



pneumoADIP Sponsored Activities  
in Bangladesh



# Preface

## *Streptococcus pneumoniae* in Bangladesh

We use several modalities to study and measure the broad impact of pneumococcal disease in Bangladesh. The Bangladesh Demographic and Health Survey, a representative sample drawn from the entire country, has identified pneumonia as the leading cause of death among children less than 5 years old in Bangladesh. Extrapolating mortality rates in the survey to the Bangladesh population, an estimated 90,000 children over 1 month and less than 5 years of age die from pneumonia every year in Bangladesh. Surveillance in seven hospital, supported by pneumoADIP, has identified a wide range of pneumococcal serotypes leading to hospitalization and pneumonia as a leading cause of pediatric hospitalization. Urban community-based surveillance, supported by pneumoADIP, demonstrated an incidence of invasive pneumococcal disease equivalent to the incidence noted in the control group in the Gambia pneumococcal vaccine trial where pneumococcal vaccine was associated with decreased mortality. Rural surveillance, supported by pneumoADIP, demonstrated that serious invasive pneumococcal disease is common in rural areas. Inside this brochure are more details on these projects, and many lessons learned from these platforms. Together these data provide a strong scientific case for the importance of preventing pneumococcal disease to child health in Bangladesh, and therefore the potential benefit of an effective vaccine. Upon vaccine introduction continued surveillance can assess the impact of pneumococcal vaccination on child health and circulating serotypes.

### **PneumoADIP Bangladesh Team**



# Surveillance for Invasive *Streptococcus pneumoniae* Among Children in Bangladesh: Antimicrobial Susceptibility & Serotype Distribution

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## Background

Strategies for prevention of pneumococcal deaths and complications include case management with appropriate antimicrobial therapy and immunization against predominant serotypes.

**Aims:** Evaluation of antimicrobial susceptibility and serotype distribution of circulating *Streptococcus pneumoniae* strains among the under five children of Bangladesh.

**Design:** Multicentre Network of 7 hospitals in urban and rural area of Bangladesh. Children aged <5 years, diagnosed as the case of pneumonia, severe pneumonia, very severe diseases or meningitis and consent given to draw blood were enrolled.

Pneumococcus strains were isolated and identified by site laboratories and sent to reference laboratory for confirmation and serotyping.

Strains isolated from CSF or from blood, with significant leucocyte count in CSF, were defined as meningitis strains. Rests were labeled as pneumonia/sepsis cases.

## Progress

Establishment of a network of 7 hospitals in three different districts of Bangladesh in January 2004. Technicians at each laboratory supporting the 7 hospitals were oriented in blood and CSF culture techniques. Between May 2004 and May 2007, blood cultures were done on 17,969 patients and CSF cultures on 3,765 patients.

## Results

*S. pneumoniae* strains were isolated from 139 patients, including 91 with meningitis and 48 with pneumonia/sepsis. Out of 139 strains, rates of non-susceptibility for pneumococcal isolates were 6%, 6% and 72% to penicillin, chloramphenicol and cotrimoxazole, respectively.

Analysis of pneumococcal cases by age group showed the prevalence of pneumococcal diseases in early age of childhood; 53% of cases were within six months of birth, and 74% and 88% of the cases were within 12 and 24 months of life respectively (Figure-1).

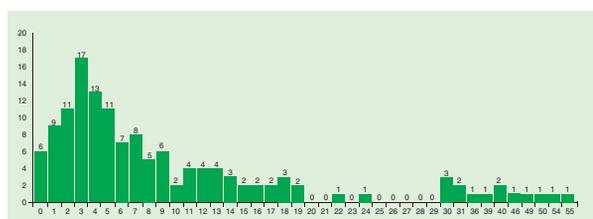


Figure 1: Age group distribution of invasive pneumococcal diseases (N=139) by months

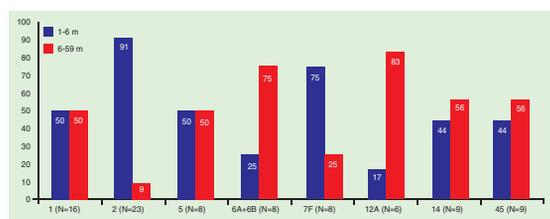


Figure 2: Distribution (%) of predominant serotypes by Age group

Distributions of some serotypes are remarkably different in early and late childhood. Specifically serotype 2 and 7F are predominant in early lifetime, contrast to serotype 6 and 12A who were common in older children. On the other hand serotype 1, 5, 14 and 45 are evenly distributed between age groups (Figure 2).

Distribution of serotypes from the present study indicates that the currently available and upcoming 7-, 10-, and 13-valent vaccines would cover 23% (95% CI 16%, 30%), 45% (95% CI 37%, 53%) and 48% (95% CI 40%, 56%) of pneumococcal diseases over all. Nonetheless, vaccine coverage would be different for meningitis and pneumonia cases, 22% Vs 23%, 40% Vs 54% and 44 Vs 61% for 7, 10 and 13 valents in that order (Figure 3).

There were 37 different serotypes with predominance of 2 (17%), 1 (11%), 14 (7%), 5 (6%), 7F (6%), 12A (5%) and 45 (5%) (Figure 4).

Serotypes of isolates from meningitis and pneumonia cases were different, specially for types 2 (25% (P<0.001) Vs 0%), 7F (8% Vs 2%), 1 (9% Vs 15%), and 5 (3% Vs 11%). Serotype 2 has emerged as the most predominant strain and all of them were from meningitis cases, indicating high virulence of these strains.

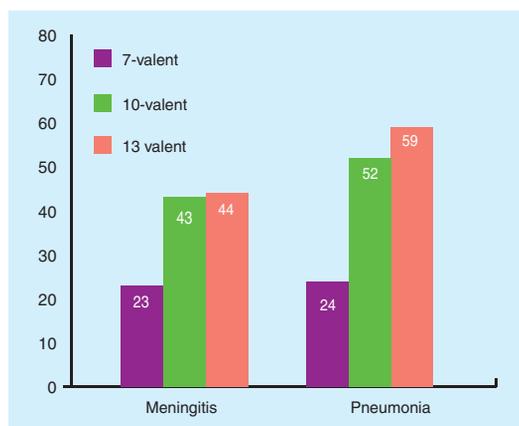


Figure 3: Serotype coverage of Pneumonia and Meningitis cases by 7, 10 and 13 valent vaccine.

