

Original Article

Isolation Of Potential Pathogenic Bacteria From Nasopharynx From Patients Having Cough For More Than Two Weeks.

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Abstracts

This cross sectional study was done from January 2009 to June 2010 in Microbiology department of Dhaka Medical College on isolation of potential pathogenic bacteria from nasopharynx from patients having cough for more than two weeks. The nasopharyngeal swabs were collected from 290 patients for culture and 101 blood samples were collected for slide agglutination test against anti- Bordetella antibody. In case of culture of nasopharyngeal swabs, 117 (73.12%) samples yielded growth of single organism, 16 (10%) yielded mixed growth and 27 (16.88%) samples yielded no growth in children. In adults, 85 (65.39%) samples yielded growth of single organism, 1 (0.77%) yielded mixed growth and 44 (33.84%) samples yielded no growth. Highest incidence (33.33%) of *S. pneumoniae* was found in age group of 1-5 years, highest incidence (32.20%) of *N. meningitidis* was found in age group of 18-25 years and highest incidence (32%) of *Staph. aureus* was found in age group of 25-50 years. One (0.63%) *B. pertussis* was isolated from a child and 5 (3.84%) *B. parapertussis* were isolated from adults. In this study, 116 (83.45%) samples yielded growth in lower socioeconomic group and 101 (70.62%) yielded growth in the samples of middle socioeconomic group. Among the study participants, 31(30.70%) were positive and 70 (69.30%) were negative for antibody against *B. pertussis*. In anti-Bordetella antibody positive cases, 14 (93.33%) children had duration of cough for 2-3 weeks, 7 (43.75%) adults had duration of cough for 3-4 weeks and 1 (6.25%) adults had more than 6 weeks.

Key words: Potential pathogenic bacteria, nasopharyngeal swabs, Bordetella species, Charcoal blood agar media.

Introduction

Infections of the respiratory tract is a major cause of morbidity and mortality throughout the world¹. Cough is the most frequent symptom of respiratory infection of which about 12% of the general population have chronic cough. Pertussis infection is one of the causes of persistent cough. Approximately 13%-20% have cough in adolescents and adults due to *B. pertussis* infection²⁻³. Adults with waning

vaccine-induced immunity are increasing and also suffering from pertussis⁴. Recently, an increase in reported cases of pertussis in adolescents and adults has been noted in many countries despite high immunization rates in children. The clinical presentation in adolescents, adults and vaccinated individuals may be atypical, with paroxysmal cough of short duration or simply a persistent cough⁵⁻⁶. The affected adolescents and adults act as reservoirs of the disease to the vulnerable population of infants⁷. The nasopharynx is densely colonized by a broad variety of microorganisms, including commensal bacteria as well as potential pathogens. However, when the condition of the host is altered, microorganism may invade adjacent sites and/or invade the bloodstream, causing disease. On the other hand, the

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nasopharynx is a major source of secretions containing bacteria that spread between individuals and may become pathogens⁸.

Material and Methods:

This cross sectional study included 290 patients of all age groups attending at Medicine and Pediatrics OPD of Dhaka Medical College and Dhaka Shishu Hospital with symptom of cough for more than two weeks. Data regarding age, sex, monthly income, level of education, DPT vaccination and duration of cough were collected by using a predesigned data collection sheet. Specimens of nasopharyngeal swab were collected and were plated immediately in different culture media. All the Charcoal blood agar media were incubated at 35°C in aerobic condition up to 7 days and adequate humidity was maintained by placing a flask of water on the floor of incubator and inspection was done regularly every morning. All organisms were identified by their colony morphology, staining characteristic, hemolysis on blood agar, pigment production, motility and other relevant biochemical tests as per standard methods. Isolated organism of *B. pertussis* was confirmed by using specific anti- *B. pertussis* antiserum. Detection of Anti-*B. pertussis* IgG/IgM antibody in serum by slide agglutination test.

Result

A total of 290 patients with history of cough for more than 2 weeks were selected for the study. The highest number of patients were 48 (16.55%) in the age group of 1-4 years followed by 45 (15.52%) in the age group of 5-9 years (Table I).

Figure 1. shows the sex distribution of the study population. Among 290 patients, 122 (42%) were males and 168 (58%) were females with a male female ratio of 3:4.

Table III shows the distribution of the study subjects by duration of cough. Among the participants 158 (54.50%) had cough for 2-3 weeks, 75 (25.90%) had cough for 3-4 weeks, 21 (7.20%) had cough for 5 - 6 weeks and 36 (12.40%) had cough for more than 6 weeks.

Table IV shows the distribution of the respondents by history of vaccination against pertussis. Among the study subjects 183 (63.10%) received vaccination, 60 (20.70%) didn't receive the vaccination and 47 (16.20%) couldn't recall if they had vaccination.

