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Bacteriology of Acute Respiratory Infections in Children

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Bacteriological investigations were carried out on 151 children (80 males and 71 females) suffering from acute respiratory infections (ARI) to And out bacteria associated with ARI. Fifty one children presenting with upper respiratory infections (URI) and 100 with lower respiratory infections (LRI) seen at the outpatient department of the Institute of Child Health and Hospital for Children, Madras, were included in this study. In all, 56% of the children yielded any one or a mixture of bacteria that could be potential or probable pathogens of ARI. Nonfermenting gram negative bacilli (NFGNB) were the predominant organisms isolated (27%) followed by non-typable ampicillin resistant *Haemophilus influenzae* (13%) and β. haemolytic streptococci groups C and G (11%). The other bacteria isolated in this study were *Klebsiella pneumoniae* (7%), *Streptococcus pneumoniae* (3%), Neisseria sps. pure (3%) and *Staphylococcus aureus* (1%). The isolation rate of NFGNB was maximum (47%) when the duration of illness exceeded 7 days. Mixed infections of potential or probable pathogens were observed in 11 patients which included NFGNB + *K. pneumoniae* (2); *H. influenzae* + NFGNB (2); β– haemolytic streptococci + *H. influenzae* (2); β– haemolytic streptococci + *K. pneumoniae (1) ; S. aureus* + *K. pneumoniae* (1) ; Neisseria sp. + *K. pneumoniae* (2) and NFGNB + β– haemolytic streptococci + *H. influenzae* (1).

Acute respiratory infections (ARI) in children constitute a major health problem causing considerable mortality in developing countries and morbidity in developed countries. It has been estimated that in most developing countries children under 5 years of age represent about 15% of the total population but over 50% of all deaths occur in this age group. In these countries ARI along with diarrhoea are identified as the major killer diseases and the ARI related mortality is accounted to be about 50 times greater than that in developed countries[1]. In rural India, the mortality rate due to ARI in children under one year was reported to be 2707 per 100,000[2] as compared to 141 per 100,000 in the United Kingdom[3].

The aetiology of ARI is very complex since it could be caused by a variety of agents such as viruses, bacteria, mycoplasmas and chlamydiae. It is difficult to

associate any particular agent as responsible for an episode of ARI since one agent may predispose the subject to infection with another and different agents may cause similar clinical symptoms. Diagnosis is further complicated because some potential pathogens may reside in the respiratory tract of normal healthy individuals.

World Health Organization has identified ARI as a priority area for research and has suggested the following studies : (I) the correlation between clinical syndromes and aetiological agents, (2) the relative frequency of occurrence of viruses and bacteria as aetiological agents, and (3) the monitoring of the drug sensitivity pattern of the bacterial pathogens isolated.

A pilot bacteriological study was conducted in children suffering from ARI to find out the causative organisms. The results are reported.

Materials and Methods

Subjects : From October 1985 to March 1986, 151 children below 6 years of age presenting with signs and symptoms of ARI and attending the outpatient department of the Institute of Child Health, Madras, were included in the study. All the children were free from any other acute bacterial illnesses like diarrhoea or meningitis. Except for 11 children none gave a history of prior treatment.

Specimens : Throat swabs, nasal swabs, nasal secretions and laryngeal swabs were collected, coded and transported on wet ice to the laboratory within 3 hours of collection.

Culture and Identification : Isolation and biochemical characterization were done according to standard methods[4-7]. The β. haemolytic streptococci were serogrouped by the staphylococcal slide coagglutination method[8]. Serotyping of *Haemophilus influenzae* was done by slide agglutination using type specific capsular antisera.

Antibiogram : The antibiotic susceptibility testing of the isolates was done on Mueller-Hinton agar by the disc-diffusion method[9].

Results

Of the children included in the study, there were 80 males and 71 females. 59% of the children were below 2 years of age.

Of the 151 children studied, 84 (56%) yielded any one or a mixture of bacteria which may be considered potential or probable pathogens of ARI, very often along with other commensal flora (Table 1). The remaining 67 children (44%) yielded only a mixture of normal flora which included Neisseria species (Heavy), diphtheroids (scanty), and β– haemolytic streptococci with none predominating over the others.

Table 1 : Distribution of children by organisms isolated, clinical diagnosis and duration of illness.

