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Original Article

Microbiology Section

Haemophilus Influenzae, an Underdiagnosed Cause of Respiratory Tract Infections

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ABSTRACT

Background: *Haemophilus influenzae* is an obligate human parasite that is transmitted from person to person by the respiratory route. This organism causes several diseases which range from chronic respiratory to serious invasive infections. *Haemophilus influenzae* is prevalent worldwide, and the morbidity and mortality of the associated disease increases with the complications and therefore a laboratory diagnosis is a vital necessity.

Aims and Objectives: The intention of the present study was to determine the prevalence of *Haemophilus influenzae* in patients who were suffering from respiratory tract infections and to study its antibiotic sensitivity pattern.

Methods: Samples were collected from144 patients with upper and lower respiratory tract infections, who were segregated by their age groups. All the samples were analyzed in a specific manner, which included gram staining, culture and biochemical reactions for the identification of the organism. Antibiotic sensitivity testing was performed for the isolated organism. Based on the findings, the results were analyzed.

Results: The results showed a prevalence of 18.05%, with the maximum numbers of isolates in patients of the age group of 0-10 years and the least number of isolates in patients of the age group of 21-30 years. The antibiotic sensitivity pattern showed a high susceptibility to the commonly used antibiotics.

Interpretation and Conclusion: An overall finding showed that the prevalence of *Haemophilus influenzae* in patients with respiratory tract infections was 18.05%, with a higher prevalence in the paediatric age group. Specific isolation of the organism and sensitivity testing are essential to enable the proper administration of antibiotics for early treatment and prevention.

Key Words: *Haemophilus influenzae*, X and V factor, Levinthal agar, Haemophilus spp.culture, Satellitism

INTRODUCTION

Members of the genus, Haemophilus, form part of the indigenous flora of the mucous membrane of the human upper respiratory tract and the mouth. However, some species such as *Haemophilus influenzae* causes diseases which range from chronic respiratory infections to serious invasive infections. *Haemophilus influenzae* type b (Hib) was the single most important cause of meningitis in children in the US before the availability of vaccines [1]. *Haemophilus influenzae* is an important cause of acute, recurrent and persistent infections of the human respiratory tract. This organism is found in 8% of the pneumonias. More than 95% of the invasive infections are associated with type b encapsulated H. influenzae. The non-encapsulated strains also play an important role in chronic bronchitis, and they are the most common cause of pneumonia in adults. These strains are also an important cause of lower respiratory tract infections in patients with cystic fibrosis [2].

Chronic obstructive pulmonary disease is frequently associated with exacerbations, owing to bacterial infection. Antibiotic therapy is effective in accelerating a recovery from the exacerbations and in preventing an early clinical deterioration. As a result, the antimicrobial agents which are used to treat exacerbations should be active against the strains of H. influenzae that are present in the lower airways [3]. Children are frequent carriers of *Haemophilus influenzae* in the nasopharynx, and the rate of the carriage is high in infancy. Its colonization may subsequently lead to the development of infectious diseases which are caused by *Haemophilus influenzae*. It has also recently been reported that β-lactamase negative ampicillin-resistant (BLNAR) strains have increased in some countries, although the global prevalence still remains low [4].

From the diagnostic perspective, the reason for the lack of data on the burden of *Haemophilus influenzae* in developing countries was mainly the failure of the detection of this fastidious organism in the laboratory. The specimens which are collected are sputum, throat swab, ear and conjunctival scrapings, CSF, blood and other body fluids. Most of the laboratories speciate the genus, Haemophilus on the basis of haemolysis and the X and V factor requirements. Biochemical tests are useful for species identification and for the differentiation of the biotypes. The additional useful methods are the commercial identification system, immunoserologic and serological identification, identification by molecular methods and the epidemiologic typing systems [5]. This study was undertaken to determine the prevalence of *Haemophilus influenzae* in patients who were suffering from respiratory tract infections and to study its antibiotic sensitivity pattern.

MATERIALS AND METHODS

This study was carried out in the K.S. Hegde Medical Academy, Deralakatte, on 144 cases of respiratory tract infections. The patients were from different age groups.

Specimen collection:

- Sputum was collected into a sterile container with proper aseptic precautions.
- Throat swabs were collected aseptically. Sterile Dacron or Alginate cotton swabs were used.

- The samples were sent to the laboratory immediately for processing.

Isolation of bacilli and its identification:

- The samples which were received in the Microbiology Laboratory were processed immediately. The sputum sample was homogenized by shaking it with sterile water and glass beads for 15-30 minutes for better results [6].
- Gram staining showed pale, gram negative, small coccobacilli.

Culture: The homogenized sputum sample or the throat swabs were streaked onto blood agar, chocolate agar, Levinthal's agar (selective media), chocolate agar containing 5.0 µg of Vancomycin per ml (CHOC-V), chocolate agar containing 5.0 µg of Vancomycin per ml, 300 µg of Bacitracin per ml, and 1.0 µg of Clindamycin per ml (CHOC-VBC) and Muller-Hinton agar with the X and V factor discs. The inoculated plates were kept in a candle jar and were incubated at 37^{0}C for 24-48 hrs [7].

The plate of Levinthal's agar, CHOC-V and CHOC-VBC showed small (0.5-1.0 mm), β haemolytic, smooth, translucent, greyish, convex colonies with an entire edge and a mousy odour.

