men, Spn, and Hi; its introduction into the diagnostic routine has contributed to increase the detection of those agents, by 85%, 52%, and 20%, respectively, compared with the bacterial culture.¹³ In the qPCR, the use of the *bexA* target gene aiming the six encapsulated types (a, b, c, d, e, f), has been suggested for detecting Hi; it has been largely used in detection, and surveillance of invasive Hi.¹⁸⁻²¹

However, despite the qPCR high sensitivity and specificity to Hi, Sam & Smith (2005) reported some issues regarding the use of *bexA* target to detect Hie and Hif types, due to the probe's possible failure to recognize region I of the *bexA* target *cap locus*.²² Furthermore, Kroll *et al.* have found a 16.5% difference between nucleotides of the *bexA* gene in Hib strains from different origins.²³ Consequently, the use of the *bexA* target gene implies reduced notifications of diseases by Hi, due to the proven effectiveness of the vaccine against Hib, which has allowed the emergence of other spreading types, and HiNt.^{1,9-11,24,25}

In order to overcome the limitations of *bexA*, several studies were carried out in search of new target genes, aiming to improve the molecular diagnosis of Hi.^{23,26,29} Wang *et al.* (2011) have documented the use of the *hpd* target gene as a promising alternative, able to detect all types of Hi, as well as HiNts.³⁰

Within such a context, ALI has recommended changing the *triplex* qPCR (Men, Spn, and Hi) composition, in order to heighten the sensitivity of the Hi component detection. The *triplex* qPCR with *hpd* was assessed, and implemented in the ALI diagnostic routine in August 2012.³¹

In view of the above, this paper aimed to assess the Hi genotypes spreading in Sao Paulo State, based on the qPCR results obtained from the Laboratory for Molecular Diagnosis of Bacterial Infections, in the Immunology Center at ALI (LMDBI-ALI), from samples of suspected BM and BP cases within a period of 10 years. In order to do so, we have captured secondary data, stored in a digital system, comparing the period before (2010 to July 2012) and after (August 2012 to 2019) the replacement of the target gene responsible for detecting Hi in the through qPCR.

METHODOLOGY

We have retrieved from the Hospital Information Management System (HIMS), within the period from 2010 to 2019, qPCR results from CSF and serum samples from suspected BM cases, or serum and pleural fluid samples, from suspected BP cases, all from São Paulo State.

In the HIMS system, we have used filters to divide the results by year, clinical suspicion, and type of testing required, such as: PCR for bacterial Meningitis (MenPCR); PCR for bacterial pneumonia (PNEPCR), and genotyping for Hi (HITIP). The collected data were examined in an Excel spreadsheet, so as they would display the nuBMer of cases; i.e., each case was computed, no matter the nuBMer of analyzed samples.

The analysis of the impact the target gene replacement had on Hi detection was split into two periods. From 2010 to July 2012, we used the *bexA* target gene, and from August 2012 to 2019, the *hpd* target gene.

All Hi-positive DNA samples by qPCR were submitted to molecular typing (HITIP). In this study, however, samples from 2010 to July 2012 screened for Hi with *bexA* target gene showing negative result in HITIP for types a, b, c, d, e, f were considered “indeterminate” (HiND), as the *bexA* target may have failed to detect Hie and Hif types in those samplings—once all samples detectable by that gene should be positive for some of the encapsulated serotypes. Likewise, samples screened by *hpd* target gene with negative result in HITIP were considered HiNt.

We have carried out this study accordingly to the National Health Council resolutions N. 441, of May 12, 2011; and N. 466, of DeceBMer 12, 2012. We have removed personal data compromising the patients’ anonymity, in order to ensure the confidential status of all information. This study was approved by ALI’s Research Ethics Committee, and registered on Plataforma Brasil with the CAAE protocol: 10760812.6.0000.0059.

