



Lower Respiratory Tract Infections by *Haemophilus influenzae*

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Received: 22-12-2025

Accepted: 11-01-2026

Available online: 23-01-2026

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Medical and Pharmaceutical Research

ABSTRACT

Background: *Haemophilus influenzae* is an important bacterial cause of lower respiratory tract infections (LRTIs), especially in elderly patients and those with underlying chronic lung disease.

Objectives: To determine the proportion of *H. influenzae* isolated from sputum samples of patients with LRTIs and to assess the antimicrobial susceptibility pattern of the isolates in a tertiary care hospital in Kerala.

Methods: A hospital-based cross-sectional study was conducted from October 2022 to May 2023. Sputum samples from suspected LRTI cases were evaluated using Bartlett's grading and processed by standard microbiological methods. Identification of *H. influenzae* was based on colony morphology, satellitism, biochemical reactions, and X and V factor requirements. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method and interpreted according to CLSI 2022 guidelines. Minimum inhibitory concentration testing for amoxicillin–clavulanic acid was performed using E-test strips.

Results: Of 2,173 sputum samples received, 903 were suitable for culture. *H. influenzae* was isolated from 39 samples (4.3%). Most isolates were from males aged 61–80 years, with acute exacerbation of chronic obstructive pulmonary disease being the predominant clinical presentation. High susceptibility was observed to ceftriaxone, chloramphenicol, tetracycline, piperacillin–tazobactam, meropenem, and amoxicillin–clavulanic acid.

Conclusion: *H. influenzae* accounted for a modest but clinically significant proportion of LRTIs, predominantly affecting elderly patients with COPD. The isolates demonstrated a largely favourable antimicrobial susceptibility profile, with low resistance rates compared to earlier regional reports. Continued surveillance and periodic antimicrobial susceptibility testing are essential to monitor emerging resistance trends and guide effective therapy

Keywords: *H. influenzae*, Lower respiratory tract infections, Antimicrobial susceptibility testing

INTRODUCTION

Haemophilus influenzae is a short pleomorphic Gram-negative bacillus and is responsible for many diseases like community acquired pneumonia, meningitis, sinusitis, epiglottitis, ophthalmic infections and otitis media [1]. It affects mostly young children and the elderly population with the highest burden of disease in low- and middle-income countries [2,3]. The organism is known to be carried in the nasopharynx of young children [4]. The encapsulated *H. influenzae* type b (Hib) strains are the major pathogens of invasive infections, while non-typeable *H. influenzae* (NTHi) accounts for majority of respiratory infections. NTHi has been found commonly in association with lung disease such as acute exacerbations of COPD [5,6]. *H. influenzae* pneumonia shows bimodal seasonal variation, with increased numbers of cases during March–May and September–December [7]. Its isolation from sputum samples could be indicative of its presence as normal flora, or it could be a pathogen, which can be differentiated by correlating with Gram's stain. Also, we have to look specifically for *H. influenzae* colonies near a streak of *Staphylococcus aureus* on blood agar plates, i.e satellitism, otherwise it could be missed. Usually, this organism is sensitive to routinely used antimicrobials, but recently resistance has been

emerging, requiring sensitivity testing to provide appropriate treatment and prevent complications such as septicaemia and meningitis. In many parts of the world, resistance among *H. influenzae* isolates to β -lactam antibiotics is most frequent followed by trimethoprim-sulfamethoxazole [2].

H. influenzae has increasingly been recognized as the second most common bacterial cause of community-acquired pneumonia (CAP), only exceeded in frequency by *Streptococcus pneumoniae*[8]. Whereas ample clinical data have been presented for patients with pneumococcal pneumonia, information on only severe and invasive cases of *H. influenzae* is available. Antibiotic *H. influenzae* resistance is the issue of concern, and it is essential to monitor the response to treatment and alter the antibiotic accordingly [9]. Considering the limited data on the burden of *H. influenzae* in developing countries [10], the present study was undertaken to determine the proportion and the antimicrobial susceptibility pattern of *H. influenzae* in patients with lower respiratory tract infections in our tertiary care hospital.

