ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 03 | March - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF HAEMOPHILUS INFLUENZAE ISOLATED FROM LOWER RESPIRATORY SAMPLES OF CANCER PATIENTS AT A TERTIARY CARE HOSPITAL.

Clinical Microbiology

Dr. Amrutha T S* Junior Resident, Tata Memorial Hospital, HBNI University, Mumbai. *Corresponding Author

Dr. Gaurav Salunke Associate Professor, Tata Memorial Hospital, HBNI University, Mumbai.

Mrs. Shamita Binod Senior Technician, Tata Memorial Hospital, HBNI University, Mumbai.

Dr. Sanjay Biswas Professor & Head of Department of Microbiology, Tata Memorial Hospital, HBNI University, Mumbai.

ABSTRACT

Background and Objective: *Haemophilus influenzae* are capsulated gram-negative coccobacilli that causes invasive respiratory tract infectios. Cancer patients being immunocompromised are more prone to a wide range of infections. CLSI approved treatment for *Hemophilus influenzae* includes Ampicillin, Ciprofloxacin, Ceftazidime, Cotrimoxazole, Imipenem and Meropenem. The aim of my study was to review the prevalence and antibiotic susceptibility pattern of *Haemophilus influenzae* and to emphasize the need for Hib vaccination in our cancer patients **Methods:** One-year retrospective analysis of lower respiratory samples of cancer patients was performed in the Microbiology department at Tata Memorial Hospital, Mumbai. *Haemophilus* influenzae identified on VITEK2 compact system and confirmed with factor V and X were included in the study. Antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion method. **Results:** The prevalence of *Hemophilus influenzae* isolated from lower respiratory samples was 7.5%, out of which 76.6% isolated from an age group of 18-65. No cases documented among paediatric age group. In our study most of the cases were isolated from Head and neck cancer and thoracic cancer patients that were 30% each, followed by Haematological cancer patients which was 21%. All the isolates of *Haemophilus influenzae* in the study were susceptible to Imipenem, Meropenem and Ceftazidime. 84.4% isolates were sensitive to Ciprofloxacin, 74.4% isolates were sensitive to Ampicillin and Cotrimoxazole sensitivity was seen 31.1% of all isolates. **Interpretation And Conclusions**. *Haemophilus influenzae* cause significant respiratory tract infections in adult cancer patients. All Isolates were sensitive to meropenem, imipenem and ceftazidime, but showed resistance to ampicillin, ciprofloxacin and cotrimoxazole. This states the importance of surveillance and highlights the need for implementing Hib vaccination protocol in our adult cancer patients.

KEYWORDS

Haemophilus influenzae, Cancer patients, Prevalence, Antibiotic susceptibility.

INTRODUCTION

Haemophilus influenzae is also known as Pfeiffer's bacillus was first discovered by Richard Pfeiffer in 1892(1). It posed a significant threat to the world health in the early 20th century causing invasive diseases especially in children(2). *Haemophilus influenzae* are pleomorphic, oxidase positive, fastidious, capsulated gram-negative coccobacilli which require factor X(hemin) and V (NAD) for their growth.

Although *Haemophilus influenzae* is a part of upper respiratory tract normal flora, it is known to cause many respiratory tract infections. Virulence factors like outer membrane proteins p2 and p5 allow bacteria to adhere to mucus, while lipo-oligosaccharide impair ciliary functions thus facilitating infections. Adhesins and pili facilitate bacterial attachment to the respiratory tract epithelial cells and endopeptidases, which cleaves and neutralize IgA, the primary antibody responsible for preventing infections. In addition, its ability to undergo phase variation and antigenic drift enable the organism to invade local tissue and persist inside mononuclear phagocytes and epithelial cells thus contribute to lung tissue invasion(3).

During the course their treatment, cancer patients experience immune system compromise due to chronic inflammation ,impaired hematopoietic lineage functions, and treatment there by damaging their immune defences and causing morbidity and mortality(4).

CLSI has recommended Ampicillin, Ciprofloxacin, Ceftazidime, Cotrimoxazole, Imipenem and Meropenem for the treatment of *Haemophilus influenzae*(5).In 1972 *Haemophilus influenzae* containing Tem1 βlactamases was identified and subsequently ROB β lactamases were discovered which was found to be the cause of 95% of ampicillin resistant isolates, highlighting their significant role in mediating resistance(6). Keeping this in mind Ampicillin resistant *H. influenzae* has been included in the medium priority list of antibiotic-resistant bacteria by WHO BPPL 2024(7). In 1985 pure polysaccharide vaccine was licensed for use which was less effective so replaced by Hib conjugate vaccine in 1987(1). Routine immunization of infants with multiple Hib vaccine is now advised which resulted in the decline of infections caused by *Haemophilus influenzae* among children.