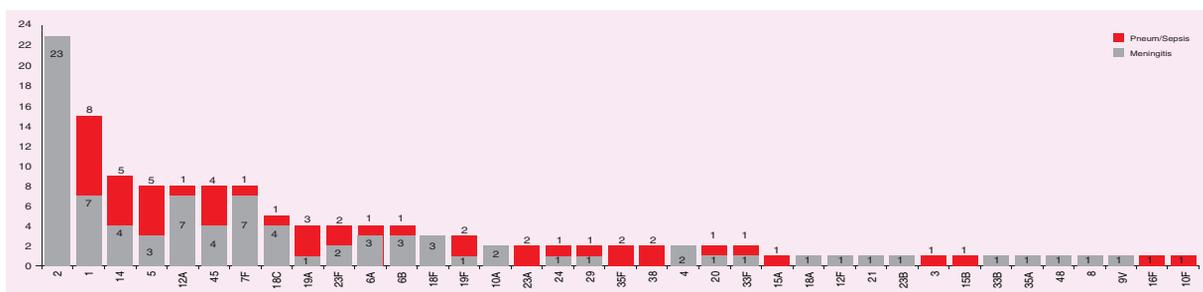


Figure 4: Over all serotype distribution in meningitis (N=91) and pneumonia (N=48) cases

## Conclusion

Non-susceptibility to cotrimoxazole is very high. Penicillin non-susceptibility is not common and thus may be a drug of choice for invasive pneumococcal diseases.

Serotype distribution is diverse with changes in predominant serotypes, when compared with earlier data from Bangladesh, the remarkable difference to the previous data indicating the value of continuous surveillance. Specifically, emergence of serotype 2, 1 and 5, and relative decline of 7F and 15 in causing invasive disease is noteworthy. This possibly puts a note of caution on interpretation of serotype replacement in any population, in post vaccination era.

Although optimal vaccine formulations would need to provide species-wide coverage or include substantially more serotypes, implementing the upcoming 10 or 13-valent vaccine would be expected to be effective against half or more of the strains causing invasive pneumococcal disease. Nonetheless, considering the birth cohort and diseases burden, relatively suboptimal coverage will have a huge impact on child mortality and public health of Bangladesh. This inference further emphasized by the observation of higher coverage in pneumonia cases by upcoming vaccines.

# Invasive Pneumococcal Disease Among Children In Rural Bangladesh: Results From A Population-Based Surveillance

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## Background

Pneumonia accounts for almost 2 million (19%) of the 10.6 million childhood deaths annually in the world. *Streptococcus pneumoniae* (pneumococcus) infections are responsible for a substantial proportion of these pneumonia deaths. Population-based data is largely lacking for most parts of the developing world.

## Methods

A population-based surveillance was conducted to estimate the incidence of laboratory-confirmed invasive pneumococcal disease in children, identify the most common serotypes, and describe their antimicrobial resistance. The surveillance was conducted in the rural community of Mirzapur, 60 kilometers north of the capital, Dhaka in Bangladesh. Pneumococcal disease surveillance in children aged 1-59 months was established in a population of ~144,000. Kumudini Hospital is a 500-bed general hospital that serves the population of Mirzapur and is centrally located in the area. Dhaka Shishu Hospital had established a laboratory at Kumudini as part of another project. This laboratory was responsible for all the field laboratory work for the surveillance (processing of blood, CSF and urine specimens, cultures).

72 village health workers (VHW) were recruited locally and trained. Each VHW was responsible for a population of about 2000, i.e., 175-190 under-5 children. VHWs made weekly home visits to the households with an under-5 children (excluding neonates), and checked on the health of the children. If a mother reported that the child has fever, cough or difficult breathing on the day of the home visit, the VHW assessed the child using an IMC-type algorithm. The child was referred to Kumudini Hospital if any of the following signs and symptoms were present:

- Possible severe pneumonia (tachypnea + danger sign)
- High fever ( $\geq 102^{\circ}\text{F}$  or  $\geq 101^{\circ}\text{F}$  if <2 months old)
- Suspected meningitis/very severe disease

Sick children were enrolled in the surveillance if (i) they were admitted to Kumudini Hospital and diagnosed with pneumonia, severe pneumonia, meningitis, or very severe disease (including high fever), and (ii) a blood or CSF specimen was obtained for culture, and (iii) the child's caretaker provided informed consent. From April 2005, sick children who were not admitted, but otherwise met the case definitions and had a blood sample collected for culture were also enrolled if consent was given.

## Sick Children Identification and Management

Variables	Jul-Dec 2004	Jan-June 2005	July-Dec 2005	Jan-June 2006	July-Dec 2006	Jan-June 2007	Total
Average # of registered children	14,443	1,416	1,557	1,660	1,437	1,865	22,378
Average # of children seen/week	8,158	10,767	11,190	10,805	10,299	10,087	10,231
Child-years of observation	3,909	5,365	5,576	5,384	5,132	5,026	30,392
# of episodes assessed by VHW	6,631	11,201	13,972	12,262	11,116	9,206	64,388
# of episodes referred to Kumudini	1,588	3,200	2,432	2,621	2,437	1,617	13,895
# of sick child visits at hospital	1,589	2,857	2,323	3,875	3,811	2,898	17,353
# of hospitalization	290	580	469	446	458	353	2,596
# of blood cultures done	91	577	787	1,251	1,952	2,267	6,925
# of CSF cultures done	7	7	12	4	6	5	41

\*The VHWs make home visits once a week and ask caretakers about child's health at that time. We are assuming that the once-a-week observation is equivalent to 7-days of observation, since the VHWs are unlikely to miss serious illnesses that may have started between the weekly visits.

## Children visiting hospital - Spn and Hib

	2004		2005		2006		2007		2004-2007					
	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun	Jul-Dec	Jan-Jun	Total	Total				
Child years	3,909	5,365	5,576	5,384	5,132	5,026	30,392	30,392	30,392	30,392				
Cultures	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF				
S. pneumoniae isolated (total)	0	0	4	1	5	0	6	0	4	0	25	1		
Invasive S. pn disease per 100,000 child-years (overall)	0	93	90	111	78	119	86	86	86	86	86			
S. pneumoniae isolated (among inpatients)	0	0	4	1	4	0	2	0	1	0	12	1		
Invasive S. pn disease per 100,000 child-years (among inpatients)	0	93	72	37	19	20	43	43	43	43	43			
H. influenzae (b) isolated	2	2	1	0	3	1	2	0	0	0	3	0	11	3
Invasive Hib disease per 100,000 child-years	51	19	54	37	0	60	36	36	36	36	36			

