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Research Article

Antibiotic susceptibility profiles of *Haemophilus influenzae* isolates collected in Dakar between 2018 and 2022

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Abstract

Haemophilus influenzae, a Gram-negative coccobacillus unique to humans, is known to cause a substantial number of respiratory tract infections, along with severe invasive conditions like meningitis and septicemia. This study aimed to ascertain the antibiotic susceptibility patterns of *H. influenzae* strains isolated from the Pasteur Institute Medical Laboratory in Dakar. A retrospective study was carried out between January 2018 and February 2022 on strains isolated from respiratory tract samples (sputum, BAL). Strains were isolated after culture on Chocolate PVX agar and incubated at 35°C in 5% CO₂ for 24 to 48 hours. *Haemophilus influenzae*-like colonies were identified by conventional methods including requirement tests for haemin (factor X) and NAD (factor V). Biochemical identification was performed using API 20 NH biochemical strips (BioMerieux, France). Disk diffusion test or Kirby-Bauer method had been used to perform antibiotic susceptibility testing on Chocolate PVX media. 132 strains of *H. influenzae* were isolated. 56% of the strains were from females and 44% from males with a mean age of 44 years. The most represented age groups were [40-59] and [≥60] with 31% each. Of the strains, 126 (95%) were from sputum and 6 (5%) from bronchoalveolar lavage (BAL). Resistance rates of *H. influenzae* isolates to ampicillin, amoxicillin+clavulanic acid, cefepime, cefotaxime, chloramphenicol, ciprofloxacin, meropenem and tetracycline were 24.7%, 22.2%, 72.7%, 9.5%, 8.1%, 8.3% and 22.1%, 22.8% respectively. Antibiotic resistance in *H. influenzae* requires continued attention with effective detection methods and a rational antibiotic use policy.

Keywords: *Haemophilus influenzae*, antimicrobial resistance, Biochemical identification, Resistance rates

Introduction

Haemophilus influenzae is a Gram-negative coccobacillus that is exclusively found in humans, primarily within the respiratory tract. The initial step in a sequence of events that can lead to disease involves nasopharyngeal colonization (Agrawal & Murphy, 2011; Murphy et al., 2009). *H. influenzae* plays a significant role in various mucosal respiratory tract infections and invasive diseases, such as bacterial meningitis. These *H. influenzae* strains are categorized as either Hi typable or non-typeable, depending on the presence of the polysaccharide capsule antigen. Typable

Hi strains are further classified into six serotypes (a to f), while the absence of the capsule is identified as NTHi (non-typeable *H. influenzae*). Before the introduction of conjugate vaccines, *H. influenzae* type b (Hib) was the leading cause of bacterial meningitis in young children in European countries (Slack, 2021).

Following the inclusion of the Hib vaccine in childhood immunization programs, there has been an overall decrease in Hi colonization and invasive Hib disease among children. However, there has been an increase in infections caused by non-encapsulated strains referred to as non-typeable *H.*

influenzae (NTHi) (Agrawal & Murphy, 2011; Madore, 1996; Takala et al., 1991). Recent data indicate an increasing virulence of these NTHi isolates and their growing resistance to various antibiotics. Furthermore, *H. influenzae* infections are influenced by various factors, including environmental conditions, population density, and climatic factors (Brown et al., 2021; Mazamay et al., 2020). Consequently, continuous monitoring of both local and global epidemiological trends is essential for the development of future disease control strategies. Surveillance of antibiotic resistance provides critical insights to assist clinicians in making informed decisions regarding empirical treatment.

Resistance to beta-lactam antibiotics appears to be attributed to the acquisition of beta-lactamase enzymes and/or the presence of well-characterized mutations in penicillin-binding protein 3 (PBP3). β -lactam antibiotics are now widely employed in the clinical treatment of *H. influenzae* infections. In several countries, empirical drugs like these have been reported to be ineffective due to resistance (Guitor & Wright, 2018). Rates of ampicillin-resistant Hi vary, ranging from less than 5% in some countries to as high as 67.9%, as observed in Canada, Japan, Italy, Shanghai, and Taiwan (Wen et al., 2020).