This highlights the importance of managing and controlling diseases that can be controlled through vaccination. The aim of my study was to review the prevalence and antibiotic susceptibility pattern of *Haemophilus influenzae* and to emphasize the need for Hib vaccination in our cancer patients.

MATERIAL AND METHODS

Nonduplicate 90 isolates of *Haemophilus influenzae* were isolated from lower respiratory patient samples received in the Microbiology department at Tata Memorial Hospital, Mumbai. All colonies whose morphology was suggestive of *Haemophilus Influenzae* were initially subjected to gram stain, catalase and oxidase test. Subsequently satellitism test was performed on colonies which were labelled or identified as gram negative cocco-bacilli and catalase, oxidase positive. Further speciation into Hemophilus Influenza was done using factor V and X(8). The identification was also confirmed by VITEK2 compact (BioMérieux) (9). Antimicrobial susceptibility of Ampicillin, Ciprofloxacin, Ceftazidime, Cotrimoxazole, Imipenem and Meropenem was performed using Kirby – Bauer disk diffusion method & results were interpreted using CLSI M100 (34th edition)(5)

Inclusion Criteria

- Gram negative cocco-bacilli which were oxidase, catalase positive, shows satellitism, growth at factor X and V and identified as *Haemophilus influenzae* in Vitek 2 compact with confidence of 95% and above were included in the study.
- Samples containing only single isolates were selected for this study.
- Clinical data suggestive of potential pathogen .

Exclusion Criteria

- Gross mixture of multiple bacterial isolates were excluded .
- No clinical data suggestive of potential pathogen.

Multi-drug Resistance

We defined multi-drug resistance as isolates demonstrated resistance

to 3 or more tested antibiotics(10).

RESULTS

Demographic, clinical diagnosis and antibiotic susceptibility profile of clinical isolates .

Given In Table No. 1

Category	Subcategory	Haemophilus influenzae. N=90
Age	<18 years	0
	18-65 years	76.6% (69)
	>65 years	23.3% (21)
Gender	Male	70% (63)
	Female	30% (27)
Clinical diagnosis	Head and neck cancer	30% (27)
	Haematological cancer	21% (19)
	Thoracic cancer	30% (27)
	Gynaecological cancer	2.2% (2)
	Gastrological cancer	6.6% (6)
	Breast cancer	4.4% (4)
	Urological cancer	5.5% (5)
Antibiotic sensitivity	Ampicillin	74.4% (67)
	Ceftazidime	100% (90)
	Ciprofloxacin	84.4% (76)
	Imipenem	100% (90)
	meropenem	100% (90)
	cotrimoxazole	31.1% (28)
Multidrug resistance	4.4% (4)	

The study was conducted in the department of microbiology from June 2023 to May 2024. During this period a total of 90 isolates of Hemophilus influenza obtained from 1195 lower respiratory samples. The prevalence of *Haemophilus influenzae* in lower respiratory samples was found 7.5%. Majority of patients were infected in the age group of 18-65 years ie;76.6% (69/90), followed by age group above 65 years 23.3% (21/90). No cases have been documented in the paediatric age group. Predominantly males were infected with Hemophilus influenza accounting for 70% (63/90) of the cases. In our study most of the cases were isolated from Head and neck cancer and thoracic cancer patients that were 30% (27/90) each. Followed by Haematological cancer patients which was 21% (19/90).

All the 90 isolates of *Haemophilus influenzae in the study* were susceptible to Imipenem, Meropenem and Ceftazidime. 84.4% (76/90) isolates were sensitive to Ciprofloxacin, 74.4% (67/90) isolates were sensitive to Ampicillin and Cotrimoxazole sensitivity was seen 31.1% (28/90) of all isolates.

Multidrug Resistance

4.4% isolates of *Haemophilus influenzae* showed multidrug resistance. The resistant antibiotics were Ampicillin, Ciprofloxacin and Cotrimoxazole.