METHODOLOGY

This hospital-based cross-sectional study was conducted in the department of microbiology at a tertiary care centre in Kerala for a period of 8 months from October 2022 to May 2023 after obtaining approval from Institutional Ethics Committee No. 137/2023 dated 18/05/2023. The study was conducted on sputum samples which were sent to the microbiology laboratory for routine bacteriology culture & sensitivity. Sputum samples from OP and IP patients suffering from respiratory infections were included in the study. History and demographics were collected from requisition forms. Sputum samples were graded according to the quality by Bartlett scoring [11], and the ones which did not have a Bartlett score of greater than 0 were rejected. The samples whose gram stain was fulfilling the Bartlett's grading, were cultured on Blood agar with a streak of *Staphylococcus aureus*, Chocolate agar and MacConkey's agar [12]. The plates were incubated at 35-37°C in the presence of 5-10% CO₂ for 18-24 hours. Isolates showed pleomorphic gram-negative bacilli on Gram's stain [Fig no:1] and satellitism, i.e., growth around *Staphylococcus aureus* streak on Blood agar [Fig no:2], were indicative of *Haemophilus*. The *Haemophilus* isolates were also identified on chocolate agar with characteristic small (0.5-1mm) smooth, translucent, convex colonies with entire edge. In satellitism test, *S.aureus* was streaked across the surface of an inoculated blood agar plate, incubated at 35-37°C for 18-24 hours in presence of 5-10% CO₂ in candlejar. The colonies of *H.influenzae* nearer to the *S.aureus* appeared larger because of the release of V factor into the medium. These procedures were done according to standard techniques [12]. Colonies which were catalase and oxidase positive, gram negative cocci in culture smear, were further proceeded with growth requirement of both X (hemin) & V (Nicotinamide adenine dinucleotide) using HIMEDIA DD020/DD021/DD022 differentiation discs. Inoculate the test organism on Nutrient agar and aseptically place X(DD020),V(DD021) and X+V (DD022) factor discs and incubate the plates at 35-37°C after 24-48 hours. Isolates that grown around X +V factor discs were confirmed to be *H. influenzae* and Antibiotic sensitivity testing was done on Blood Muller Hinton Agar.

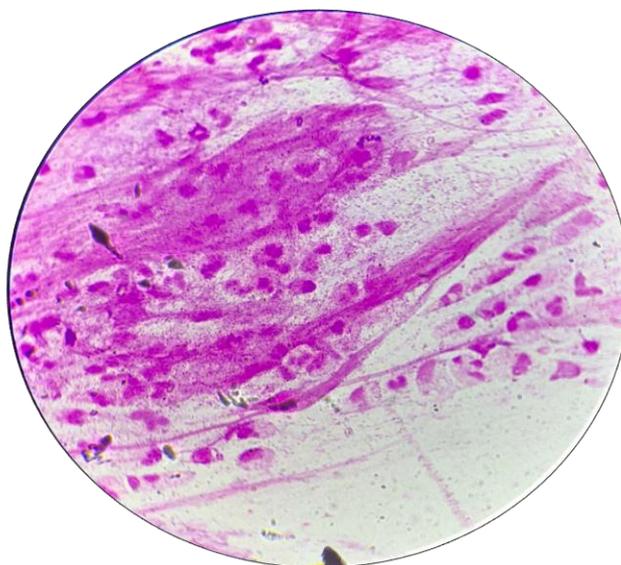


Fig no : 01 Pleomorphic Gram-negative bacilli with pus cells on Gram's stain of sputum



Fig no :02 Blood agar showing satellitism

Antibiotic susceptibility testing of the *H. influenzae* isolates to Ampicillin (10 µg), Ceftriaxone (30 µg), Cotrimoxazole(1.25/23.75µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Piperacillin-tazobactam (100/10 µg) and Meropenem (10 µg) was performed on Muller Hinton agar enriched with blood by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI M100) 2022 guidelines[13]. E test was done to determine the MIC of Amoxicillin clavulanic acid using Amoxyclav E strip EM003 0.016 – 256 mcg/ml Hi media E strips. *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), were used as controls.