Little recent information is available on the epidemiology, microbiology and antibiotic resistance of *H. influenzae* strains in sub-Saharan Africa. Our objective was to determine the susceptibility profile of *H. influenzae* strains isolated at Medical Biology Laboratory of the Pasteur Institute of Dakar.

Material and method

This retrospective study was carried out on strains isolated from respiratory tract samples (sputum, BAL) between January 2018 and February 2022 at medical biology laboratory of Pasteur Institute of Dakar.

The strains were isolated after culture on Chocolate PVX agar for bronchoalveolar lavage (BAL) and after fluidification with the Digesteur (Eurobio Scientific) for sputum and incubation at 35°C in 5% CO₂ for 24 to 48 hours. *H. influenzae*-like colonies were identified by conventional methods including requirement tests for haemin (factor X) and NAD (factor V). Biochemical identification was performed using API 20 NH biochemical strips (Bio Merieux, Marcy l'Etoile, France). Disk diffusion test or Kirby-Bauer method was used to perform antibiotic susceptibility testing on Chocolate PVX media (Bio Mérieux, Marcy l'Etoile, France) incubated for 24 hours at 37°C with 5% CO₂, according to EUCAST recommendations. The following Biorad antibiotic discs were used in our study: penicillin G (PNG, 1U), ampicillin (AM, 10 µg), amoxicillin+clavulanic acid (AUG, 2-1 µg), cefotaxime (CTX, 30 µg), cefepime (FEP, 30 µg), meropenem (MEM, 10 µg), chloramphenicol (CHL, 30 µg), nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg), rifampicin (RIF, 5 µg) and tetracycline (TET, 30 µg).

From a pure culture of 18-24 hours on agar medium, a bacterial suspension was prepared by taking 2-3 colonies mixed with 1 ml of sterile saline (0.9% NaCl) and adjusted to 0.5 Mc Farland rate. This bacterial suspension was spread on the surface of Chocolate PVX agar plates by swabbing. Antibiotic discs were placed on the agar surface by a disc dispenser with a minimum distance of 15 mm between the edge of the plate and peripheral discs and 30 mm between discs. Plates were then incubated in an oven in an aerobic atmosphere at 37°C with 5% CO₂ for 18 hours. After incubation, the results were read using the ADAGIO automated system (Biorad®) according to EUCAST recommendations. Data entry was performed using Excel version 2016 and statistical analysis was performed using STATA 14.0.

Results

Socio-demographic characteristics

A total of 132 strains of *H. influenzae* were identified. They were 56% female and 44% male with a mean age of 44 years. The most representative age groups were [40-59] and [≥60], each with 31%.

Table 1 : Socio-demographic characteristics of study population

Details	Number	%
Gender		
Women	74	56
Men	58	44
Sex ratio M/W	0.78	
Mean age (Extremes)	44 ans (1 – 84 years)	
Age group		
[0-19]	14	10.6
[20-39]	36	27.3
[40-59]	41	31.06
[≥60]	41	31.06
Total	132	100

Distribution of strains by origin of sample

Of the strains, 126 (95%) were from sputum and 6 (5%) from bronchoalveolar lavage (BAL).

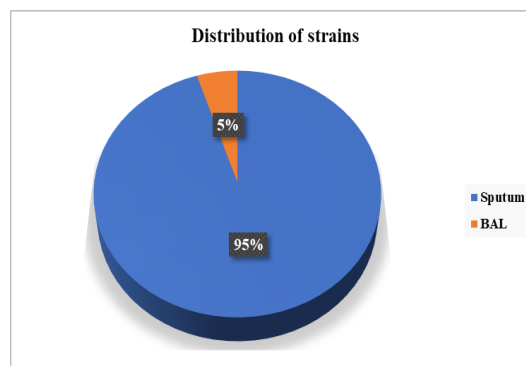


Fig.1 : Strain distribution by sample origin: majority from sputum (95%) and few from bronchoalveolar Lavage (5%)

Susceptibility profile of strains to β -lactams

The resistance rates of *Haemophilus influenzae* isolates to penicillin G, ampicillin, amoxicillin+clavulanic acid, cefotaxime and meropenem were 37.9%, 24.7%, 22.2%, 22.1% and 9.5% respectively.