DISCUSSION

This study primarily focuses on determining the prevalence of *Haemophilus influenzae* from lower respiratory samples in immune-compromised cancer patients and evaluates the anti-microbial susceptibility pattern. It also aims to assess the necessity of vaccination in these cancer patients from our institute to alleviate the burden caused by *Haemophilus influenzae.*

Marks Dworkin and et al(11) in their study from January 1996 to December 2004 reported a rise in the incidence of diseases caused by *Haemophilus influenzae* from 0.4 to 1.0 especially in adults aged over 65 years. The study also highlighted a shift in epidemiological characteristics of the disease from being primarily observed in children to frequently affecting adults. In another study conducted by F Sanchez et al(12), it was observed that 6.5% all patients admitted with pneumonia was due to *Haemophilus influenzae* and predominantly seen in males (69%) with a mean age was 67±16.8. Findings of these studies align with our study, where the prevalence of Haemophilus influenzae was 7.5% with 76.6% isolated from an age group of 18-65 years followed by age group above 65 years 23.3% and no cases were reported from paediatric age group, also 70% of the cases observed in male patients when compared to females (30%). Since 2011, Hib immunization is a part of national immunization programme in India which explains the reason for *Haemophilus influenzae* infection free paediatric patients in our hospital.(13)

In the paper published by C.T Reiger(14) the study found that adult patients with solid tumour have high incidence of Hib infection compared to children, likely due to the protective effects of childhood immunization. It also recommended that the immune response to vaccine is diminished during chemotherapy. Therefore, patients vaccinated during chemotherapy should be revaccinated 3 months after completing treatment, while those vaccinated 14 days before starting of chemotherapy do not require revaccination unless specially indicated. In unvaccinated patients having lymphoma, multiple myeloma and myeloproliferative neoplasms Hib vaccine is advisable, with evidence suggesting that earlier administration during the disease course leads to better immune response. In patients with acute leukaemia, the use of Hib vaccine primarily used in children and in adult undergoing stem cell transplants.

In our study 30% of isolates were from head and neck and thoracic cancer followed by haematological adult cancer patients. This marks the importance of vaccination in adult cancer patients.

Determining the antibiotic susceptibility pattern is crucial to assess the effectiveness of ongoing antibiotic therapy. In this study, we evaluated the antibiotic susceptibility of 5 groups of drugs and highlighted the diminished effectiveness of clinically significant antibiotics.

Globally the ampicillin resistance varies from 28.1% to 34.4% and which was found to be linked to the presence of bla TEM-1 gene(15) (10). In the study conducted by Hamid Vaez(16) in Iran in 2019 ampicillin resistance was found to be 54.8%.

In comparison, ampicillin resistance in current study was found to be 25.6% .

In the study conducted by Hamid Vaez (16)in Iran in 2019 the resistance to ciprofloxacin was found to be 30.8%. Our study the resistance was found to be 15.6%.

The resistance for ampicillin and Ciprofloxacin was found to be relatively low in our current study could be attributed to the strict antibiotic stewardship and the judicious use of these antibiotics in our hospital.

From around the globe, the average resistance of cotrimoxazole has seen to be less than 30% (10) (15) with an exception of 53% reported by Hamid Vaez(16) from Iran in 2019. In our study cotrimoxazole resistance was found to be 68.9% which is significantly higher. Cotrimoxazole is routinely used in prophylactic and therapeutic doses in immune-compromised patients to prevent and treat opportunistic infections respectively. This probably explains the higher resistance in our patients. Revision of hospital prophylactic guidelines and judicious use of antibiotic is needed.

In our study all the isolates were susceptible to meropenem and Ceftazidime which is in line with the global studies(15). To ensure these drugs remains viable for future applications, it is essential to adopt strict antimicrobial stewardship for safeguarding these drugs from resistance.

The introduction of *Haemophilus influenzae* vaccine has led to dramatic decline in invasive *Haemophilus* infections among children, but the disease continues to present as more complicated challenges in adults (17). At our institute, the significant prevalence highlights the urgent need for revised Hib vaccination protocol, which though currently absent, could effectively reduce the burden of Haemophilus influenzae infections in our cancer patients

CONCLUSION

Haemophilus influenzae cause significant respiratory tract infections in cancer patients. All Isolates were sensitive to meropenem, imipenem and ceftazidime but showed resistance to ampicillin, ciprofloxacin and cotrimoxazole which are commonly used drugs for the treatment. This states the importance of surveillance to detect prevalence and antibiotic susceptibility pattern of *Haemophilus influenzae* and highlights the need for implementing Hib vaccination protocol in our adult cancer patients.