## Pneumococcal serotype distribution of invasive isolates by age

Serotype	Age group (in months)					Overall
	1-11	12-23	24-35	36-47	48-59	
1	0 (0)	1 (11)	1 (100)	0 (0)	0 (0)	2 (8)
5	4 (29)	1 (11)	0 (0)	0 (0)	0 (0)	5 (19)
10F	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	1 (4)
12A	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (4)
14	2 (14)	3 (33)	0 (0)	1 (100)	0 (0)	6 (23)
18B	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	1 (4)
18C	1 (7)	1 (11)	0 (0)	0 (0)	0 (0)	2 (8)
19A	2 (14)	1 (11)	0 (0)	0 (0)	0 (0)	3 (11)
35B	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)
38	2 (14)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8)
45	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)
NA	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)

\* Numbers in parentheses: percent distribution within each age group

## Summary

- The surveillance findings suggest substantial burden of pneumonia and meningitis among under-5 children in rural Bangladesh.
- Invasive pneumococcal diseases (IPD) contribute substantially to childhood morbidity in rural Bangladesh.
- *Streptococcus pneumoniae* can also cause invasive but non-severe disease in children, and IPD incidence can be seriously under-reported if such conditions are overlooked.
- The emerging high resistance to co-trimoxazole should be addressed.
- The serotype distribution should help guide appropriate pneumococcal conjugate vaccine formulation.
- The burden of pneumococcal disease and the potential beneficial impact of pneumococcal vaccines in this community would be best evaluated with a prospective "vaccine probe" study.

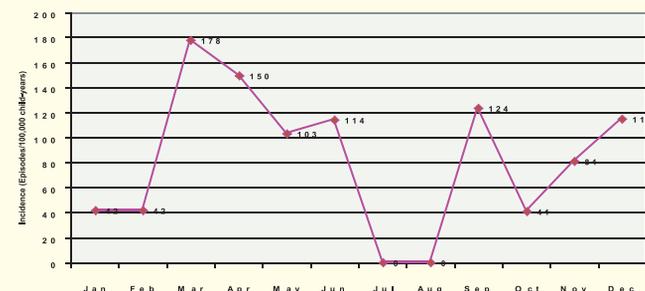
## Sick Children by Diagnosis (Reporting period: July 2004 - June 2007)

### Referred by Health Worker (n= 13,895)

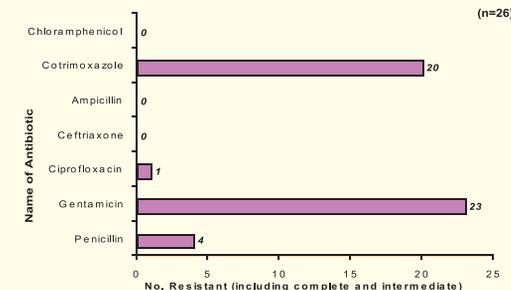
Suspected meningitis or very severe disease	2029 (67/1000 child-years)
Possible severe pneumonia or very severe disease	5020 (165/1000 child-years)
High fever/ possible bacteremia	8967 (295/100 child-years)
Possible pneumonia	9411 (310/1000 child-years)

- Pneumonia was the single most common form of illness (38%) observed among 2,596 hospitalizations.

## Seasonality in incidence of invasive pneumococcal disease (mean incidence/month)



## Antimicrobial Resistance of S. pneumoniae Isolated From Under-5 Children of Rural Surveillance Area (Period: July 2004 - June 2007)



# Emergence of *Streptococcus pneumoniae* Serotype 2 meningitis in Bangladesh: epidemiological and molecular perspectives

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7-valent pneumococcal conjugate vaccine (PCV-7) has been remarkably successful in reducing invasive pneumococcal diseases, however, there is concern that non-vaccine serotypes may replace the pathological niche of vaccine strains. Information on secular trends of serotype distribution prior to routine use of PCV-7 is lacking.

As part of our hospital based surveillance on pneumococcal meningitis in Bangladesh, we observed the emergence of serotype-2 during the last decade. The proportion of pneumococcal meningitis attributed to serotype-2 ranged from 5.9% to 41.3% during 2001 to 2007 (N=60; 35 by culture and 25 by PCR), and has been the most commonly isolated serotype in meningitis patients since 2004. It was only the sixth most common serotype in 1999-2000 (5/65), was isolated only once between 1996 and 1998 (1/48) and was not isolated at all during 1993-1995 (0/39) (Fig-1).