RESULTS

Between 2010 and 2019, we assessed 19,645 suspected BM cases of Men, Spn, or Hi at the LMDBI-ALI. Of these cases, 5,663 (28.8%) were positive for the bacterial agents analyzed through qPCR: 3,091 (15.7%) for Men; 2,267 (11.5%) for Spn; and 305 (1.6%) for Hi. To these 305 BM by Hi cases, we have added 70 more from the state's countryside, totaling 375 Hi-positive cases, which were submitted to genotyping (HITIP). Of that total, 78 were from the 2010-July 2012 period (*bexA*), and 297, from the August 2012-2019 period (*hpd*).

The frequency of the genotypes detected in the 375 BM by Hi, in both periods, is shown on Table 1. We could notice the HiNts were responsible for about one third of the BM caused by Hi after the *hpd* target gene was introduced. Furthermore, the replacing *hpd* target gene has allowed us to detect Hie and Hif, as we could correct the failure in detecting those genotypes, leading to a 3% increase in genotyping.

Table 1. Frequency of *H. influenzae* genotypes detected through qPCR in CSF or serum DNA samples of 375 bacterial meningitis suspected cases, in the period from 2010 to 2019, divided 2012 between July and August, due to the replacement of the *bexA* target gene by *hpd* in the lab routine.

| Bacterial Meningitis by <i>H. influenzae</i> | | | | |
|--|------------------------------|-------------|-------------------------------|-------------|
| Genotypes (n) | <i>bexA</i> (2010-July 2012) | | <i>hpd</i> (August 2012-2019) | |
| | N. of cases | Frequency | N. of cases | Frequency |
| a (128) | 31 | 39.7% | 97 | 32.7% |
| b (123) | 35 | 44.9% | 88 | 29.6% |
| c (0) | - | - | - | - |
| d (3) | 1 | 1.3% | 2 | 0.7% |
| e (2) | - | - | 2 | 0.7% |
| f (7) | - | - | 7 | 2.3% |
| ND (11) | 11 | 14.1% | - | - |
| Nt (101) | - | - | 101 | 34.0% |
| TOTAL (375) | 78 | 100% | 297 | 100% |

Captions: Hi = *Haemophilus influenzae*; ND = not defined Hi; Nt = non-typable (unencapsulated) Hi

Regarding age, the frequencies of BM caused by Hi are distributed on Figure 2. When grouping the ages, we could see a greater frequency of BM by Hi among children under 5 years old, accounting for 59% of cases. Also, in the analysed samples samplings, there was a higher prevalence of Hia among children under 5 (77.3%; 99/128), and of HiNt among people over 40 years old (46.0%; 52/113).

Figure 1. Proportion of *H. influenzae* genotypes detected in 375 bacterial meningitis cases in the period from 2010 to 2019, divided 2012 between July and August, due to the replacement of the *bexA* target gene by *hpd* in the qPCR assay at LMDBI-ALI. [Nt = non-typable (unencapsulated) Hi; ND = not defined Hi].