Acknowledgements

We would like to thank all the technical staff of the bacteriology section.

REFERENCES

1. CDC. Epidemiology and Prevention of Vaccine-Preventable Diseases. 2024 [cited 2025 Jan 19]. Chapter 8: Haemophilus influenzae. Available from: https://www.cdc.gov/pinkbook/hcp/table-of-contents/chapter-8-haemophilus-influenzae.html
2. Heinz E. The return of Pfeiffer's bacillus: Rising incidence of ampicillin resistance in Haemophilus influenzae. Microb Genomics. 2018;4(9):e000214.
3. King P. Haemophilus influenzae and the lung (Haemophilus and the lung). Clin Transl Med. 2012 Jun 14;1:10.
4. Kamboj M, Bohlke K, Baptiste DM, Dunleavy K, Fueger A, Jones L, et al. Vaccination of Adults With Cancer: ASCO Guideline. J Clin Oncol Off J Am Soc Clin Oncol. 2024 May 10;42(14):1699–721.
5. M100 Ed34 | Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition [Internet]. [cited 2025 Jan 19]. Available from: https://clsi.org/standards/products/microbiology/documents/m100/
6. H. Influenzae (01-10) [Internet]. [cited 2025 Jan 19]. Available from: http://www.antimicrobe.org/b67rev.asp
7. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance [Internet]. [cited 2025 Jan 19]. Available from: https://www.who.int/publications/i/item/9789240093461
8. Procop GW, Church D, Hall G, Janda WM, Koneman EW, Schreckenberger PC, et al. Koneman's color atlas and textbook of diagnostic microbiolgy. 7 ed. Philadelphia, Pa: Lippincott, Williams & Wilkins; 2017. 1586 p.
9. Valenza G, Ruoff C, Vogel U, Frosch M, Abele-Horn M. Microbiological Evaluation of the New VITEK 2 Neisseria-Haemophilus Identification Card. J Clin Microbiol. 2007 Nov;45(11):3493–7.
10. (PDF) Haemophilus influenzae and Haemophilus parainfluenza in Cystic Fibrosis: 15 Years Experience [Internet]. [cited 2025 Jan 19]. Available from: https://www.researchgate.net/publication/284196127_Haemophilus_influenzae_and_Haemophilus_parainfluenza_in_Cystic_Fibrosis_15_Years_Experience
11. Dworkin MS, Park L, Borchardt SM. The changing epidemiology of invasive Haemophilus influenzae disease, especially in persons > or = 65 years old. Clin Infect Dis Off Publ Infect Dis Soc Am. 2007 Mar 15;44(6):810–6.
12. Sánchez F, Mensa J, Martínez JA, Angrill J, Marcos MA, Marco F, et al. [Pneumonia caused by Haemophilus influenzae. Study in a series of 58 patients]. Rev Espanola Quimioter Publicacion Of Soc Espanola Quimioter. 1999 Dec;12(4):369–74.
13. Nandi A, Deolalikar AB, Bloom DE, Laxminarayan R. Haemophilus influenzae type b vaccination and anthropometric, cognitive, and schooling outcomes among Indian children. Ann N Y Acad Sci. 2019 Aug;1449(1):70–82.
14. Rieger CT, Liss B, Mellinghoff S, Buchheidt D, Cornely OA, Egerer G, et al. Anti-infective vaccination strategies in patients with hematologic malignancies or solid tumors-Guideline of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Medical Oncology (DGHO). Ann Oncol Off J Eur Soc Med Oncol. 2018 Jun 1;29(6):1354–65.
15. Potts CC, Rodriguez-Rivera LD, Retchless AC, Buono SA, Chen AT, Marjuki H, et al. Antimicrobial Susceptibility Survey of Invasive Haemophilus influenzae in the United States in 2016. Microbiol Spectr. 2022 May 10;10(3):e02579-21.
16. Vaez H, Sahebkar A, Pourfarzi F, Yousefi-Avarvand A, Khademi F. Prevalence of Antibiotic Resistance of Haemophilus Influenzae in Iran- A Meta-Analysis. Iran J Otorhinolaryngol. 2019 Nov;31(107):349–57.
17. Rubach MP, Bender JM, Mottice S, Hanson K, Weng HYC, Korgenski K, et al. Increasing incidence of invasive Haemophilus influenzae disease in adults, Utah, USA. Emerg Infect Dis. 2011 Sep;17(9):1645–50.