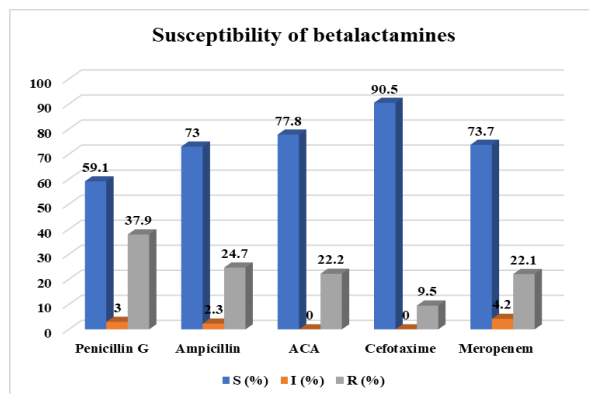


Fig. 2: Susceptibility profile of strains to β -lactams

Susceptibility profile of strains to other antibiotics

For other antibiotic families, high resistance rates were noted for rifampicin (48.9%) and tetracycline (22.8%). For chloramphenicol, ciprofloxacin and nalidixic acid 8.1%, 8.3% and 16% of strains were resistant respectively.

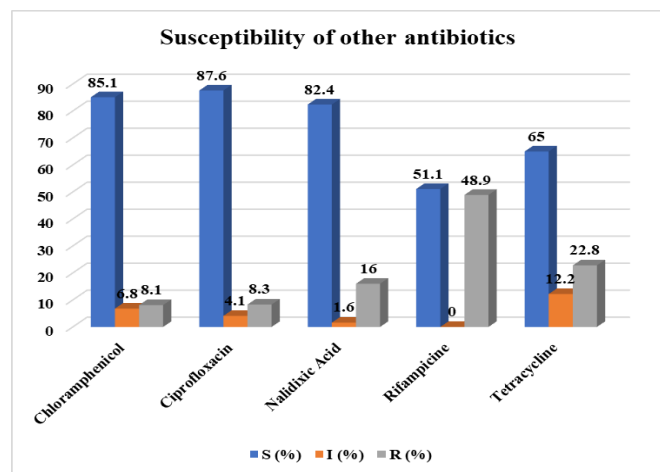


Fig. 3: Susceptibility profile of strains to other antibiotics

Discussion

The data gathered through our study offer a current snapshot of the local epidemiology of *H. influenzae* infections, encompassing aspects such as patient demographics (sex and age), specimen origin, and antibiotic sensitivity. *H. influenzae*, a Gram-negative bacterium, is a known culprit

behind respiratory tract infections, particularly affecting children (Kehl & Dowzicky, 2015). Following the introduction of the Hib conjugate vaccine, there has been a decline in the prevalence of invasive *H. influenzae*, consequently leading to a shift in the epidemiological landscape of Hi. Nevertheless, it's important to note that the promotion and implementation of Hib conjugate vaccines date back to the 1980s, contributing to substantial variations in Hi epidemiological patterns across the globe.

All strains in this study were derived from respiratory samples, mainly sputum samples. The performance of the chromogenic cefinase test could have allowed the identification of 3 resistance mechanisms. In our study, 59.1% of the strains showed a wild-type phenotype to β -lactams. A study conducted from 2014 to 2019 at the Assistance Publique des Hôpitaux de Marseille found 74.7% of wild-type strains to beta-lactams (Monegat, 2020).

However, 24.7%, 22.2% and 9.5% were resistant to ampicillin, amoxicillin-clavulanic acid and cefotaxime respectively and 8.33% (11/132) of strains were resistant to penicillin, ampicillin and amoxicillin-clavulanic acid.

Regarding ampicillin resistance, our results are consistent with other European studies, reporting similar levels of ampicillin resistance with 24% in France in 2017 and 21.9% in Germany between 2016 and 2019 (Deghmane et al., 2019; Nürnberg et al., 2021). A lower prevalence was observed elsewhere, such as in Portugal and Spain with 15.2% and 17.6% ampicillin resistant respectively (Lâm et al., 2015; Li et al., 2017; Wen et al., 2023).