	Total no. of children	No, in whom organisms isolated	No. of organisms isolated [a]						
			Non Fermenting gm neg bacilli	β– haemolytic streptococci	*K. pneumoniae*	*Haemophilus influenzae*	Pneumococci	*Staphylococcus aureus*	Neisseria sp. (pure growth)
(a) By clinical diagnosis									
URI (unspecified)	51	29	15 (29) [b]	6 (12)	2 (4)	10 (20)	0	1	0
LRI (unspecified)	77	42	19 (25)	9 (12)	7 (9)	8 (10)	2	0	4 (5)
LRI (specified)*	23	13	6 (26) (5)[c]	2	1	2	2	0	0
(b) By duration (days) of history of illness									
1 – 3	55	32	13 (24) (2)	9 (16)	2 (4)	7 (13)	I	0	2
4 – 7	66	34	13 (20)	5 (8)	7 (11)	10 (15)	3 (5)	1	2
> 7	30	18	14 (47) (3)	3 (IO)	1	3 (10)	0	0	0
Total	151	84	40 (26) (5)	17 (11)	10 (7)	20 (13)	4 (3)	1	4 (3)

* Asthmatic bronchitis, Bronchopneumonia, Bronchitis, Acute bronchiolitis, Primary complex, PUD and pleural effusion.

a Children having more than one type of organisms are added under each type.

b Figures in parentheses indicate percentages to the total no. of children.

c Figures in parentheses given below the numbers indicate pure growth.

Of the 96 isolations obtained from the 84 children yielding probable pathogens of ARI, 40 (42%) were non-fermenting gram negative bacilli (NFGNB), 20 (21%) *Haemophilus influenzae,* 17 (18%) β– haemolytic streptococci, 10 ((10%) *Klebsiella pneumoniae,* 4 (4%) *Streptococcus pneumoniae,* 4 (4%) Neisseria and 1 (1%) coagulase positive *Staphylococcus aureus.*

The rates of isolation of NFGNB, the most frequently isolated bacteria in this study, were similar in upper respiratory tract infection (URI) and lower respiratory tract infection (LRI), being 29% and 25% respectively. Five patients yielded NFGNB in pure culture all of whom had presented with LRI. Like NFGNB, the rates of isolation of β– haemolytic streptococci were similar in URI and LRI (12% and 11% respectively). *Klebsiella pneumoniae* were more often isolated from LRI (8%) than from URl cases (4%) whereas *Haemophilus influenzae* were more often isolated from URI (20%) than from LRI cases (10%). However, in both the cases the difference was not statistically significant. All the 4 isolations of pneumococci and that of Neisseria in pure culture, were from patients who presented with LRI.

Mixed growth of potential or probable pathogens of ARI were observed in 11 patients. These were NFGNB + *K. pneumoniae (2),* NFGNB + *H. infiuenzae* (2), β– haemolytic streptococci + *H. influenzae* (2), β– haemolytic streptococci + *K. pneumoniae* (1) and *S. aureus* + *K. pneumoniae* (1), Neisseria sp. + *K. pneumoniae* (2), and NFGNB + β– Haemolytic streptococci + *Haemophilus influenzae* (1).

The isolation of bacteria in relation to the duration of history of illness on the examination day is also presented in Table 1. it can be seen that the isolation of *NFGNB* was maximum when the duration of illness exceeded 7 days as compared to that when the duration of illness was 1-7 days. The age adjusted rates were 44% and 22% respectively (P=0.03). The isolation rate of β. haemolytic streptcocci was higher when the duration of illness was 1-3 days, the rate being 16% as compared to 8% when the duration was more than 3 days (Statistically not significant).

A study of the isolation of bacteria according to age and sex of the children showed that the NFGNB were more often isolated from children aged 2 years and above (32%) as compared to those aged below 2 years (22%) and were more predominant among males (30%) than among females (23%). However, these differences were not statistically significant. The β. haemolytic streptococci were isolated more frequently from females than from males, the rate being 17% and 6% respectively (statistically not significant).

All the NFGNB isolates were completely characterised based on growth characters and biochemical reactions and were identified as pseudomonas sps. other than *Ps. aeruginosa.* Majority of the NFGNB isolates (92%) belonged to *Pseudomonas pseudoalkaligenes alkaligenes* group. Four strains were identified as *Pseudomonas pseudomallie* group and one strain as *Ps. stutzeri.* The β– haemolytic

streptococci belonged to groups C and G. None of the *Haemophilus influenzae* isolates were typable with any of the type specific capsular antisera, a, b, c, d, e and f.

Antibiogram : The antibiotic susceptibility pattern are shown in Table 2. Almost all the strains of β– haemolytic streptococci were sensitive to most of the

Table 2 : Antibiotic susceptibility pattern of the bacteria isolated in the study