RESULTS

A total of 2173 Sputum samples were received for culture from patients with suspected LRTI in the Microbiology laboratory during the study period. Out of the total, 903 sputum samples were deemed satisfactory for doing culture and sensitivity. Among 903 satisfactory sputum samples, 39 confirmed cases of *Haemophilus influenzae* were identified [Fig no:3], accounting for an isolation rate of 4.3%. *H. influenzae* respiratory tract infections were more observed in the age group of 61-80 years (74.3%) [Table No:1] in this study. Majority of the patients presented with acute exacerbations of chronic obstructive pulmonary disease (COPD) (23, 59%) [Fig no:4] followed by pneumonia (4, 10%) in our study. Lower respiratory tract infections due to *H. influenzae* was also observed as super-added infection in patients diagnosed with pulmonary tuberculosis (3, 8%) . We have observed that females (79%) were more commonly affected than males (21%) in this study.

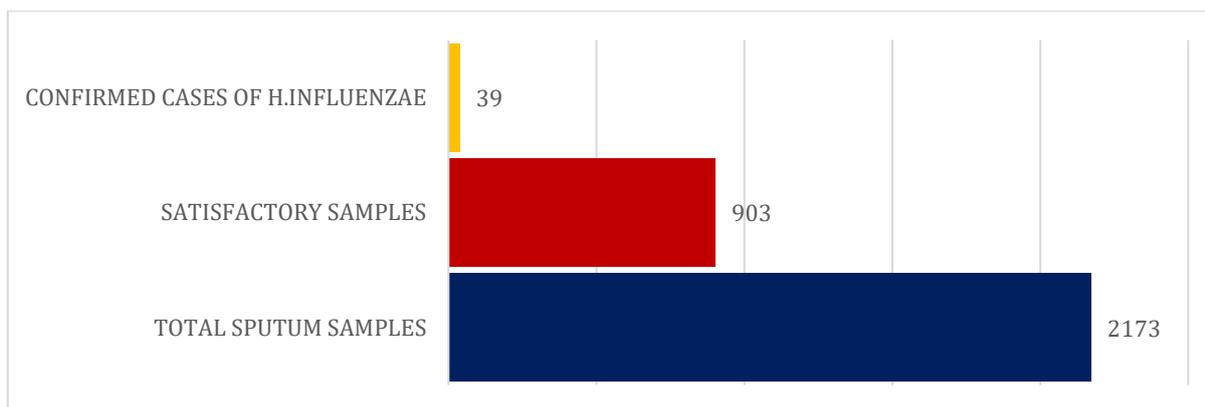


Fig no :03 Shows Number of confirmed cases of H..influenzae

Table no:1 Age wise distribution of patients from which *H.influenzae* were isolated

Age category	Number of patients from which <i>H. influenzae</i> isolated
0-10 years	0
11-20 years	2
21-30 years	2
31-40 years	0
41-50 years	4
51-60 years	5
61-70 years	13
71-80 years	13
81-90 years	0
91-100 years	0
Total	39