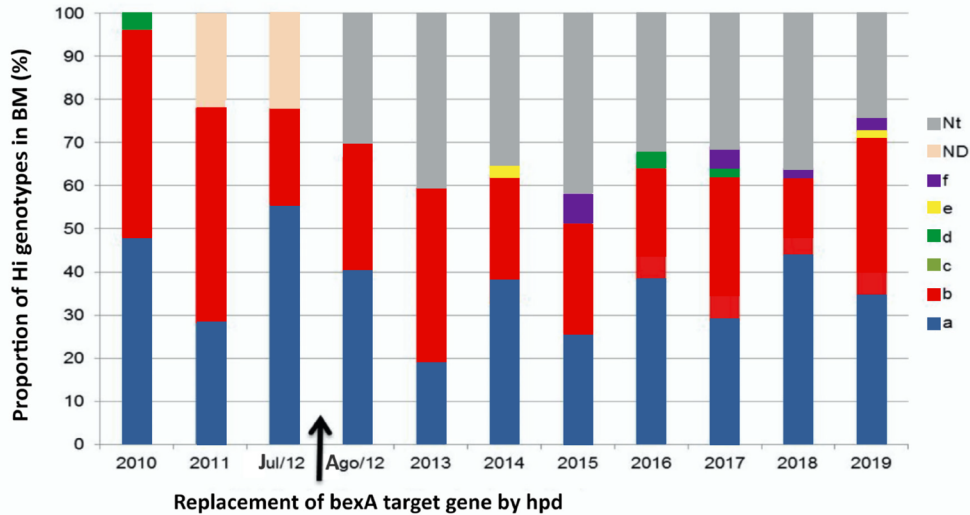
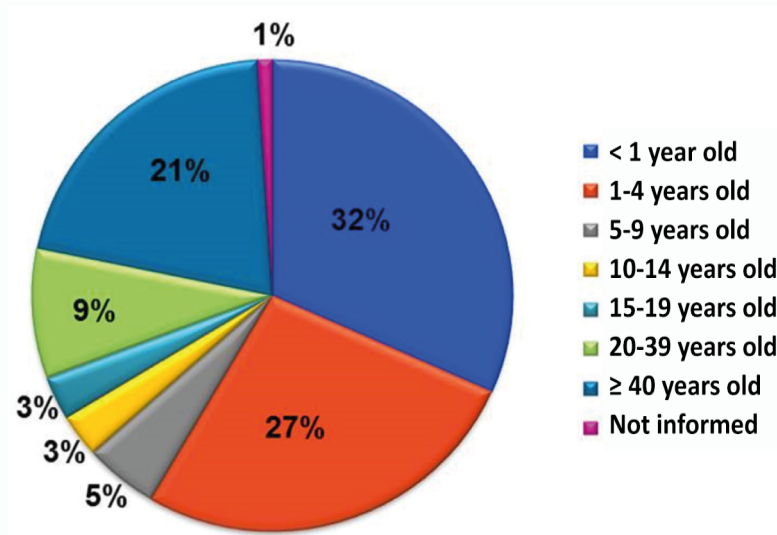


Figure 2. Distribution, by age group, of the 375 BM caused by Hi from the state of Sao Paulo, detected by qPCR in CSF or serum DNA samples at the LMDIB, 2010-2019.



Unlike BM, BP is not on the list of compulsory notifiable diseases, and etiologic agent testing is only recommended to patients showing severe condition, or bad response to previous antibiotic therapy.³² Those factors have probably had a high impact upon the reduced number of samples referred to LMDBI-ALI for diagnosing BP (2010-2019).

Regarding BP, we have analyzed 1,075 cases, of which 227 (21.1%) were positive, 843 (78.4%) negative; and 5 (0.5%) inconclusive. Out of the 227 positive cases, 210 (19.5%) were positive for Spn; and 17 (1.6%) for Hi (Table 2).

Table 2. Bacterial agents detected through qPCR in serum or pleural fluid DNA samples, and Hi genotyping of clinical suspicions of bacterial pneumonia assessed by LMDBI-ALI, in the period from 2010 to 2019, divided 2012 between July and August, due to the replacement of the *bexA* target gene by *hpd* in the lab routine.

| Year | PNEPCR | | | | | Genotipagem de Hi | | | Total |
|--------------|--------------|------------|-----------|------------|----------|-------------------|----------|----------|-----------|
| | TOTAL | SPN | HI | NEG | INC | Hia | Hib | Nt | |
| 2010 | 113 | 10 | - | 103 | | | | | |
| 2011 | 108 | 16 | 1 | 91 | | 1 | | | |
| Jan-Jul 2012 | 42 | 7 | - | 35 | | | | | |
| Ago-Dez 2012 | 19 | 4 | - | 15 | | | | | |
| 2013 | 215 | 22 | 4 | 189 | | 1 | | 1 | |
| 2014 | 84 | 20 | 1 | 63 | | | | | |
| 2015 | 56 | 7 | 2 | 47 | | | | 1 | |
| 2016 | 107 | 31 | 1 | 72 | 3 | | 1 | | |
| 2017 | 114 | 19 | 2 | 92 | 1 | 1 | | 1 | |
| 2018 | 107 | 39 | 4 | 63 | 1 | 4 | | | |
| 2019 | 110 | 35 | 2 | 73 | - | 2 | | | |
| Total | 1.075 | 210 | 17 | 843 | 5 | 9 | 1 | 3 | 13 |

Thirteen of the 17 Hi-positive samples were submitted to genotyping, and 9 (69%) of them were identified as Hia; 1 (8%) as Hib; and 3 (23%) as HiNt.

