Original article

Prevalence of acute respiratory bacterial pathogens in children in Gondar

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Abstract: A study was conducted in Gondar, North-Western Ethiopia, during 1997-1998 to determine the prevalence of bacterial etiologic agents of acute respiratory infection (ARI) in children. A total of 390 subjects were studied out of which 63% were cases from Gondar Hospital and Gondar Health Center and the rest (37%) were controls from different schools and kindergartens in Gondar Town. From each case and control throat and nasopharyngeal specimens were collected, and cultured and biochemical tests done to isolate the bacterial etiologic agents of the disease. Clinical findings, such as cough, raised respiratory rate, difficult breathing, and fever were correlated with laboratory findings. *S. pneumoniae* and *H. influenzae* type b were the dominant isolated pathogens in both throat and nasopharyngeal specimens obtained from 71% and 68% of the cases and 5% and 1% of the controls, respectively. About 20% of the cases had diarrhea as concurrent illness. Even though different bacteria are known to cause ARI, *S. pneumoniae* and *H. influenzae* type b were found to be the dominant etiologic agents of acute respiratory infection. This paper discusses the association of bacteria isolated with acute respiratory infection in children in Gondar. [*Ethiop. J. Health Dev.* 2000;14(2):191-197]

Introduction

The total population of Ethiopia is about 60 million, 16% of whom comprises children below the age of five years. Demographic data show that infant mortality rate is 105/1000 while under-five mortality rate is 172/1000 (1). Acute respiratory infections are the major causes of morbidity and mortality of under-five children in developing countries. Taking this into consideration WHO, in 1982, initiated a program for the control of ARI based on a case-management approach. One of the strategies of the program is to recommend antimicrobial drugs for children with pneumonia (2).

Acute respiratory infection morbidity and mortality rates in children are much greater in developing countries than in the privileged parts of the world (3). Pneumonia and diarrhea are the major killer diseases of children in developing countries (4). Like in other developing countries, children in Ethiopia are seriously affected by communicable diseases among which acute respiratory infection plays the major role (5).

Pneumonia can be caused by viral, fungal, and bacterial agents among which bacterial agents account for more than 50% of ARI cases. The most common bacteria are *S. pneumoniae* and *H. influenzae* which are Gram-positive diplococci and Gram negative coccobacilli, respectively. *S. pneumoniae* is carried in the throat and nasopharynx of healthy people, but infants and young children have little resistance to this bacterium (6). In association with cases of invasive *H. influenzae* type b disease the carriage rate of type b strains is considerably higher in close contacts in households and day care centers (7).

K. pneumoniae occurs in the respiratory tract

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of about 5% of normal individuals and is the causative agent responsible for a small proportion (about 3%) of bacterial pneumonia. *K. rhinocleromatis* is also known to be a serious infectious agent in the respiratory tract (8).

Study has shown that the frequency of ARI in children is 8-9 episodes per year (9). Other studies have also shown that children in general experience 4-6 acute respiratory illnesses per year during the first five years of life (10,11). Current estimates put global acute respiratory infection deaths at about five million per year which is approximately one-third of all childhood mortality. More than 90% of these deaths occur in less developed countries (12).

Blood cultures are positive in only a small proportion of children with bacterial pneumonia. As a result of these limitations, it is assumed that the bacteria carried in the throat and nasopharynx are the reservoir for strains giving bacterial pneumonia. Since pneumonia can be caused by a variety of organisms, the ideal approach to its management would be to identify the causative agents in each individual case so that an appropriate antibiotic can be prescribed. However, an etiological diagnosis of pneumonia is very difficult to establish in infants and young children, because sputum is usually not available. A bacterial cause of pneumonia can only be established by lung (or pleural fluid) tap or blood culture. Rapid immunological techniques such as enzyme-linked immunosorbent assay (ELISA), latex particle agglutination or coagglutination do not yet perform adequately for reliable bacterio-logical diagnosis in children (13).

The aim of this study is, therefore, to determine the potential bacterial pathogens that are carried in the throat and nasopharynx in children with ARI in Gondar and identify them at species level.

Methods

Patients and specimens: Throat and nasopharyngeal specimens from 246 children who were clinically suspected for ARI were collected at the out-patient departments of Gondar Hospital and Gondar Health Center. Acute respiratory infection was diagnosed when children had cough with or without difficult breathing as examined by a pediatrician. Cases with the typical symptoms of the disease, such as cough, fast or difficult breathing, and fever were included in this study while those who were under antibiotic treatment during the time of specimen collection or for the last one week were excluded from the study. All patients who came in the morning of a working day during the study period were enrolled. One hundred and forty four control samples were collected from apparently healthy children at schools and kindergartens as well as from those who came for immunization to the Hospital and Health Center.