Drug sensitivity status	Antibiotics PEN	STR	ERY	CHL	TET	CEP	KAN	AMP	GEN	COL	COT
B haemolytic streptococci (n=12)											
Sensitive	12	0	4	12	12	10	5	1	10	10	12
Mod. Sensitive	0	0	6	0	0	1	6	11	0	0	0
Resistant	0	12	2	0	0	1	1	0	2	2	0
Haemophilus influenzae (n=18)											
Sensitive	1	0	0	18	4	0	0	3	8	2	17
Mod. Sensitive	13	3	9	0	13	1	3	0	0	13	0
Resistant	4	15	9	0	1	17	15	15	10	3	1
Klebsiella pneumoniae (n = 7)											
Sensitive	0	1	0	2	2	0	5	0	6	6	2
Mod. Sensitive	1	0	0	0	0	0	0	1	0	1	0
Resistant	6	6	7	5	5	7	2	6	1	0	5
NFGNB (n=60)											
Sensitive	0	0	0	36	36	0	46	2	16	38	54
Mod. Sensitive	0	4	6	2	6	0	10	2	0	21	0
Resistant	60	56	54	22	18	60	4	56	44	1	6

a PEN : Penicillin TET : Tetracycline AMP : Ampicillin
STR : Streptomycin CEP : Cephaloridine GEN : Gentamicin
ERY : Erythromycin KAN : Kanamycin COL : Colistin
CHL : Chloramphenicol COT : Cotrimaxazole

b Some children had NFGNB isolated from more than one specimen collected at the same time.

drugs tested. In contrast, more than 50% of the *Haemophilus influenzae were* multidrug resistant and 15 (83%) of the 18 strains were resistant to ampicillin. However, these strains were highly susceptible to chloramphenicol (all the 18) and cotrimoxazole (17 of 18).

Majority of the NFGNB and *K. pneumoniae* isolates were multidrug resistant. Kanamycin, gentamicin and colistin were the most effective drugs for *K. pneumoniae* isolates. As regards NFGNB, cotrimoxazole followed by kanamycin were the most effective of the drugs tested.

There were 11 patients who gave a history of prior antibiotic treatment. Two of them yielded NFGNB, and 2, *H. influenzae.* The remaining 7 had a mixture of normal flora.

Discussion

The spectrum of bacteria obtained in our study is quite different from the conventional one where the bacteria most commonly incriminated. in ARI are *Pneumococcus, H. influenzae* type b, group A β– haemolytic streptococci and *Staphylococcus aureus* [10]. The isolation of NFGNB from such a sizable number of ARI patients has not been documented by anybody so far to our knowledge.

The mere isolation of the bacteria, even of the potential pathogens like *Haemophilus influenzae* and Pneumococcus may not always bear any aetiological significance considering their colonisation at times even in healthy individuals. The invasive nature of these bacteria may probably be demonstrated by the presence of their antigen in body fluids like urine and pleural effusion. The antigen of *Haemophilus influenzae* and pneumococci have already been demonstrated in the urine and pleural fluid of patients presenting with ARI [11,12].

The NFGNB in general are commensals of free-living saprophytes. But their increasing frequency of isolation from a variety of clinical materials[13] in various clinical conditions, necessitates a re-evaluation of their role in the human disease process. The NFGNB have been shown to have the potential to act as secondary or opportunistic pathogens in man[14].

The *Haemophilus influenzae* isolates have failed to react with any of the 6 capsular antisera tested and hence were grouped as 'non-typable'. However, the lack of encapsulation need not necessarily indicate non-pathogenicity. Recently, Wallace et al[15] examined a large number of *Haemophilus influenzae* strains isolated from CSF, blood and genital secretions and found that almost 90% were non-typable but were capable of producing invasive disease process.

All the β– haemolytic streptococci isolated were grouped as C and G. This is again a deviation from the conventional finding in that β– haemolytic streptococci

of AR1 belong to group A[10]. Groups C and G are normally seen as commensals of the respiratory tract of healthy individuals. The pathogenic potential of these isolates need to be studied further.

In general, the exact role of bacteria in the aetiopathogenesis of ARI is far from clear. It has been postulated that viruses at times initiate mild respiratory infections and predispose the subject to secondary bacterial colonisation and complication. Also endogenous bacterial infection in this situation might acquire potential to invade tissues deeper and play a role in enhancing the disease process[16]. Among the other possibilities, the viruses may also cause transient immunosuppression[17], increased adherence of bacteria to infected cells[18], or destruction of the integrity of the respiratory epithelial lining[19]. It is also quite likely that interaction of host factors, environmental conditions, nutritional status, and immunological competence etc. have a combined role in the complex aetiopathogenesis of ARI.

In this study, we have classified the ARI broadly into LRI and URI based on the signs and symptoms at the time of admission. It is true that URI have a thin barrier and often appear as associated phenomenon in the same child. It is also known that what starts as an URI is likely to progress to LRI within 48 hours.

The highlights of the antibiogram studies were the presence of ampicillin resistant strains of *Haemophilus influenzae* and multidrug resistant NFGNB and *K. pneumoniae.* The indiscriminate use of antibiotics in respiratory infections in general might have contributed for this observation. The emergence of such multidrug resistant bacteria will have serious consequences if they are capable of producing a complicated course of disease process.

More reliable procedures like detection of antigens in body fluids, along with isolation and antibiogram studies are essential in the bacteriological surveillance of ARI in children.

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