Confirmatory methods: The isolated colonies were subcultured on Muller-Hinton agar with the X and V factor discs and they showed growth only around the discs.

Satellitism: Staphylococcus aureus was streaked across a plate of blood agar on which Haemophilus influenzae was inoculated and kept for incubation. After overnight incubation, the colonies of Haemophilus influenzae were found to be large and well developed alongside the streak of Staphylococcus aureus and smaller farther away [5].

Biochemical reaction: The preliminary biochemical tests showed positive Oxidase and Catalase tests. Nitrates were reduced, phosphatase was positive and glucose was fermented.

Antibiotic sensitivity testing: The sensitivity testing of *Haemophilus influenzae* was performed by using the following antibiotics, Ampicillin (10mcg), Amoxiclav (30mcg), Doxycycline (30mcg), Erythromycin (15mcg), Co-trimoxazole (25mcg), Chloramphenicol (30mcg) and Ceftriaxone (30mcg) on chocolate agar with a haeme supplement by using the Kirby Bauer disc diffusion method [8].

RESULTS

144 samples from clinically suspected cases of lower respiratory tract infections were analyzed over a period of 1 year, of which 26 (18.05%) samples showed the growth of *Haemophilus influenzae,* with an increased prevalence in the age group of 0-10 yrs (42.3%) and the least prevalence in the age group of 21-30 years (11.53%), as summarized in [Table/Fig-1]. *Haemophilus influenzae* showed high susceptibility to most of the routinely used antibiotics, with Ceftriaxone (92.3%) being the most effective one and Cotrimoxazole (69.23%) being the least, as tabulated in [Table/Fig-2].

DISCUSSION

Haemophilus influenzae is a strict parasite of humans, which is found principally in the upper respiratory tract. Although the type of infectious diseases which are caused by *Haemophilus influenzae* has changed considerably in recent years because of the widespread and routine immunization of children against the type B organisms, *Haemophilus influenzae* remains to be a significant pathogen. Humans are the only natural hosts for *Haemophilus influenzae*. Therefore, the maintenance of this organism in the human population depends on a person-to-person transmission, which efficiently occurs via respiratory droplet spreads. Both the nontypeable strains and Hib spread easily via person to person transmission [6]. In our study, the prevalence of *Haemophilus influenzae* and its antibiotic sensitivity pattern have been described. 144 samples from clinically suspected cases of lower respiratory tract infections were analyzed over a period of 1 year, of which 26 (18.05%) samples showed the growth of *Haemophilus influenzae,* with an increased prevalence in the age group of 0-10 yrs (42.3%) and the least prevalence in the age group of 21-30 years (11.53%). Among the 26 isolated samples, 18 (69.23%) gave identifiable growth on chocolate agar, and all the 26 isolates gave visible growth on special media (CHOC-V,CHOC-VBC). In a study which was done by Fang et al, it was observed that the isolation rate of *Haemophilus influenzae* could be improved by using selective media [9]. A similar study which was conducted in another part of India by B. K. Das, et al, in the year 2000–2001, on school going children, found a prevalence rate of 27.16% [10]. A related study which was carried out by Ayyagari et.al.showed a prevalence of 21.9% in patients with respiratory tract infections [11]. The present study revealed that the highest prevalence was in the age group of 0-10 years (42.3%) and that the least prevalence was in the age group of 21-30 years (7.69%).

Klebsiella pneumoniae and *Streptococcus pneumoniae* were the most common pathogens, while *Haemophilus influenzae* was the third commonest pathogen which was responsible for lower respiratory tract infections in our study. This finding is almost similar to that of a study which was conducted by Karen C, Carroll, where *Hameopholus influenzae* was commonly isolated after Streptococcus pneumoniae [12].

The present study showed a high sensitivity of *Hameopholus influenzae* to the commonly used antibiotics, with a high sensitivity to ceftriaxone (92.30%) and the least to ampicillin, chloramphenicol (73.07%) and cotrimoxazole (69.23%). Similar results were observed in various other studies which were done by Nag V L et al [13],

Age in Years	Total Test Samples				Culture Positive for H. Influenzae			
	Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%
0–10	19	13	16	11	5	19	6	23
11–20	11	8	7	5	2	8	1	4
21–30	16	11	8	6	1	4	1	4
31–40	23	16	12	8	2	8	2	8
41–50	19	13	13	9	3	12	3	12
Total	88	61	56	39	13	50	13	50

[Table/Fig-1]: Distribution of test samples and culture positive samples based on age and gender

Antibiotic	S	%	I	%	R	%
Ampicillin	19	73.07	2	7.69	5	19.23
Amoxiclav	22	84.61	1	3.84	3	11.53
Ceftriaxone	24	92.30	2	7.69	0	0
Chloramphenicol	19	73.07	3	11.53	4	15.38
Cotrimoxazole	18	69.23	1	3.84	7	26.92
Doxycycline	23	88.46	2	7.69	1	3.84
Erythromycin	23	88.46	1	3.84	2	7.69

[Table/Fig-2]: Antibiotic susceptibility pattern of H.influenzae isolates
S = Sensitive, I = Intermediate, R = Resistant

Marina Cerquetti et al [14], Mariana Castanheira et al [15] and Michael R Jacobs et al [16].

CONCLUSION

The study showed a high prevalence of *Haemophilus influenzae* in paediatric patients (0–10 years) with respiratory tract infections. Prompt diagnosis and proper antibiotic treatment is essential to prevent complications.

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