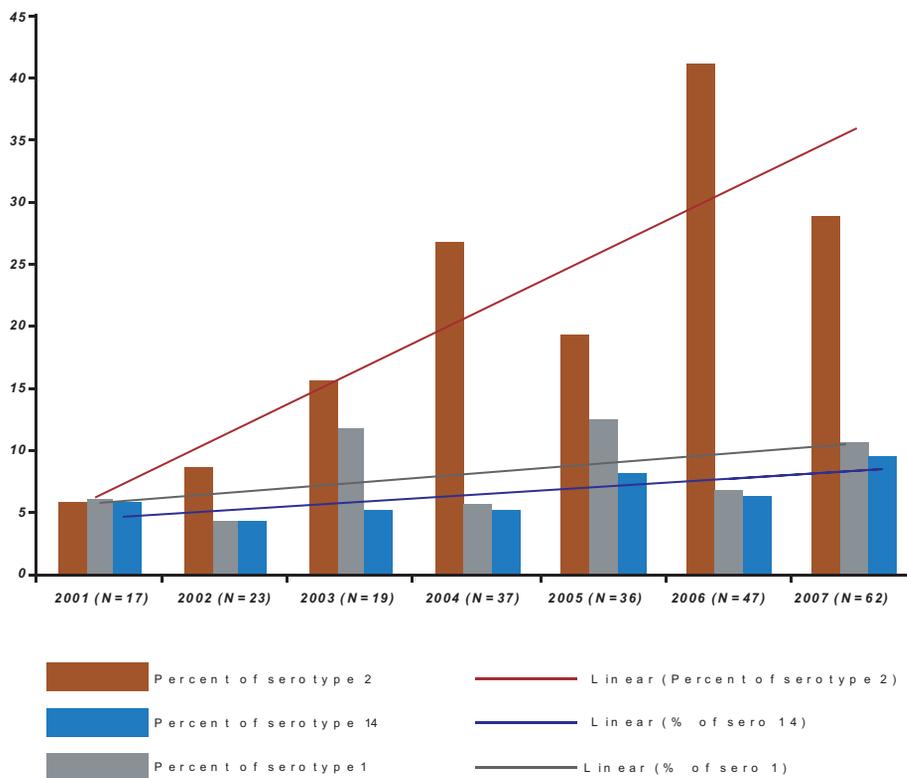


Figure-1: Gradual increase by year of Serotype 2

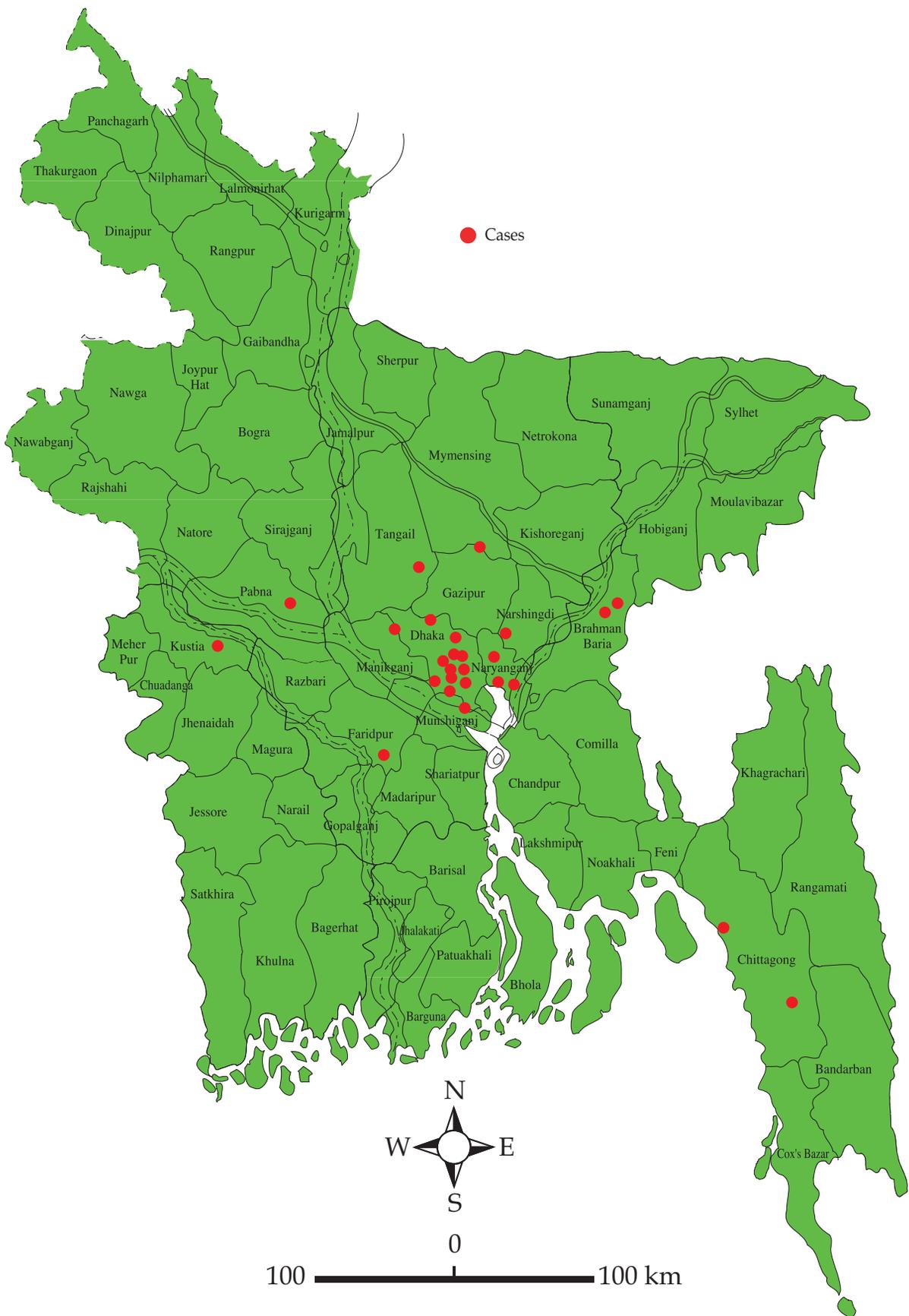


Figure-2: Location of Serotype 2 Cases in GIS Map

Mapping of geographic positions of the cases showed no clustering by locality or family (Fig-2).

This type was mostly prevalent in younger children; 88% of cases occurred in infants <6 months of age (median 3m); in contrast to 50% (median 7m) of other serotypes (P=0.003) (Fig-3).

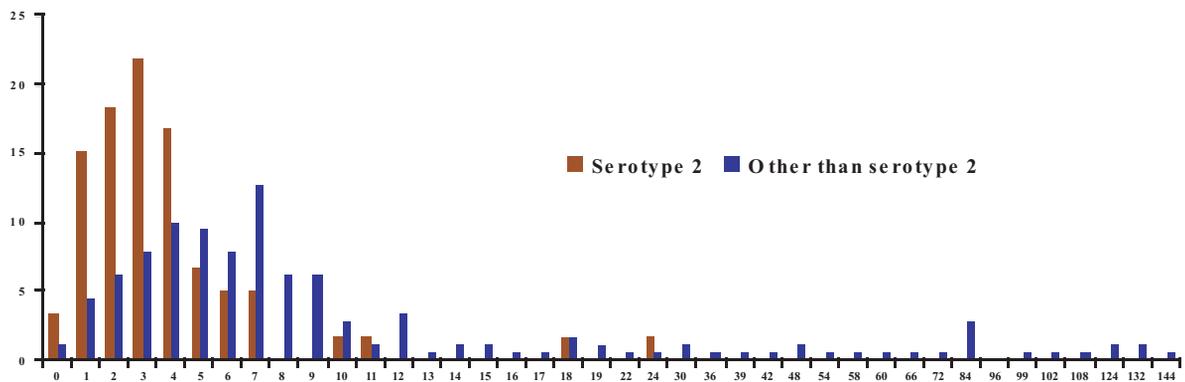


Figure-3: Age (in month) distribution of Serotype 2 vs others

Serotype 2 is also uniquely susceptible to cotrimoxazole (67%; MIC-90 = 1.5 µg/ml) in comparison to other serotypes (18%; MIC 90 = 16.0 µg/ml) (Fig-4).