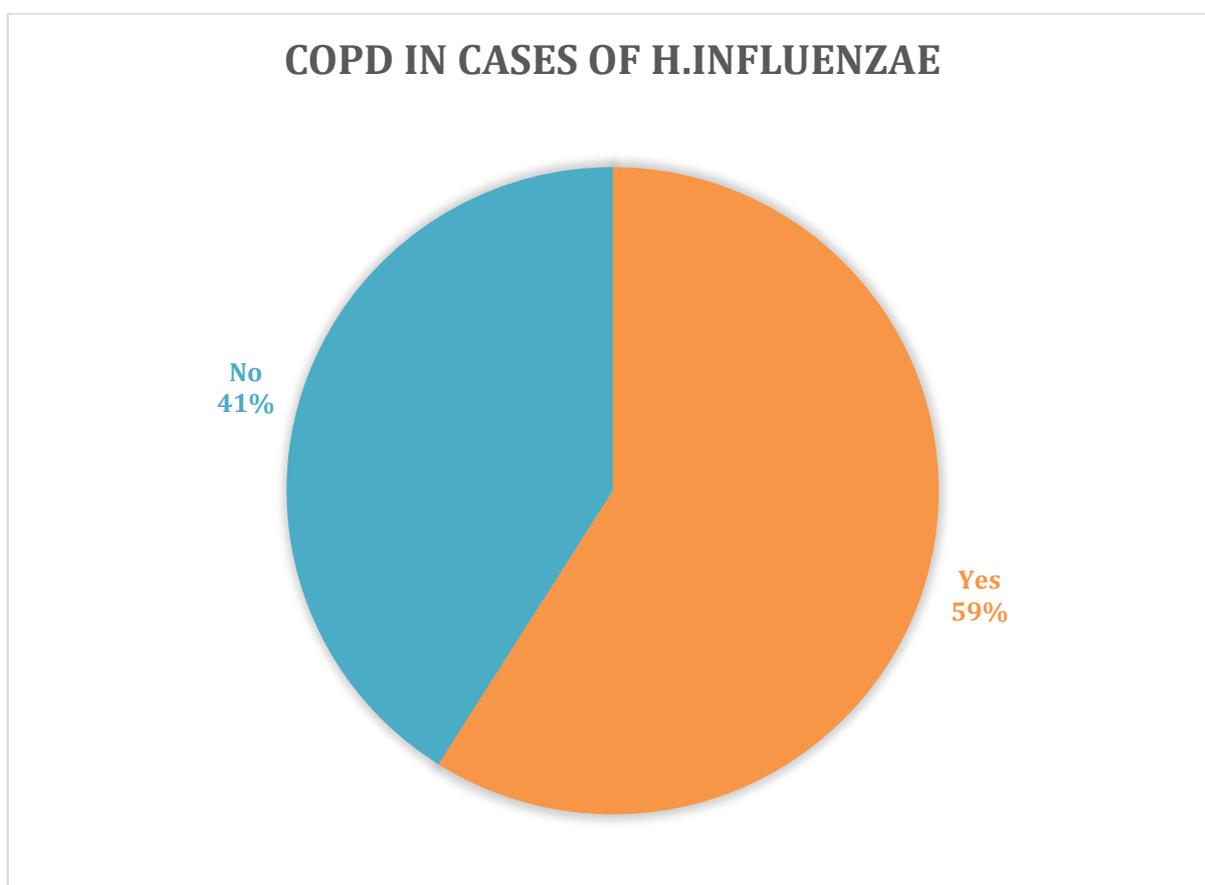


Fig no :04

GENDER WISE DISTRIBUTION OF HAEMOPHILUS CASES

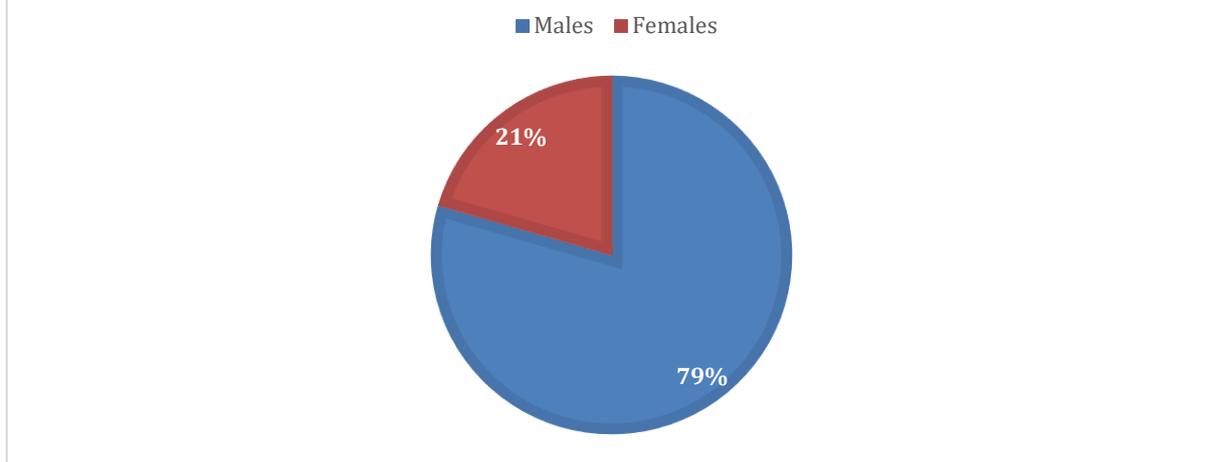


Fig No: 05 Gender wise distribution of patients from which *H.influenza* were isolated