The controls were all children who did not have any of the clinical symptoms of ARI who were also examined by a pediatrician. For this purpose a questionnaire was prepared and filled by a nurse for each case and apparently healthy child to collect information on age of subjects, clinical features of the patients, family size, etc. by asking the parents and from the clinical records of the patients.

Collection and transportation of specimens: Throat swabs were collected from tonsillar areas using sterile cotton swabs on wooden applicator sticks while nasopharyngeal specimens were collected from the posterior nares with flexible wires having calcium alginate tips. The swabs collected were placed immediately in Amies transport medium (Difco) and brought to the bacteriology laboratory of Gondar College of Medical Sciences where laboratory investigations were performed.

Laboratory analysis: The specimens collected were primarily inoculated on blood agar base (Oxoid) supplemented with 5% sheep blood to isolate the bacteria such as *S. pneumoniae, S. aureus* and beta hemolytic streptococci Group A. GC agar base(Oxoid) with 2% hemoglobin (BBL) and 1% isovitalex (Biomerieux) as well as chocolate agar plates were used to allow the growth of *Haemophilus species*. MacConkey agar with 0.01% crystal violet (Oxoid) and different biochemical media were also used to isolate Gram-negative bacteria such as *K. pneumoniae* and *K. rhinoscleromatis* that cause ARI. All plates were incubated at 37°C for 24 hours after which typical colonies were isolated and identified morphologically and by their hemolytic activity on blood agar plate.

Pure colonies of those suspected for *S. pneumoniae* were picked out from the blood agar plate and inoculated on tryptone soya yeast (TSY) broth. They were then uniformly scattered on blood agar plate after which a 6-mm optochin disk was placed immediately and the plate was again incubated at 37°C for 24 hours for the identification of *S. pneumoniae* with a zone of inhibition of ≥ 14mm in diameter around the disk. Bile solubility test was also done whereby pure colonies were suspended in 10% sodium deoxycholate in a test tube. Clearing of the suspension within 5 to 15 minutes showed that the organism is *S. pneumoniae*. In addition, slidex pneumo kit (Biomerieux) test was carried out to confirm that the organism is *S. pneumoniae*.

The *Haemophilus species* were tested using the XV growth factor (Oxoid) requirements. Pure colonies from the GC agar base supplemented with the hemoglobin and isovitalex as well as from the chocolate agar plates were picked and inoculated on TSY broth after which they were uniformly scattered on nutrient agar plate. The XV factor disks were placed immediately on the nutrient agar plate with the bacteria. The plate was incubated at 37°C for 24 hours. The organisms that grew around the XV disks were identified to be *H. influenzae*. The *H. influenzae* type b antiserum was also used to confirm that the organism isolated is *H. influenzae* type b.

Isolates were identified as beta hemolytic streptococci Group A strains using bacitracin susceptibility test on blood agar plate where a zone of inhibition of ≥ 15 mm in diameter is observed around the 6-mm bacitracin disk. Other isolates were identified as *S. aureus* by their colonial morphology, hemolytic activity on blood agar plate, and their catalase as well as coagulase positive test results.

The pure colonies isolated from MacConkey agar plate were inoculated on nutrient broth and different biochemical media in order to be identified as different Gram-negative bacteria that could be associated with ARI.

Gram stain was done whenever it was deemed necessary.

Sample size determination and data analysis: The sample size for this study was determined using EPI-INFO software program. The results of the study were entered into a computer system using Dbase program and statistical analysis was made using SPSS PC +

Results

Table 1 indicates that 216 (87.8%) of the cases who came to the OPD of Gondar Hospital and Gondar Health Center with symptoms of ARI were below the age of five years. Out of the total, 45 (18.3%) cases were from large families while 183 (74.4%) of all cases live in a single room with crowded conditions. Fifty (20.3%) of the cases who came to the Hospital and Health Center had a family member who was having ARI problem. This table also indicates that mothers who are believed to be more responsible in taking care of their children are more commonly illiterate than fathers.

Table 1: General characteristics of the study population, Gondar, 1997-1998.