Table V shows the vaccination status against pertussis (DPT) among the 183 participants with history of vaccination, 148

(80.90%) had completed the full course of vaccination, 20 (10.90%) completed up to 2nd dose, 15 (8.02%) received only the 1st dose and 2 (1.09%) cases did not complete vaccination schedule.

Table VIII shows results of culture of nasopharyngeal swab. In children, 117 (73.12%) samples yielded growth of single organism, 16 (10%) yielded mixed growth and 27 (16.88%) samples yielded no growth. In adults, 85 (65.39%) samples yielded growth of single organism, 1 (0.77%) yielded mixed growth and 44 (33.84%) samples yielded no growth. P statistically significant.

Table IX shows isolation of different organisms among children and adults. The most common isolated organism was *Neisseria* among children (n=69; 43.12%) and *Staphylococcus* among adults (n=43; 33.08%).

Table X shows distribution of growth according to age groups. Among the study population, highest incidence (33.34%) of *S. pneumoniae* was found in age group of 1-5 years and highest incidence (32.20%) of *N. meningitidis* was found in age group of 18-25 years and highest incidence (32%) of *Staph. aureus* was found in age group of 25-50 years.

Table XI shows distribution of socioeconomic condition and isolation of organisms. Among the nasopharyngeal samples of study population, 116 (83.45%) samples yielded growth and 23 (16.55%) samples yielded no growth in lower socioeconomic condition. One hundred one (70.63%) yielded growth and 42 (29.37%) yielded no growth in the samples of study population of middle socioeconomic. P statistically significant.

Table XII shows the distribution of study participants by serology result. Among the study participants, 31(30.70%) were positive and 70 (69.30%) were negative for antibody against *B. pertussis*.

Table XIII shows duration of cough among anti-Bordetella antibody positive cases. Among study population, 14 (93.33%) children had duration of cough for 2-3 weeks, 7 (43.75%) adults had duration of cough for 3-4 weeks and 1 (6.25%) adults had more than 6 weeks.

Table I: Distribution of the study participants by age (n =290)

Age group	Frequency	Percentage
< 1 years	37	12.75
1 – 4 years	48	16.55
5 - 9 years	45	15.52
10 - 18 years	30	10.34
19 - 29 years	40	13.79
30 - 39 years	20	06.81
40 - 49 years	27	09.33
50 - 59 years	17	05.84
>=60 years	26	09.06
Total	290	100.0

Figure 1: Distribution of the study participant’s by sex

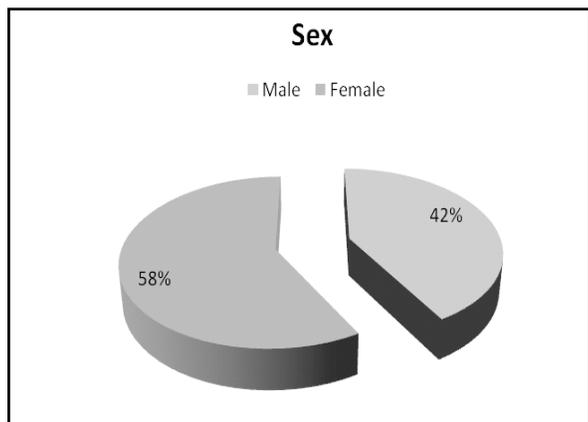


Table III: Duration of cough among the study population

Duration of cough	Frequency	Percentage
2 - 3 weeks	158	54.50
3 - 4 weeks	75	25.90
5 - 6 weeks	21	7.20
> 6 weeks	36	12.40
Total	290	100.00

Table IV: History of DPT vaccination

History of vaccination	Frequency	Percentage
Vaccinated	183	63.10
Non- vaccinated	60	20.70
Couldn't recall	47	16.20
Total	290	100.00

Table V: Status of vaccination among 183 population

Status of vaccination	Frequency	Percentage
1 dose	15	08.20
2 doses	18+2*	10.90
3 doses	148	80.90
Total	183	100.00

*2 cases did not complete vaccination schedule.