The main mechanism used by Hi to resist ampicillin is production of β -lactamase to destroy antibiotics. Another mechanism is mutation of penicillin binding protein 3 (PBP3), which decreases susceptibility to ampicillin and other β -lactam antibiotics. β -lactam resistance phenotype mediated by second mechanism is referred to as non- β -lactamase producing ampicillin resistance (BLNAR). Although β -lactamase production is the main mechanism of ampicillin resistance (Lâm et al., 2015; Li et al., 2017; Wen et al., 2023), BLNAR strains are the most frequently isolated and β -lactamase-producing Hi are rare (Shiro et al., 2015). This increase in ampicillin resistance is due in particular to the emergence of BLNAR isolates carrying several amino acid substitutions in the three functional motifs of PBP3 and are responsible for ampicillin resistance (Tristram et al., 2007). These results highlight the need to maintain active surveillance of non-invasive strains as this may call into question use of amoxicillin as first-line treatment for ENT infections.

Resistance to amoxicillin/clavulanic acid accounted for 22.2%, which is different from the 1.7% in Germany and 1.9% in the USA (Nürnberg et al., 2021). Indeed, resistance to amoxicillin/clavulanic acid could be associated mainly with ampicillin-resistant strains without beta-lactamase

production (BLNAR) with several substitutions in the PBP3 protein.

Cefotaxime resistance accounted for 9.5% of isolates similar to the 6.4% found in China (Wang et al., 2019) but different from worldwide prevalences with 1.0% in Italy (Giufre et al., 2023) and 0.90% in Germany (Nürnberg et al., 2021). Monitoring by sequencing of the *ftsI* gene encoding the PBP3 protein remains an effective means of monitoring the emergence of 3rd generation cephalosporin resistant strains. The latter therefore remain the empirical treatment of choice for invasive *H. influenzae* infections.

We identified isolates resistant to ciprofloxacin in 8.3% of isolates and 16% to nalidixic acid. Emergence of fluoroquinolone resistance in recent years has also been observed in Spain, where 4.4% of isolates were weakly resistant to ciprofloxacin (Carrera-Salinas et al., 2021). *H. influenzae*'s resistance to fluoroquinolones has been observed in elderly subjects or those with chronic bronchial disease treated with fluoroquinolones repeatedly or for long periods (Kuo et al., 2014; Tristram et al., 2007). This resistance is most often found in non-encapsulated, non-invasive strains.

Resistance to chloramphenicol and tetracycline was 8.1% and 22.8% respectively. This resistance is related to frequently reported plasmid-mediated production of chloramphenicol acetyltransferase (van Klingeren et al., 1977). Furthermore, a relative permeability barrier due to the loss of an outer membrane protein has also been proposed in chloramphenicol resistance (Burns et al., 1985).

The limitations of this study were the lack of serotyping data for isolates and vaccination data for patients. With the widespread use of the Hib vaccine, the number of Hi b infections has gradually decreased, while infections caused by non-typeable Hi strains have gradually increased (Langereis & de Jonge, 2015; Naito et al., 2018). Accurate determination of the minimum inhibitory concentration (MIC) and the cefinase test would also have allowed better characterisation of the isolates.

Conclusion

H. influenzae infections remain a major problem due to their diversity and potential severity. We found a resistance rate in our series of 24.3% ampicillin resistance, 22.2% amoxicillin-clavulanic acid resistance and 9.5% of strains were resistant to 3rd generation cephalosporins. Although data on the distribution of Hi serotypes in our region before and after vaccination are not available, it is important to study non-typeable *H. influenzae* (NTHi) infections which have become common and cannot be prevented by Hib vaccination.