In the BP samples, the replacement of the *bexA* target gene by *hpd* resulted in an increase 23% in Hi detection by HiNTs (3/13) identification. Among the detected genotypes, Hia was predominant (69%) among the examined cases.

Regarding distribution by age group, children under 5 years old had 84.6% of BP cases caused by Hi, distributed into 23.1% (3/13) among infants under 1, and 61.5% (8/13) among children between 1 and 4 years old. Individuals aged between 5 and 14 years old represented 15.4% of the total BP by Hi cases.

DISCUSSION

The vaccine against Hib has enabled the Hi epidemiological scene to be changed worldwide. Since immunoprevention is serotype-specific, it has the effect of reducing the

incidence of invasive diseases and nasopharynx colonization by type b, without interfering with other Hi variants.^{6,9,11,33} In fact, after the vaccine was introduced in the Brazilian National Immunization Program (NIP), in 1999, Hib strain isolating have decreased in approximately 40%.¹⁰

The correct identification of Hi serotypes plays a central role in monitoring the spreading variants; it also helps in understanding the Hi epidemiology, as well as in taking actions to control Hi-caused illnesses. Within that context, highly sensitive, specific, and fast diagnostic tests are the key to successful therapies and patient care, favoring immediate actions to control and contain outbreaks and epidemics.

Molecular assays aiming mainly Hib had to be reassessed in order to improve Hi diagnosis, using a broad spectrum target gene, as we expected the emergence of non-b variants and HiNTs.⁹

In their BM study with sentinel surveillance, conducted between 2006 and 2009, Sacchi *et al.* (2011) proposed, at ALI, the *bexA* target gene-based *triplex* qPCR assay.¹³ That test, able to detect only encapsulated variants, detected only 3 Hi-positive cases in 660 samples, possibly due to the limitations of the *bexA* component.¹³

Considering that fact, and based on previous studies,^{30,34} ALI (which is the Brazilian national reference laboratory for BM diagnosis) has proposed an improvement in Hi detection, by suggesting the *bexA* target gene should be replaced by *hpd* in the *triplex* qPCR assay, in order to boost laboratorial Hi surveillance. Indeed, this *hpd*-modified *triplex* qPCR assay proved to be more efficient in increasing Hi detection in clinical samples during the assessed period. The modified assay was implemented in ALI's diagnostic routine in 2012.

This study compared the Hi detection ability of *triplex* qPCR assay using *bexA* (2010 to July 2012) versus *hpd* (August 2012 to 2019) in clinical samples from patients with suspected BM and BP.

A Brazilian study by Zanella *et al.* (2011), with 3,910 Meningitis-causing Hi isolates referred to ALI for serotyping, reported the Hib was responsible for 98% of Meningitis by Hi cases in the pre-vaccine period (1990-1999); that percentage was reduced to 59% after the vaccine was introduced (2000-20008). In the post-vaccine period, the authors noted an increase in the occurrence of non-b serotypes (1% to 19%) and HiNTs (2% to 22%).¹⁰ In the present study, which was carried out in São Paulo State, in the second post-vaccine decade (2010-2019), a higher nuBMer of non-b serotypes detections, as well as HiNTs, was observed in both BMs and BPs, due to the Hib decline fostered by vaccination. Hia

represented 34% (128/375), and HiNts, 27% (101/375) of the assessed BM cases from 2010 to 2019; of the BP cases, Hia represented 69% (9/13), and HiNts, 23% (3/13), in the same period. McNeil *et al.* (2021) draw attention to the increased nuBMer of invasive Hia and HiNt-caused diseases, in the period from 2011 to 2018, in the American pediatric population, with no apparent increase in the other capsulated Hi.³³

In regards to age, we could notice a greater vulnerability to invasive Hi infections among children under 5, who represented 59% of suspected BM cases, and 85% of BP cases. Such observation confirms the available data on the Sao Paulo State Epidemiological Surveillance (Sinan/DDTR/CVE/CCD/SES-SP) website, which reports a 57% prevalence of Hi infections in that age group over the past ten years.