Table VIII: Results of culture of nasopharyngeal swab (n=290)

	No growth	Single growth	Mixed growth	Total
Children	27 (16.88)	117 (73.12)	16 (10.00)	160(100)
Adults	44 (33.84)	85 (65.39)	1 (0.77)	130(100)
Total	71 (24.48)	202 (69.66)	17 (5.86)	290(100)

Table IX: Isolation of different organisms among children and adults

Growth	Children			Adults		
	Single	Mixed	Total	Single	Mixed	Total
<i>B. pertussis</i>	1	0	1(0.63)	0	0	0 (0.00)
<i>B. parapertussis</i>	0	0	0 (0.00)	5	0	5 (3.84)
<i>Staph. aureus</i>	5	4	9(5.62)	16	0	16(12.31)
<i>S. pneumoniae</i>	15	2	17(10.62)	4	0	4(3.08)
<i>N. meningitidis</i>	25	3	28(17.50)	3	0	3(2.31)
<i>H. influenzae</i>	1	0	1(0.63%)	0	0	0 (0.0)
<i>Staphylococcus</i> (Coag -ve)	12	10	22(13.75)	26	1	27(20.77)
<i>Streptococcus</i> (others)	24	6	30(18.75)	14	0	14(10.77)
<i>Neisseria</i> (others)	34	7	41(25.62)	16	1	17(13.08)

Figures in parentheses represent percentage

Note- *Streptococcus* (other) - Other than *S. pneumoniae*

Neisseria (others) - Other than *N. meningitidis*

Table X: Distribution of growth according to age groups

Growth	<1year	1-5 years	5-10 years	10- 18 years	18-25 years	25-50 years	>50years	Total
<i>S. pneumoniae</i>	6(28.58)	7(33.34)	2(9.52)	2(9.52)	0(0.0)	2(9.52)	2(100)	21(100)
<i>N. meningitidis</i>	3(2.22)	3(9.78)	5(16.12)	8(25.80)	10(32.20)	2(6.44)	2(6.44)	31(100)
<i>Staph. aureus</i>	1(4)	2(8)	2(8)	3(12)	5(20)	8(32)	4(16)	25(100)
<i>Neisseria- others</i>	7(12.06)	5(25.8)	6(10.35)	8(13.80)	10(17.24)	8(13.80)	4(6.89)	58(100)
<i>Streptococcus-others</i>	8(18.18)	8(18.18)	5(11.37)	8(18.18)	3(6.82)	5(11.37)	7(15.90)	44(100)
<i>Staphylococcus</i>	5(10.20)	8(16.35)	4(8.16)	5(10.20)	5(10.20)	17(34.69)	5(10.20)	49(100)
Coa(-)ve								

Figures in parentheses represent percentage

Table XI: Isolation of organisms in different socioeconomic groups (n=290)

Socioeconomic condition	Isolation		
	No growth	Growth	Total
Low income group	23 (16.55)	116 (83.45)	139 (100)
Middle income group	42 (29.37)	101 (70.63)	143 (100)
High income group	6 (75)	2 (25)	8 (100)
Total	71 (24.49)	219 (75.51)	290 (100)

Figures in parentheses represent percentage

Table XII: Results of anti-Bordetella antibody test in children and adults (n=101)

	Children	Adults	Total
Serology positive	15 (30)	16 (31.38)	31 (30.70)
Serology negative	35 (70)	35 (68.62)	70 (69.30)
Total	50 (100)	51 (100)	101(100)

Figures in parentheses represent percentage

Table XIII: Duration of cough among anti-Bordetella antibody positive cases (n=101)

	2-3 weeks	3-4 weeks	5-6 weeks	>6 weeks	Total
Children	14 (93.33)	1 (6.67)	0 (0.00)	0 (0.00)	15 (100)
Adults	5 (31.25)	7 (43.75)	3 (18.75)	1 (6.25)	16 (100)

Figures in parentheses represent percentage

Chi-square – 9.04, df 3, p = 0.023 (statistically significant)



Fig-5: Pure culture of Bordetella spp. in the charcoal blood agar media.

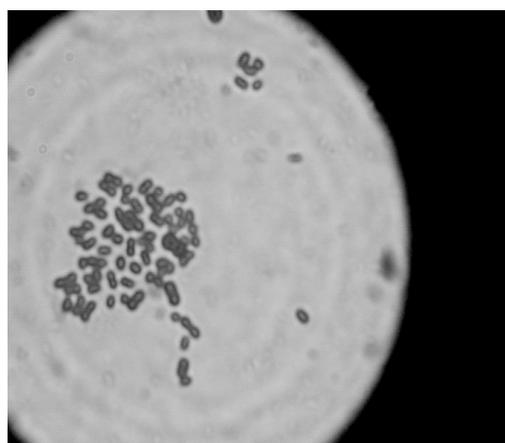


Fig 6: Gram stained smear showing Bordetella spp. from culture.

Discussion

In the present study, culture of 290 nasopharyngeal swabs samples yielded growth in 219 (75.51%) samples. In children, 73.12% yielded growth, mixed growth was 10% and no growth was 16.88% (Table-VIII). In adults, 65.39% yielded growth, mixed growth was 0.77% and no growth was 33.84% (Table-VIII). Isolation of organisms was more in children than adults. This difference was statistically significant (p= 0.047). Carriage of potentially pathogenic bacteria decreased with age in this study. In Spain, Garcia-Rodriguez and Martinez (2002) reported that the prevalence of carriage of pathogenic bacteria in healthy individuals decreased with age⁹. These bacteria are often present in nasopharyngeal samples taken from healthy children, but rarely from people of more than 16 years of age.