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Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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References

- Agrawal, A., & Murphy, T. F. (2011). Haemophilus influenzae Infections in the H. influenzae Type b Conjugate Vaccine Era. *Journal of Clinical Microbiology*, 49(11), 3728-3732.
- Brown, N. E., Blain, A. E., Burzlaff, K., Harrison, L. H., Petit, S., Schaffner, W., Smelser, C., Thomas, A., Triden, L., Watt, J. P., Pondo, T., Whaley, M. J., Hu, F., Wang, X., Oliver, S., & Soeters, H. M. (2021). Racial Disparities in Invasive Haemophilus influenzae Disease-United States, 2008-2017. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 73(9), 1617-1624.
- Burns, J. L., Mendelman, P. M., Levy, J., Stull, T. L., & Smith, A. L. (1985). A permeability barrier as a mechanism of chloramphenicol resistance in Haemophilus influenzae. *Antimicrobial Agents and Chemotherapy*, 27(1), 46-54. <https://doi.org/10.1128/AAC.27.1.46>.
- Carrera-Salinas, A., González-Díaz, A., Calatayud, L., Mercado-Maza, J., Puig, C., Berbel, D., Cámara, J., Tubau, F., Grau, I., Domínguez, M. Á., Ardany, C., & Martí, S. (2021). Epidemiology and population structure of Haemophilus influenzae causing invasive disease. *Microbial Genomics*, 7(12), 000723.
- Deghmane, A.-E., Hong, E., Chehboub, S., Terrade, A., Falguières, M., Sort, M., Harrison, O., Jolley, K. A., & Taha, M.-K. (2019). High diversity of invasive Haemophilus influenzae isolates in France and the emergence of resistance to third generation cephalosporins by alteration of *ftsI* gene. *The Journal of Infection*, 79(1), 7-14.
- Giufre, M., Cardines, R., Marra, M., Carollo, M., Cerquetti, M., & Stefanelli, P. (2023). Antibiotic Susceptibility and Molecular Typing of Invasive Haemophilus influenzae Isolates, with Emergence of Ciprofloxacin Resistance, 2017–2021, Italy. *Microorganisms*, 11(2)
- Guitor, A. K., & Wright, G. D. (2018). Antimicrobial Resistance and Respiratory Infections. *Chest*, 154(5), 1202-1212. <https://doi.org/10.1016/j.chest.2018.06.019>.
- Kehl, S. C., & Dowzicky, M. J. (2015). Global assessment of antimicrobial susceptibility among Gram-negative organisms collected from pediatric patients between 2004 and 2012: Results from the Tigecycline Evaluation and Surveillance Trial. *Journal of Clinical Microbiology*, 53(4), 1286-1293.
- Kuo, S.-C., Chen, P.-C., Shiau, Y.-R., Wang, H.-Y., Lai, J.-F., Huang, W., & Lauderdale, T.-L. Y. (2014). Levofloxacin-resistant haemophilus influenzae, Taiwan, 2004-2010. *Emerging Infectious Diseases*, 20(8), 1386-1390.
- Lâm, T.-T., Claus, H., Elias, J., Frosch, M., & Vogel, U. (2015). Ampicillin resistance of invasive Haemophilus influenzae isolates in Germany 2009-2012. *International Journal of Medical Microbiology: IJMM*, 305(7), 748-755. <https://doi.org/10.1016/j.ijmm.2015.08.028>.
- Langereis, J. D., & de Jonge, M. I. (2015). Invasive Disease Caused by Nontypeable Haemophilus influenzae. *Emerging Infectious Diseases*, 21(10), 1711-1718.
- Li, J.-P., Hua, C.-Z., Sun, L.-Y., Wang, H.-J., Chen, Z.-M., & Shang, S.-Q. (2017). Epidemiological Features and Antibiotic Resistance Patterns of Haemophilus influenzae Originating from Respiratory Tract and Vaginal Specimens in Pediatric Patients. *Journal of Pediatric and Adolescent Gynecology*, 30(6), 626-631.
- Madore, D. V. (1996). Impact of immunization on Haemophilus influenzae type b disease. *Infectious Agents and Disease*, 5(1), 8-20.
- Mazamay, S., Bompangue, D., Guégan, J.-F., Muyembe, J.-J., Raoul, F., & Broutin, H. (2020). Understanding the spatio-temporal dynamics of