The other encapsulated serotypes, Hic, Hid, Hie, and Hif, which sporadically cause Hi infections, represented 3.2% of all BM cases, and were absent in the BP cases assessed in this study. Among them, Hif was the most frequent in this study, as well as in other investigations reported on literature.^{6,33,35} The frequency of those genotypes in our study is similar to that of isolate non-a/b Hi strains in BM cases in Brazil in the post-vaccine period (2000-2008), as documented by Zanella *et al.* (2011):¹⁰ 0.2% Hic; 0,9% Hid ; 0.5% Hie; and 2.4% Hif. The results of the present study confirm the 10-year records of Hi laboratorial surveillance (2010-2019) in Latin American countries, whose results show less than 1% of clinical cases associated with Hic, Hid, or Hie (the 2010-2012 records are available on the epidemiological reports from Rede SIREVAII: https://www3.paho.org/hq/index.php?option=com_content&view=article&id=5536:2011-sireva-ii&Itemid=3966&lang=en; the 2013-2019 records can be found on ALI bulletins: <http://www.ial.sp.gov.br/ial/publicacoes/boletim>). A study conducted in Israel by BaBMerger *et al.* (2014)³⁶ showed similar results: a low frequency of clinical cases associated with non-a/b Hi.

We should mention that, although serotypes e and f are not very often in invasive Hi infections, in the present study, the triplex qPCR with hpd target gene was able to detect both serotypes, fostering a 3%-increased Hi detection in BMs.

HiNts have been ever present in the post-vaccine period. Such an increase had already been reported in several Latin American countries³⁷. In this study, HiNts represented one-third of the BM cases (34%), and 23% of the BP cases assessed through the modified qPCR assay; those data are equivalent to the ones Zanella *et al.* have obtained: they have detected 33% HiNts among 371 isolate strains in the post-vaccine period (2000-2008), in the state of Sao Paulo¹⁰. All those data ratify the importance of replacing the Hi target gene in the triplex qPCR assay, which, previously, could not detect those strains.

A study conducted by King *et al.* (2012) described HiNts as less virulent, rarely causing invasive diseases, considered as primary mucosa pathogens.³⁸ On the other hand, Slack (2015) described HiNt as responsible, in invasive infections, for 37% of no apparent cause bacteremia; 27% of pneumonia; and 12% of Meningitis¹¹. And other studies have reported increased nasopharynx HiNt colonization in pediatric population receiving conjugate vaccine against Spn, which indicates an increasing HiNt participation in both invasive diseases and carriage.^{39,40}

A possible contributing factor to the increased invasive HiNt diseases in the pre-vaccine period was the improvement in diagnosis, by using broad spectrum target genes in molecular assays—which has enabled the detection of both encapsulated and unencapsulated variants—, as well as assays identifying the several Hi genotypes, which previously focused only on Hib.⁹

Such as scenario makes the continuous improvement in diagnostic tests is necessary, as laboratory confirmation of Hi-caused disease is essential for keeping constant Hi surveillance—enabling the assessment of possible vaccine failures and/or changes in the average age of affected individuals, in order to adopt new strategies and public policies for the epidemiological control of diseases.

CONCLUSION

Replacing the *bexA* target gene with *hpd* in the molecular assay of triplex format, developed by LMDBI-AIL, has resulted in 37% and 23% more efficient detection of Hi-caused BM and BP cases, respectively, due to the increased detection of Hie, Hif, and HiNt. Such an observation has enabled increased detection of non-vaccine variants (predominantly Hia, followed by HiNt), besides assisting in epidemiologically monitoring the emergence of other encapsulated serotypes.

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Progress in laboratory-based *Haemophilus influenzae* surveillance of bacterial meningitis and pneumonia, by real-time PCR, in Sao Paulo State (2010-2019)

Gonçalves MG, Higa FT, Fukasawa LO, Barros LDA, Salgado MM

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