In this study, *Staph. aureus* was isolated in 12.31% adults and 5.62% children. In Sweden, Nilsson and Ripa (2006) reported that the rate of *Staph. aureus* isolation from anterior nares were 31% from patients admitted to an orthopedic ward¹⁰. In this study, *Staph. aureus* was isolated in 20% among 18-25

years and 32% among 25-50 years. Teenagers and male individuals were more likely to be carriers of *Staph. aureus*¹¹. Melles *et al.* (2006) demonstrated that passive smoking showed a significant association with carriage of a specific *Staph. Aureus*¹².

Among the study population, *S. pneumoniae* was isolated in 10.62% children and 3.08% adults (Table-IX). In Sweden, Gunnarsson *et al.* (1996) reported that isolation of *S. pneumoniae* was 6-19% in healthy children and 0.8% in healthy adults¹³. Syrjanen *et al.* (2001) had shown that carriage of *S. pneumoniae* increased during respiratory tract infections¹⁴. In the present study, peak incidence (33.33%) of *S. pneumoniae* was found in the age group of one to 5 years, followed by 28.57% among children less than one year (Table-X). In Mexico, Butler *et al.* (2007) reported that 29.9% *S. pneumoniae* was isolated from subjects aged 2 months to 6 years¹⁵.

In the present study, *N. meningitidis* was isolated in 17.50% children and 2.31% adults (Table-X). Similar high prevalence was observed by Cunningham *et al.* (2001) and reported that pharyngeal carriage of *N. meningitidis* was 10-23% from direct plating of nasopharyngeal swabs¹⁶. In this study, carriage rate was 3.22% in less than one year age group and increased up to 42% in the age group of 5-18 years and 18-25 years and then decreased to 6.44% in older age groups. Blackwell *et al.* (1990) reported that the carriage rate was shown to be less than 3% in children younger than 4 years and increased to 24-37% in the age group of 15-24 years and then carriage rate decreased to less than 10% in older age-groups¹⁷⁻¹⁸.

Isolation of PPB was more (83.45%) in low socioeconomic status than middle (70.63%) and high (25%) socioeconomic status (Table-XI). Anianson *et al.* (1992) reported that low socio-economic status had been identified as a risk for colonization and carriage of respiratory pathogens. Probably cramped and poor housing associated with lower access to health care are the factors¹⁹.

B. pertussis was isolated from only one child suffering from protein energy malnutrition (PEM). This child had taken only two doses of DPT vaccine. Isolation of *B. pertussis* indicates that the organism is in the community. Meticulous effort should be given to isolate this organism in the community level especially in malnourished children. In the present study, *B. parapertussis* was isolated in 5 (3.84%) adults.

Similar high prevalence was observed by He *et al.* (1998). They reported that culture positivity of *B. parapertussis* was 3.4% in children and adults²⁰.

In this study, *B. pertussis* was not isolated in culture in adults. However, 31.37% of adults were serology positive. In United State, Wright *et al* (1995) demonstrated that no subject was culture positive for *B. pertussis*, but 21% subjects met the serological criteria for pertussis infection²¹. The laboratory diagnosis of pertussis in adults is difficult because of the usual delay in suspicion of the disease²².

Maximum adults (43.75%) with positive serology for IgG/IgM against *B. pertussis* had cough for 3-4 weeks and 14 (93.33%) seropositive children had duration of cough for 2-3 weeks (Table-XIII). Cherry (1998) studied on prolonged cough illness in adolescents and adults and indicated that between 12% and 32% was the results of *B. pertussis* infection²²⁻²⁵.

Previous research in several countries had shown that pertussis is endemic among the adolescents and adults²⁶⁻²⁷. It is suggested that a universal program of adolescent and adult boosters would decrease the circulation of *B. pertussis* in these age groups and possibly could lead to the elimination of the organism from the population. With the availability of vaccine, booster doses in adolescents have been introduced in Canada, Austria, Australia, France, Germany and the US, and many other countries are considering similar expansion of their immunization programs at present.

Conclusion

Potential pathogenic bacteria (PPB) present in nasopharyngeal samples were more in children than adults. The carrier rate of PPB in the nasopharynx decreased with age. However, the carrier rate of PPB was more in the low socioeconomic condition. Pertussis may be a cause of persistent cough (>3 weeks) in adults. The affected adolescents and adults act as reservoirs of the disease to the vulnerable population of infant and children. For children presenting to primary health care center with a cough lasting for more than 2 weeks, a diagnosis of whooping cough should be considered.

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