Characteristics	Cases n(%)	Controls n(%)	p-value
Age of subjects (in years)			
0 - 1	96 (39.0)	42 (29.2)	
2 - 5	120 (48.8)	64 (44.4)	p<0.05
6 - 14	30 (12.2)	38 (26.4)	
Family size			
3 - 5	133 (54.1)	72 (50.0)	
6 - 7	68 (27.6)	36 (25.0)	p>0.1
8+	45 (18.3)	36 (25.0)	
Number of rooms in the household			
1	183 (74.4)	71 (49.3)	
2	28 (11.4)	32 (22.2)	p<0.01
3+	35 (14.2)	41 (28.5)	
Presence of people affected by ARI in the family			
Yes	50 (20.3)	9 (6.2)	
No	196 (79.7)	135 (93.8)	p<0.01
Educational status of father			
Illiterate	30 (12.2)	6 (4.2)	
Read and write only	58 (23.6)	20 (13.9)	
Elementary	37 (15.0)	26 (18.0)	p<0.05
Secondary+	121 (49.2)	92 (63.9)	
Educational status of mother			
Illiterate	65 (26.5)	20 (13.9)	
Read and write only	37 (15.0)	16 (11.1)	
Elementary	38 (15.4)	26 (18.0)	p<0.01
Secondary+	106 (43.1)	82 (57.0)	

Table 2 shows that fever (≥ 38°C) was present in 242 (98.4%) of the cases. According to the information obtained from parents of ARI case 86 (35.0%) sick children had problem to swallow food. It was also found that 62 (41.3%) of the cases above one year of age and 60 (62.5%) infants below one year of age had respiratory rates of ≥ 40/min and ≥ 50/m,n respectively. One hundred and forty nine (60.6%) and 16 (6.5%) of the cases had crepitations and bronchial sound, respectively.

Table 3 indicates that different potential pathogenic bacteria were isolated from 227 (92.3%) and 225 (91.5%) of the cases in their throat and nasopharynx, respectively.

Table 2: Clinical features of ARI cases, Gondar, 1997-1998

Presence of clinical features	n(%)
History of cough	246 (100)
History of fever	246 (100)
Recorded fever (≥38°C)	242 (98.4)
History of difficulty to swallow food	86 (35.0)
Respiratory rates ≥40/min (children above one year)	62 (41.3)
Respiratory rates ≥50/min (infants 0-1 year)	60 (62.5)
Crepitations	149 (60.6)
Bronchial sound	16 (6.5)

Table 3: Comparison of bacterial isolates by percentage in ARI cases and apparently healthy controls, Gondar, 1997-1998.

Bacteria isolated	Percent of isolation: Throat swab			Nasopharyngeal swab		
	cases no(%)	Controls n(%)	p-value	Cases n(%)	Controls n(%)	P-value
S. pneumoniae	142 (57.8)	6 (4.2)	P<0.01	132 (53.8)	2 (1.4)	P<0.01
H. influenzae type b	33 (13.4)	1 (0.7)	P<0.01	34 (13.8)	0 (0.0)	P<0.01
β-strep. Group A	2 (0.8)	0 (0.0)	P<0.01	2(0.8)	0 (0.0)	P<0.01
S. aureus	12 (4.9)	6 (4.2)	P<0.01	7(2.8)	3 (2.1)	P<0.01
Ohter bacteria*	38 (15.4)	6 (4.2)	P<0.01	50 (20.3)	9 (6.3)	P<0.01
No bacteria isolated	19 (7.7)	125 (86.7)	P<0.01	21 (8.5)	130 (90.2)	P<0.01
Total	246 (100.0)	144 (100.0)		246 (100.0)	144 (100.0)	

Note - **K. pneumoniae, K. rhinoscleromatis, E. coli,* Beta streptococci non Group A

Among these isolates, strains of *S. pneumoniae* together with *H. influenzae* type b accounted for 175 (71.2%) and 166 (67.6%) in throat and nasopharyngeal swabs, respectively. Other bacteria like *K. pneumoniae*, *K. rhinoscleromatis*, beta hemolytic streptococci non Group A, *S. aureus* and *E. coli* were also found to be associated with ARI in children.