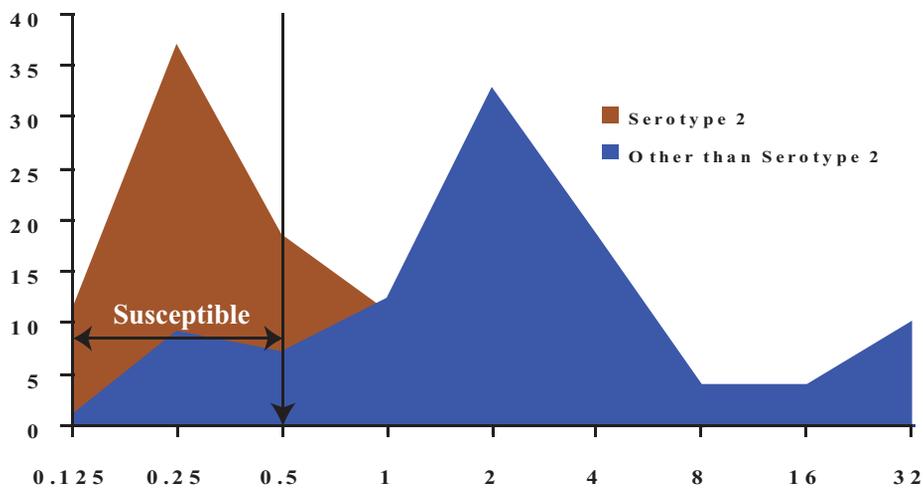


Figure-4: Susceptibility of Serotype 2 to Cotrimoxazole

Molecular analysis of the isolates by PFGE revealed that all the strains are clonal. Further investigation of 30 strains by MLST showed that all of them belong to ST\_CC 74 (Table-1).

No. of Isolates	Year of Isolation	ST_CC	MLST (ST)	aroE	gdh	gki	recP	spi	xpt	ddl
19	2003-2007	CC74	74	2	13	4	1	6	6	14
11	2003-2007	CC74	New	2	13	4	5	new	6	14

Table-1: Multi-locus Sequence Typing of Sero-2 Isolates.

Meningitis caused by serotype 2 also was associated with significantly more sequelae than other types together; 31% Vs 9% ( $P < 0.001$ ) (Fig-5).

This uncommon serotype has emergence in the absence of any identifiable selective pressure. Therefore, the 'replacement' of serotypes in the post vaccination era should be interpreted with caution. These findings emphasize the need for continuous surveillance for pneumococcal serotypes, not only for vaccine formulation but also to find the true definition of replacement disease.

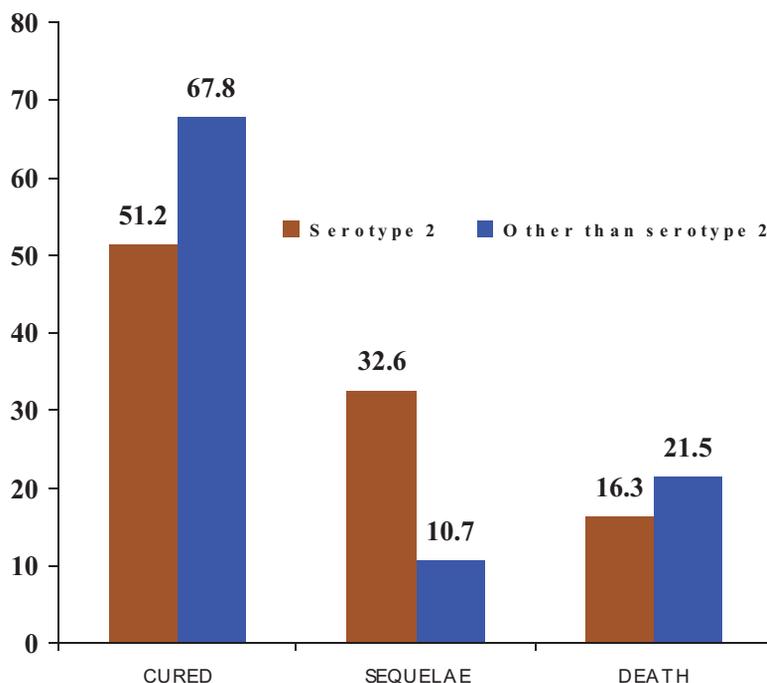


Figure-5: Hospital outcomes of Meningitis cases of Serotype 2 Vs Others

# Pneumonia burden and severity among children < 5 years of age in Bangladesh

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## Pneumonia contributes substantially to childhood death in Bangladesh



### Background

- Limited information is available on severity and risk factors associated with pneumonia in Bangladesh
- A variety of case definitions were previously used to define the burden of pneumonia resulting into different estimates
- This study used the algorithm adopted by the Accelerated Development of Introduction Plan for Pneumococcal Vaccine (PneumoADIP) to define pneumonia and other severe childhood illnesses

### Objective

- To better understand the burden and severity of pneumonia among children < 5 years
- To assess risk factors of deaths from pneumonia among children < 5 years

## Methods : A multicentre hospital based surveillance was conducted in Bangladesh in 2004-2007

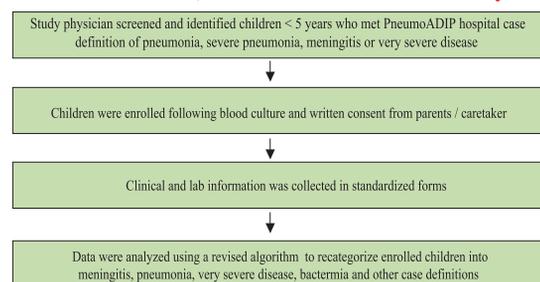
### Study sites

	Type of hospital	Number of pediatric beds
Dhaka (Capital city)	Two government teaching hospitals	45, 36
	Two private pediatric hospitals	349, 80
Chittagong (second large city)	One government teaching hospital	100
	One private pediatric hospital	200
Mirzapur (rural)	One private general hospital	85

### Location of hospitals



### Patient selection, enrollment and data analysis



## Results : Burden, severity and risk factors of pneumonia observed in seven hospitals

Pneumonia is the most frequent discharge diagnosis in the hospitals among children < 5 years of age

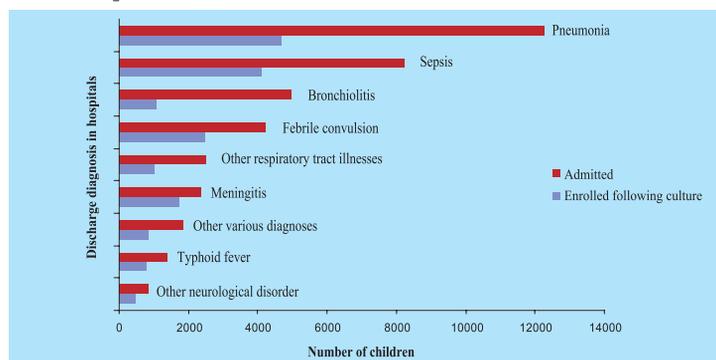


Figure 1: Number of children admitted (N = 38,664) and enrolled (N = 17,187) under various hospital diagnoses

Forty percent (40%) of children with pneumonia were treated with an antimicrobial agent before hospitalization.