Out of the 39 isolates, 34 (87%) from inpatient samples and 5 (13%) were from outpatient samples. The rate of *H.influenzae* infections in the current study was more in the months of January (38.5%) followed by December (20.5%) and March (15.4%) [Fig no: 06]. The isolates were most sensitive to Ceftriaxone (97.43%), Chloramphenicol (97.43%), Tetracycline (97.43%), Piperacillin & Tazobactam (100%), Meropenem (100%) [Fig no: 07]. Among the 39 isolates only 10 were tested for Amoxicillin-clavulanic acid (MIC) susceptibility. All the 10 isolates were susceptible to Amoxicillin-clavulanic acid. Majority of the Haemophilus isolates were found susceptible to the antibiotics tested. The resistance was observed to ampicillin was (5.13%), Azithromycin (7.7%), Ciprofloxacin & Cotrimoxazole (10.3% each) [Figno:-07].

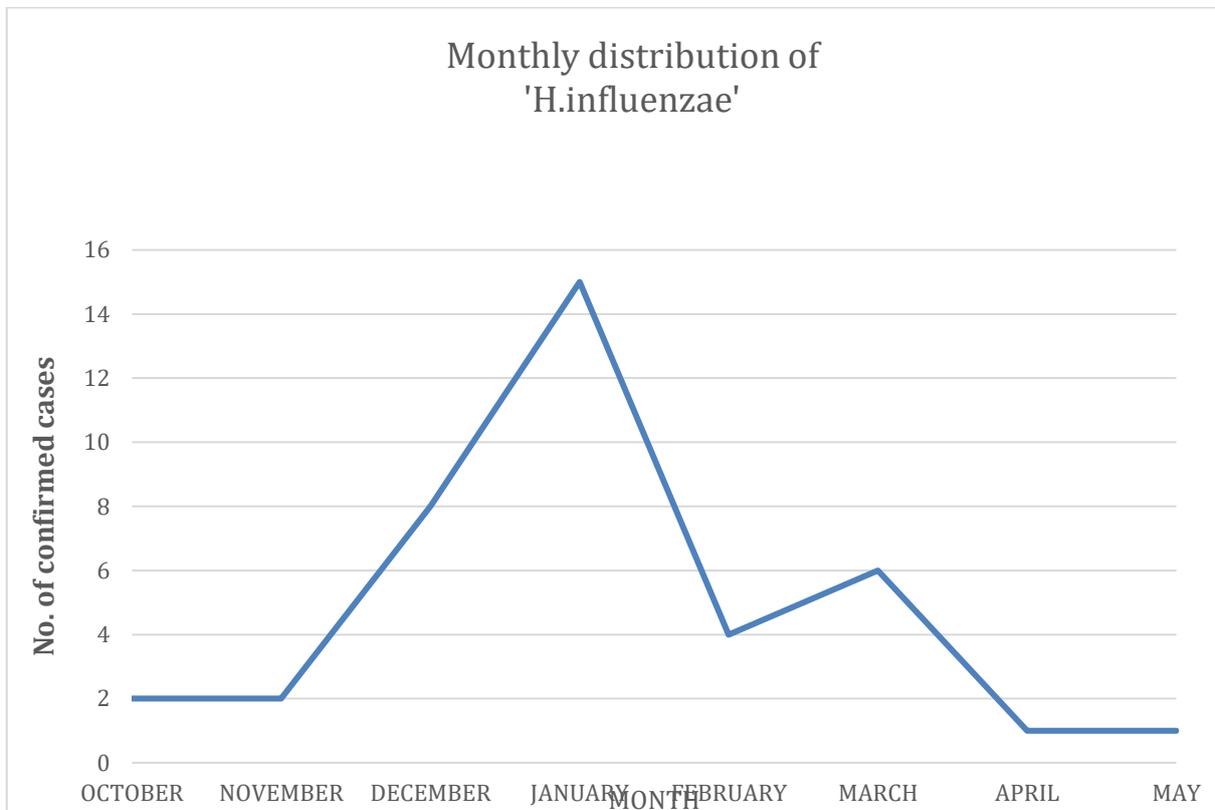


Fig no :06 Monthly distribution of *H.influenzae* cases

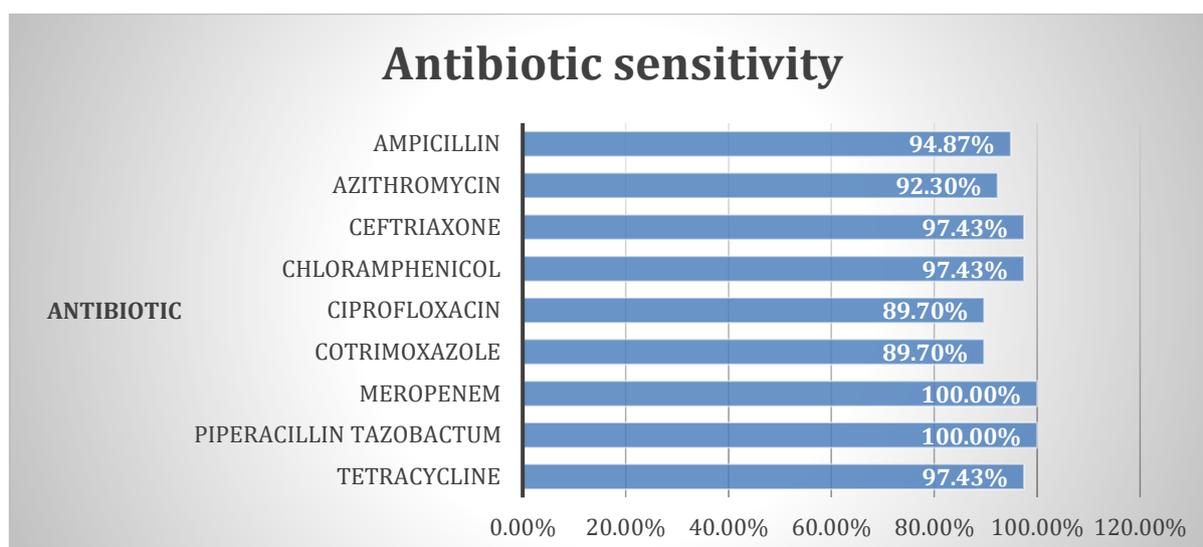


Fig no :07 Antibiotic susceptibility pattern of the isolates tested

DISCUSSION

H. influenzae though a component of normal pharyngeal flora, is well recognized cause of community acquired respiratory infection. A combination of bacterial pathogenic features and deficiency of host immune mechanism may permit the bacterium to establish infection in the lower respiratory tract results in clinical illness [14].

Out of 903 samples from patients with suspected LRTI, significant growth of *H. influenzae* was observed in 39 (4.3%) samples. The frequency of respiratory tract infection due to *H. influenzae* in our study was 4.3% which was comparatively lower than the reports of Rai R et al. (18.05%), Mishra SK et al. (21%), Ayyangari et al. (21.9%) and Khan et al. (27.6%) [15-18]. Some Indian studies by Shenoy et al. (6.14%) [19], Sai Keerthana et al. 6.6% [20] & Fuji et al. (5.9%) [21] showed comparable infection rates with the present study. *H. influenzae* was a recognized etiological agent in 6-10% of community acquired pneumonia [22]. The low prevalence in our setup could be mostly due to widespread immunization against Hib and also difference in geographical distribution of the organism and host immune conditions. According

to Slack MP “in an era when *H. influenzae* type b conjugate vaccine was widely used, the incidence of Hib as a cause of community acquired infection had dramatically reduced” [23].