Table 4: **Association of clinical features with the bacteria isolated from ARI cases, Gondar, 1997 - 1998.**

Clinical features	Status	No bacterial growth	Growth of all bacteria	N	p-value
Difficulty in swallowing	Yes	6 (7.0)	80 (93.0)	86	
	No	13 (8.1)	147 (91.9)	160	P>0.1
Recorded fever (≥38°C)	Yes	17 (7.0)	225 (93.0)	242	
	No	1 (25.0)	3 (4.8)	4	P<0.05
Respiratory rates/min (children above 1 year)	≥40	3 (75.0)	59 (95.2)	62	
	<40	9 (10.2)	79 (89.8)	88	P>0.1
Respiratory rates/min (infants 0-1 year)	≥50	4 (6.7)	56 (93.3)	60	
	<50	3 (8.3)	33 (91.7)	36	P>0.1
Crepitations	Yes	7 (4.7)	142 (95.3)	149	
	No	12 (12.4)	85 (87.6)	97	P<0.05
Bronchial sound	Yes	1 (6.3)	15 (93.7)	16	
	No	19 (8.3)	211 (91.7)	230	P>0.1

Both *S. pneumoniae* and *H. influenzae* type b were also isolated from seven (4.9%) and two (1.4%) controls in throat and nasopharynx, respectively ($p<0.01$). Association of clinical features with the bacteria isolated from ARI cases is shown in Table 4. Except for recorded fever (≥38°C) and crepitations, the other clinical features and the bacteria isolated from the ARI cases do not have any significant relationships. Table 5 indicates that 87 (35.3%) of the cases had different concurrent illnesses among which diarrhea accounts for 48 (19.5%).

Table 5: **Concurrent illnesses of ARI cases, Gondar, 1997-1998.**

Concurrent illness	n(%)
Diarrhea	48 (19.5)
Conjunctivitis	26 (10.6)
Diarrhea + conjunctivitis	5 (2.0)
Measles	3 (1.2)
Other skin lesions	4 (1.6)
Oral thrush	1 (0.4)
No concurrent illness	159 (64.7)
Total	246 (100.0)

Discussion

The results of this study show that children below the age of five years are more likely to carry potential bacterial pathogens associated with ARI than older children. This study indicates that educational status of the mothers is found to be an important risk factor for ARI in children. A study (14) had shown that mothers who are illiterate may face problems in taking care of their children and consequently the children may be exposed to ARI. Another study (15) had also shown that living in a single room crowded with ARI infected member, especially with bacteria like *S. pneumoniae,* the other members of the family may be easily infected by these bacteria. This situation has also been shown in our study.

All the cases included in this study had cough. According to the World Health Organization (16), children with cough in the presence of fast breathing are assumed to have pneumonia. The bacteria carried in the upper respiratory tract are the microbial population reservoir agents that are believed to give respiratory infection problems in the community (17). Acute respiratory infection involves the upper respiratory tract first and then may progress to involve the lower respiratory tract leading to lung infection.

Pneumonia is the most common lower respiratory tract infection. This disease is mainly caused by *S. pneumoniae* and it is associated with high morbidity and mortality rates.

As indicated in this study more than 90% of the cases were infected by different bacteria in which *S. pneumoniae* and *H. influenzae* type b are the two major etiologic agents of ARI. Our study showed that in 57.8 % of throat swabs and 53.8% of nasopharyngeal swabs, *S. pneumoniae* was the predominant isolate.

Recent studies have also shown that the risk of invasive disease by *S. pneumoniae* is estimated to be twenty times greater in small children if they are attending day care centers than if they are taken care of at home or at family day care (18). Studies also indicate that pharyngeal carriage of *S. pneumoniae* is very common in small children (19). Diseases usually caused by *S. pneumoniae* develop early in the course of acquisition of the carrier state (20).

This study also showed that ARI caused by *H. influenzae* type b is a significant threat to children in Gondar. Studies indicate that about five million children below the age of five years in the developing countries each year die from *H. influenzae* type b pneumonia (21). Other studies also show that nasopharyngeal colonization by *H. influenzae* type b is strongly associated with the development of other infections such as otitis media (22), and the same strain of this bacterium may be carried in the nasopharynx for several weeks or more after which it is replaced by a new strain with different antigenic characteristic (23).

Our study showed that Gram-negative bacteria like *K. pneumoniae, K. rhinoscleromatis,* and *E. coli* were found to be the etiologic agents of ARI.

Gram-negative bacillary pneumonia caused by these species is a major cause of morbidity and mortality (24). *S. aureus* was also found to cause ARI. This bacterium has been shown to cause high morbidity and mortality in children (25).

Further studies on mechanisms of infection by *S. pneumoniae* and *H. influenzae* and association with ARI in children are recommended.

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