Findings	< 2 months N = 1,405	2-11 months N = 2,897	12-59 months N = 1,258
Duration between illness onset and admission, mean(± sd) days	4 (4)	5 (4)	5 (5)
Treated with an antimicrobial agent prior to admission in hospital, n (%)	394 (28)	1305 (45) <sup>§</sup>	537 (43) <sup>§</sup>
Total bacteria isolated (bacterial isolation rate), n (%)	102 (7)	116 (4)	45 (4)
Proportion of <i>S.pneumoniae</i> , n (%)	0	5 (3)	5 (11)
Proportion of <i>H. influenzae</i> , n (%)	0	4 (4)	1 (2)
Death (Case fatality ratio), n (%)	142 (10)	123 (4) <sup>§</sup>	27 (2) <sup>§¶</sup>
Children died within 2 days of admission	94 (66)	81 (66)	13 (48)

<sup>§</sup> P< 0.05 when compared to children < 2 months of age; <sup>¶</sup> P< 0.05 when compared to children 2-11 months of age

Table 1: Findings of children with pneumonia were enrolled in the surveillance following a blood culture (N=5,560)

Children presenting with a pneumonia danger sign were more likely to die in hospitals

Clinical features	Children died in hospital N = 150	Children survived in hospital N = 4005	OR	95% CI	P value
Age < 12 months	123 (82)	2774 (69)	2.0	1.3-3.2	<0.001
Delay between illness onset and admission, median (range) days	5 (2-58)	4 (1-68)	---	---	<0.001
Findings on clinical examination by the study physicians on admission, n (%)					
Cough	95 (63)	2749 (69)	0.8	0.5-1.1	0.18
Difficult breathing	115 (77)	3200 (80)	0.8	0.5-1.2	0.35
Tachypnoea	125 (83)	3787 (95)	0.3	0.2-0.5	<0.001
Chest indrawing	119 (79)	2662 (67)	1.9	1.3-2.9	0.001
Cyanosis*	7 (5)	40 (1)	4.9	1.9-11.5	<0.001
Inability to drink or breastfeed*	44 (29)	230 (6)	6.8	4.6-10.1	<0.001
Lethargy/ prostration*	53 (35)	243 (6)	8.5	5.9-12.1	<0.001
Convulsion*	4 (3)	16 (0.4)	6.8	2.3-20.7	0.005
Severe malnutrition *	27 (18)	181 (5)	4.6	2.9-7.4	<0.001
Bacterial isolation in blood, n (rate %)	17 (11)	144 (4)	3.4	2.0-5.8	<0.001

\*Pneumonia danger sign: Cyanosis, inability to drink, lethargy/prostration, convulsion. \*WAZ < -3SD

Table 2: Risk factors associated with deaths among children 2-59 months of age admitted in hospital with pneumonia according to PneumoADIP case definitions (2004-2007)

### Conclusion

- Pneumonia was the leading cause of admission in hospitals for children < 5 years
- Thirty two percent (32%) of enrolled children had pneumonia according to PneumoADIP case definitions
- The low isolation rate of Spn and Hib among infants with pneumonia might be attributable to high background use of antimicrobial agents (40%) prior to admission in hospitals
- There is substantial delay for pneumonia admission. Children die shortly after admission in hospitals
- Infancy, presence of a pneumonia danger sign on admission, culture confirmed bacterial pneumonia, severe malnutrition and delayed care seeking at hospital were highly associated with deaths from pneumonia among children 2-59 months of age

### Recommendation

- Multiple risk factors associated with pneumonia case fatality suggest multiple strategies, including vaccines, to reduce pneumonia-related and overall child mortality
- Using a standardized classification for pneumonia diagnosis through a well maintained surveillance network would help to monitor impact of vaccines and other preventions strategies in Bangladesh

### Acknowledgement

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## Use of sequential multiplex PCR to identify the serotype distribution of culture negative pneumococcal meningitis: implications for vaccine design

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Knowledge about the serotype distribution of *Streptococcus pneumoniae* causing invasive diseases is fundamental for designing an appropriate vaccine. However, conventional methods of serotyping are cumbersome and expensive, and information on pneumococcal serotypes is available from few laboratories.

PCR-based serotyping has been proposed as a simpler approach, however, it has not been reported previously from Asia where serotypes are diverse and different from other settings.

Further, serotype information in this region is quite incomplete, as most pneumococcal meningitis cases are culture negative and diagnosed by latex agglutination testing, thus precluding identification of serotype.

During 2004 to 2007, we identified 358 cases of pneumococcal meningitis; 136 (38%) were culture positive. The culture-negative cases (N=222, 62%) were detected either by latex agglutination (N=92) or immunochromatographic testing (ICT) (N=130), and the serotype remained unknown (Fig-1).

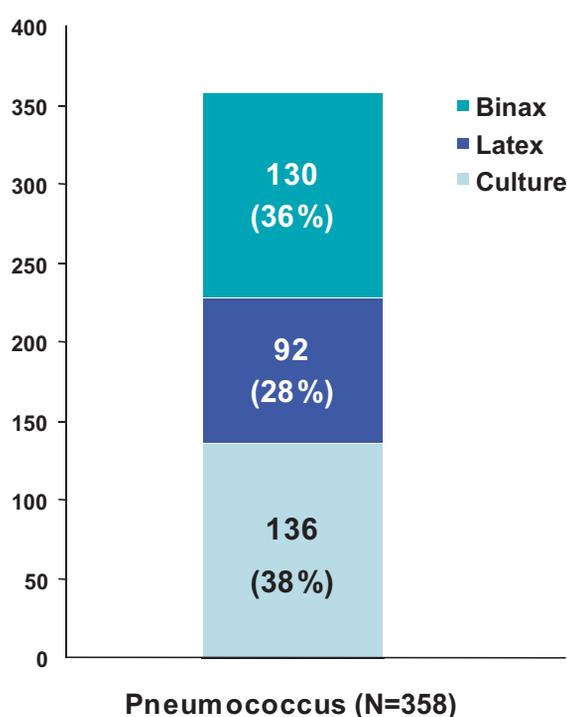


Figure-1: Pneumococcal meningitis (Jan 2006 - Nov 2007)

Thirty-six serotype-specific primers, 7 newly designed and 29 available previously, were arranged in 7 groups for sequential multiplex PCR analysis, providing cumulative coverage for 40%, 66%, 79%, 85%, 89% and 92%, respectively, of prevalent Bangladeshi serotypes. Five multiplex reactions detected 90% of all known Bangladeshi serotypes (Fig-2).