In the present study, more number of cases were found in the age group of 61-80 years (74.3%), and males (79%) were commonly affected than females (21%). This could be due to occurrence of more number of COPD cases in our study group. In another study done in Kerala also showed >60 yrs as the commonest age group [24]. Whereas other Indian studies showed different opinions. Shenoy et al., found commonest age group affected is 41-60 yrs (41%) followed by >60 yrs (38%). In another Indian study 92% of *H. influenzae* were isolated from patients above 20 years of age and 7.7% from below 20 years [20]. Children less than 10 years (42.3%) were the commonly affected age group, followed by 41-50 yrs in another Indian study by Rai R et al. [15]. Khan et al found that 56% of the case were in the age group 1-10 yrs, 29.4% from 11-20 yrs, in a study conducted at Nepal in contradicting to our study [18]. An Indian study by B. K. Das, et al, in the year 2000–2001, on school going children, found a prevalence rate of 27.16% [25]. In our study none of the isolates were from the children less than 10 yrs, only 2 (5.1%) from 11-20 yrs. This could be due to the polysaccharide protein conjugate Hib vaccines have the capacity to reduce nasopharyngeal carriage of the *H. influenzae* in the target age group. The addition of Hib conjugate vaccines to infant immunization programmes had resulted in a dramatic decline in the Hib infection through direct and indirect (herd) protection.

Males (79%) were commonly affected than females (21%) which is mirroring the findings of Shenoy et al., (68.5%), Sai Keerthana et al, (61.2%), Luong DC et al., (59.8%) and Puig C et al., (69.6%) [19,20,26,27]. Major comorbidity associated was acute exacerbations of chronic obstructive pulmonary disease (COPD) (23.59%) followed by pneumonia (4, 10%) and pulmonary tuberculosis (3, 8%) in our study. Sai Keerthana et al in an Indian study found out that 33% of the inpatients were associated with COPD, 26.2% with Bronchial asthma and 18.44% were provisionally diagnosed as pyrexia of unknown origin [20]. COPD is an important risk factor for the development of *Haemophilus influenzae* pneumonia and their association was 25 to 30% percent in other studies by Jain et al., and Urwin et al., [28,29] similar to our study.

Similar to another study we also found that 87% of the isolates from inpatients [20]. The rate of *H. influenzae* infections in the current study was more in the months of January (38.5%) followed by December and March. The strains isolated were also more during winter months of October to January in another Indian study [20].

Antibiotic Susceptibility Profile

Ampicillin:

H. influenzae isolates in this study demonstrated 94.8% susceptibility to ampicillin, reflecting a favourable susceptibility profile. This finding corresponds closely with that of Shenoy et al, Rekha Rai et al., Sai Keerthana et al, and Nair G. et al [Table no:2]. The resistance to Ampicillin in our study (5.13%) is significantly lower than earlier Indian reports (9.7–81.2%) [Table no:2]

Table no:2- Ampicillin resistance in *Haemophilus influenzae* reported in previous studies.

Prior studies	Percentage of Resistance
Saikia KK et al.,[4]	81.25
Das BK et al.,[25]	56.8
Mojgani N et al.,[30]	43.6
Jain A et al.,[28]	22.9
Mohd-Zain Z et al.[2]	29.4
Rai R et al.[15]	19.23
Imad K et al.,[31]	19
Puig C et al.,[27]	10.5
Shenoy et al.,[19]	9.74
Nair G et al.,[24]	16.67
Sai Keerthana et al.,[20]	14.6
Present study	5.2

Amoxicillin – clavulanic acid

Susceptibility of Amoxicillin clavulanic acid for the tested isolates(10/10) was 100% which corresponds closely with the finding of Shenoy et al[19].

Azithromycin:

A high susceptibility rate of 92.3% was recorded for azithromycin, aligning well with previous reports from Sai Keerthana et al , Saikia KK et al confirming the continued potency of macrolides[20,4].In contrast, the IBIS multicentric study using erythromycin demonstrated lower efficacy [10].