- meningitis epidemics outside the belt: The case of the Democratic Republic of Congo (DRC). *BMC Infectious Diseases*, 20(1), 291.
- Monegat, M. (2020). *Épidémiologie des infections à Haemophilus influenzae de 2014 à 2019 à l'Assistance Publique des Hôpitaux de Marseille. Sciences pharmaceutiques. Fjdumas- 02961330*. Université d'Aix-Marseille.
- Murphy, T. F., Faden, H., Bakaletz, L. O., Kyd, J. M., Forsgren, A., Campos, J., Virji, M., & Pelton, S. I. (2009). Nontypeable *Haemophilus influenzae* as a pathogen in children. *The Pediatric Infectious Disease Journal*, 28(1), 43-48.
- Naito, S., Takeuchi, N., Ohkusu, M., Takahashi-Nakaguchi, A., Takahashi, H., Imuta, N., Nishi, J., Shibayama, K., Matsuoka, M., Sasaki, Y., & Ishiwada, N. (2018). Clinical and Bacteriologic Analysis of Nontypeable *Haemophilus influenzae* Strains Isolated from Children with Invasive Diseases in Japan from 2008 to 2015. *Journal of Clinical Microbiology*, 56(7), e00141-18.
- Nürnberg, S., Claus, H., Krone, M., Vogel, U., & Läm, T.-T. (2021). Cefotaxime resistance in invasive *Haemophilus influenzae* isolates in Germany 2016-19: Prevalence, epidemiology and relevance of PBP3 substitutions. *The Journal of Antimicrobial Chemotherapy*, 76(4), 920-929.
- Shiro, H., Sato, Y., Toyonaga, Y., Hanaki, H., & Sunakawa, K. (2015). Nationwide survey of the development of drug resistance in the pediatric field in 2000–2001, 2004, 2007, 2010, and 2012: Evaluation of the changes in drug sensitivity of *Haemophilus influenzae* and patients' background factors. *Journal of Infection and Chemotherapy*, 21(4), 247-256.
- Slack, M. P. E. (2021). Long Term Impact of Conjugate Vaccines on *Haemophilus influenzae* Meningitis: Narrative Review. *Microorganisms*, 9(5), 886.
- Takala, A. K., Eskola, J., Leinonen, M., Käyhty, H., Nissinen, A., Pekkanen, E., & Mäkelä, P. H. (1991). Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. *The Journal of Infectious Diseases*, 164(5), 982-986.
- Tristram, S., Jacobs, M. R., & Appelbaum, P. C. (2007). Antimicrobial resistance in *Haemophilus influenzae*. *Clinical Microbiology Reviews*, 20(2), 368-389.
- Van Klingeren, B., van Embden, J. D., & Dessens-Kroon, M. (1977). Plasmid-mediated chloramphenicol resistance in *Haemophilus influenzae*. *Antimicrobial Agents and Chemotherapy*, 11(3), 383-387.
- Wang, H.-J., Wang, C.-Q., Hua, C.-Z., Yu, H., Zhang, T., Zhang, H., Wang, S.-F., Lin, A.-W., Cao, Q., Huang, W.-C., Deng, H.-L., Cao, S.-C., & Chen, X. (2019). Antibiotic Resistance Profiles of *Haemophilus influenzae* Isolates from Children in 2016: A Multicenter Study in China. *The Canadian Journal of Infectious Diseases & Medical Microbiology = Journal Canadien des Maladies Infectieuses et de la Microbiologie Médicale*, 2019, 6456321.
- Wen, S., Feng, D., Chen, D., Yang, L., & Xu, Z. (2020). Molecular epidemiology and evolution of *Haemophilus influenzae*. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 80, 104205.
- Wen, S., Mai, Y., Chen, X., Xiao, K., Lin, Y., Xu, Z., & Yang, L. (2023). Molecular Epidemiology and Antibiotic Resistance Analysis of Non-Typeable *Haemophilus influenzae* (NTHi) in Guangzhou: A Representative City of Southern China. *Antibiotics*, 12(4), Article 4.

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