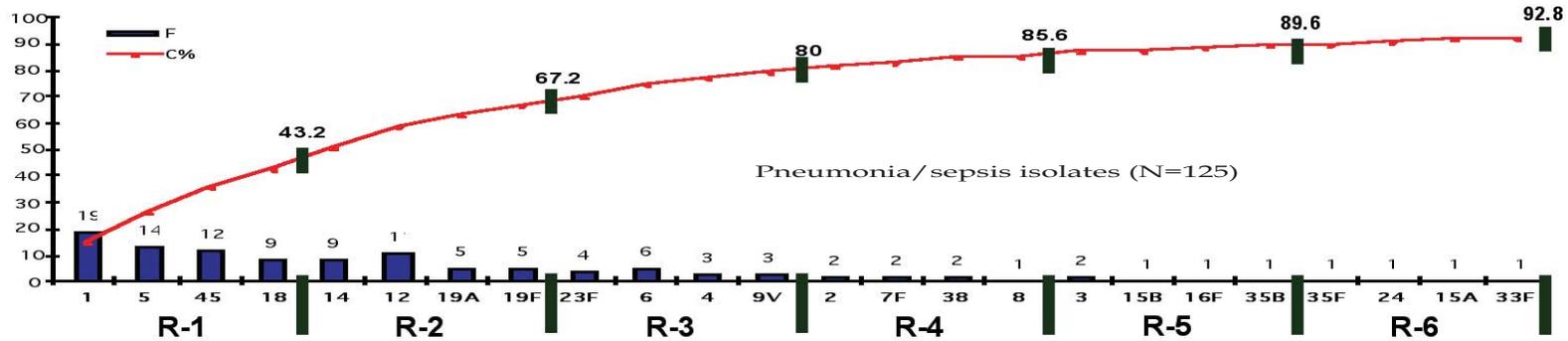
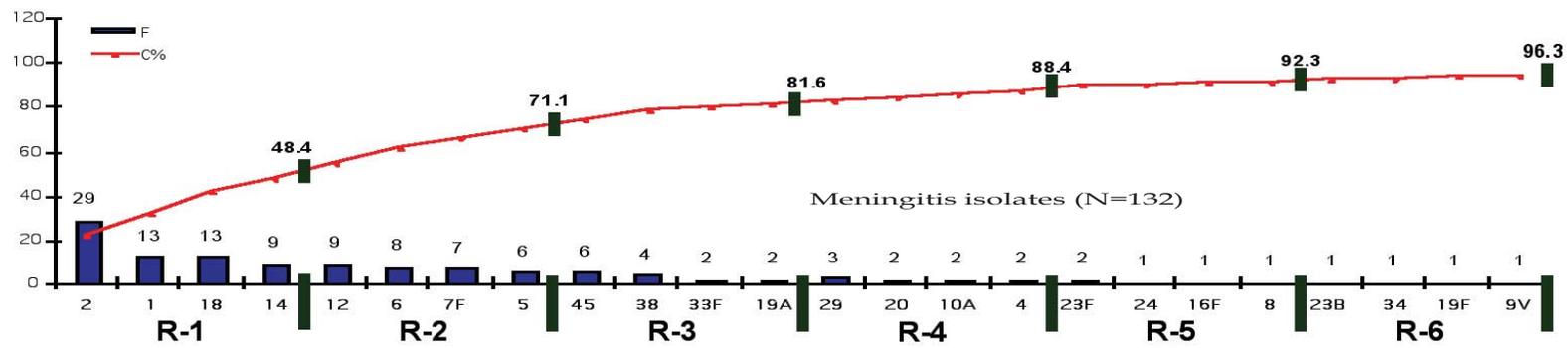
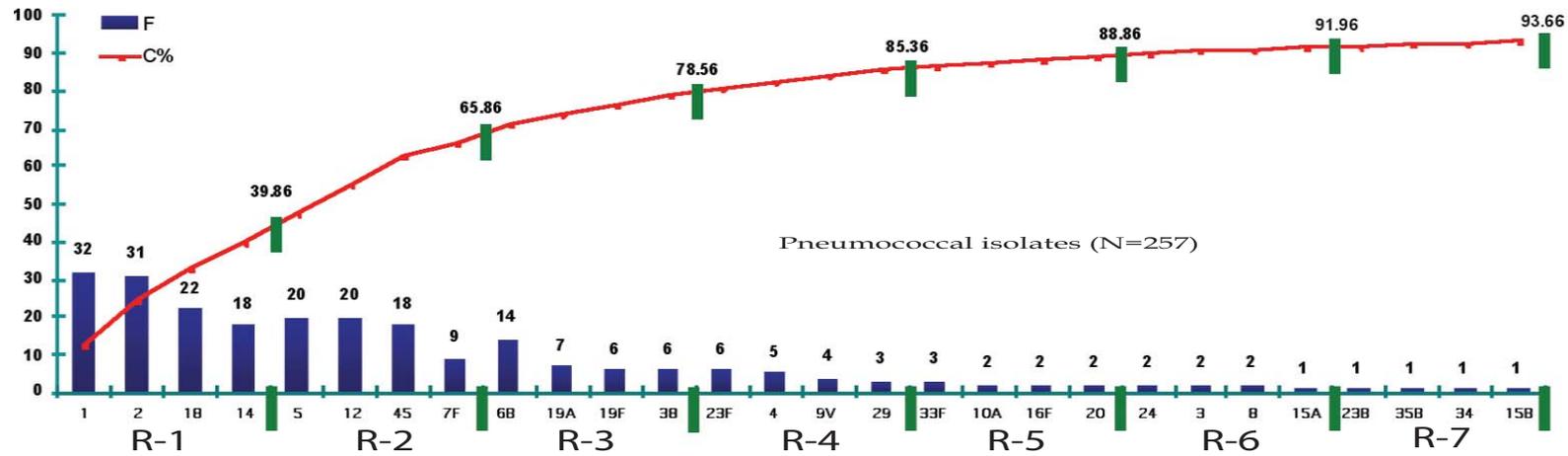


Figure-2: Multiplex PCR scheme and their coverage for pneumococcal serotypes of Bangladesh



Examination of 157 available culture-negative CSF specimens, using a separate format of multiplex reactions, revealed serotype information for 51 additional cases, 37 and 14 from 68 and 89 latex and ICT positive specimens respectively (Fig-3). These serotypes were different from those of isolates, in respect of their prevalence and ranking. PCR-based serotyping cost 1/3 3rd of conventional serotyping and the results showed 100% concordance with available strains tested by quellung reaction.