Ceftriaxone:

Ceftriaxone maintained excellent activity, with 97.43% of isolates susceptible. This finding in our study corresponds closely with the reports of Padmaja Ananth Shenoy et al.(2016) (99.42%), Nair G. et al. (2020) (95.83%), as well as Rekha Rai et al. (2012) (92.3%)[19,24,15].

Conversely, Saikia KK et al. (2012) observed 36.25% resistance to cefotaxime[4]. The consistently high efficacy of ceftriaxone across multiple studies supports its role as a first-line therapy for invasive *H. influenzae* infections, with minimal evidence of emerging resistance.

Chloramphenicol:

A susceptibility rate of 97.43% was observed for chloramphenicol, mirroring the 100% and 85.2% susceptibilities reported by Nair G. et al[24].Older studies such as those by the IBIS group and Saikia KK et al. reported higher resistance (60% and 38.75%, respectively)[10,4]. The current high sensitivity may reflect reduced clinical usage of chloramphenicol, leading to lower selective pressure and preservation of its efficacy.

Ciprofloxacin:

Ciprofloxacin showed 89.7% susceptibility. Similarly Salman Khan et al. found 83.8% susceptibility[18] in another Indian study. Although Saikia KK et al. recorded slightly higher resistance[4].

Cotrimoxazole:

A notable 89.7% susceptibility was detected for cotrimoxazole. Nair G. et al. (2020) reported only 25% while the IBIS group (2002) and Salman Khan et al. (2015) observed high resistance (55% and 47.1%)[24,10,18]. Rekha Rai et al. (2012) found 73.08% susceptibility[15].

Meropenem:

All isolates were susceptible to meropenem (100%). No carbapenem resistance was noted in any comparative study, affirming the continued reliability of meropenem as a therapeutic agent for resistant or invasive *H. influenzae* infections.

Piperacillin–Tazobactam:

Complete susceptibility (100%) was observed for piperacillin–tazobactam. The consistent efficacy of β -lactam/ β -lactamase inhibitor combinations across studies highlights their importance in combating β -lactamase-mediated resistance.

Tetracycline:

Tetracycline exhibited 97.43% susceptibility, corresponding closely with the reports of Sai Keerthana and Appalaraju²⁰ (2017) (96–97%). Rekha Rai et al.¹⁵ (2012) reported 88.46% susceptibility, while Salman Khan et al.¹⁸ (2015) noted moderate resistance (41.2%).

The persistently high susceptibility rates across various studies suggest that tetracycline remains an effective agent, with limited resistance development in *H. influenzae* populations.

The antibiotic susceptibility profile from the current investigation demonstrates a generally favorable pattern, with high sensitivity to multiple therapeutic classes including Ampicillin, third-generation cephalosporins, β -lactam/ β -lactamase inhibitor combinations, macrolides etc. The overall resistance burden appears lower than that reported in earlier regional data, possibly reflecting improved antimicrobial stewardship practices and rational prescribing. However, ongoing surveillance remains essential to monitor emerging resistance trends.

LIMITATION

Capsular serotyping and molecular work was not done to financial constraints. While performing the antibiotic susceptibility testing, β -lactamase detection test was not done

CONCLUSION

The present study highlights *Haemophilus influenzae* as an important etiological agent of lower respiratory tract infections in a tertiary care setting in Kerala, predominantly affecting elderly patients and those with underlying chronic lung diseases such as chronic obstructive pulmonary disease. The overall isolation rate was relatively low, possibly reflecting the impact of widespread Hib vaccination and regional epidemiological differences.

Despite the favorable susceptibility pattern observed, the emergence of resistance to certain antibiotics underscores the need for continued microbiological surveillance, routine susceptibility testing, and judicious antibiotic prescribing. Regular monitoring of resistance trends, along with timely modification of treatment protocols, is essential to ensure optimal patient outcomes and to prevent the development and spread of antimicrobial resistance among *H. influenzae* isolates.

Funding: NIL

Conflict of Interest: NIL

Acknowledgement: We would like to extend our gratitude to Dr Joana Mary Magdaline, Professor & HOD, Microbiology, Govt medical college Ernakulam for the support and guidance.

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