PCR-based serotyping is simple, cost-effective and free of cross-reactivity. Its application on culture negative cases determined 27% more serotype information on meningitis cases compared to isolates alone. Wider use of this approach could improve the understanding of serotype distributions especially in settings with high use of antibiotic prior to care-seeking.

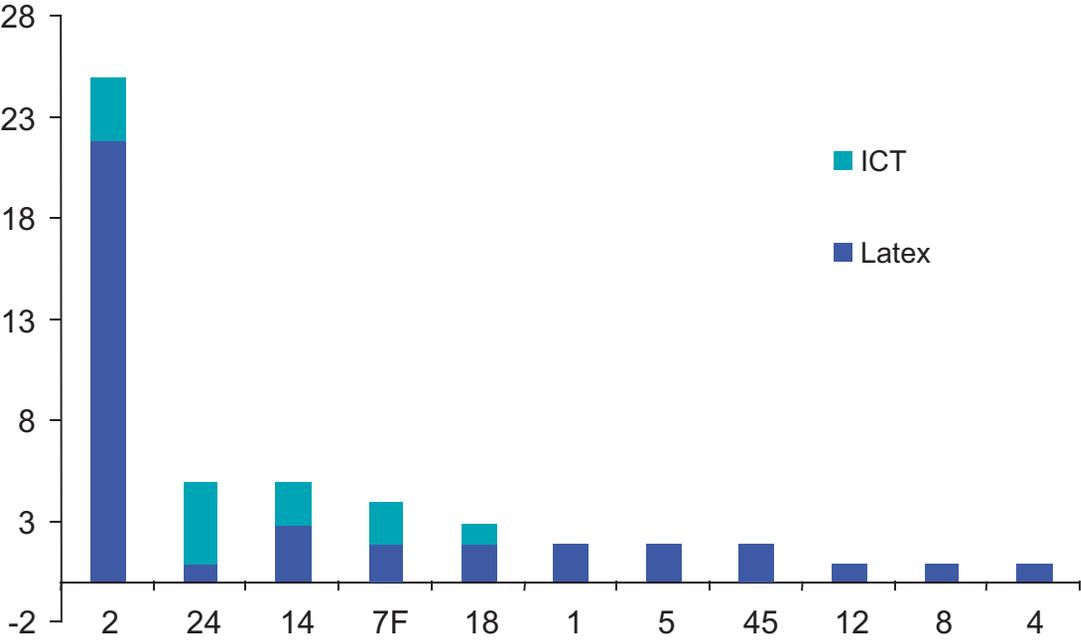


Figure-3: Serotype distribution of culture negative cases (N=51)

# Neurological and Developmental Sequels in Pneumococcal Meningitis Cases



Samir K Saha, Naila Z Khan, Nawshad U Ahmed, Ruhul Amin, M Hanif, Shamim Qazi, Paul Kilgore, Abdullah H Baqui and Meningitis Study Group, Bangladesh.

## Background

To better delineate the impact of pneumococcal diseases on survived children and on their families, it is important to assess the long term impact of pneumococcal meningitis. A multidisciplinary team in Bangladesh has been systematically evaluating the pneumococcal meningitis cases to understand the short and long term impact of the disease.

## Methods

### Patients:

★ Laboratory confirmed pneumococcal meningitis cases from Dhaka Shishu Hospital were enrolled for short and long term follow-up.

- Prospective Cohort
  - Prospectively assembled between January 2006 and March 2007
  - Subjected to short term follow up within 30-40 days of discharge
- Retrospective Cohort
  - Selected from the cases discharged prior to January 2005
  - Subjected to long term follow up, within 6-24 months from the date of discharge.



### Assessments:

#### ★ Clinical

- Complete physical examination
- Complete physical examination
- Measurement of OFC

#### ★ Neurological

- Hearing
  - Screened by oto-acoustic emissions (EOA)
  - Hearing loss above 40 dB were further tested by auditory brain stem response
  - Hearing deficit was graded as Mild (26-40), Moderate (41-60 dB), Severe (61-80 dB) and Profound (>80 dB)
- Vision
  - Papillary reaction to light
  - Ability to fix and follow the bright objects
  - Movement of the eyeballs
  - In case of abnormality, detail examination of fundus by pediatric ophthalmologist

#### ★ Developmental

- Mental
  - Bayley Scales of Infant Development (BSID)
- Psychomotor
  - Personal/social
  - Fine motor adaptive
  - Gross motor
  - Language
- Impact on family
  - Families of pneumococcal meningitis cases were anthropological assessed to understand the impact of the disease episode on other family members.

### Controls:

- ★ Age, sex, socioeconomic status and area of residence matched healthy children, without any history of meningitis were taken as controls.
- ★ The evaluators, at 30-40 days and 6-24 months were blinded about the prior history of illness of these cases.



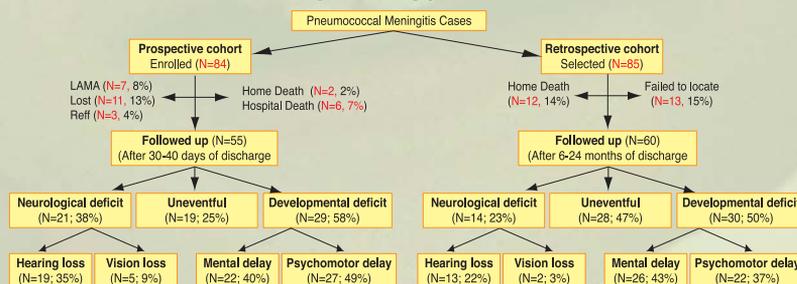
## Results

The prospective cohort included 55 pneumococcal meningitis cases who were followed up at 30-40 days after discharge. Neurological and developmental assessment of these cases revealed 35%, 9%, 40% and 49% had hearing, vision, mental and psychomotor deficits respectively.

Similar assessment of 60 retrospectively collected cases, after 6-24 months, revealed 22%, 3%, 43% and 37% had hearing, vision, mental and psychomotor deficits respectively. (Fig 1).

In comparison, only 2% of the 60 control children had developmental, mental and psychomotor delay. None of the control children had hearing or visual impairment.

Figure 1: Study profile



## Discussion

Data from this study showed that profound hearing loss and developmental delays are evident in 22% and 50% of the cases, even after more than 6 months of the disease episode. In addition, 9% and 4% of the cases of prospective and retrospective cohort had vision loss. The delineation of these high rates of residual disability is important to fully assess the impact of pneumococcal meningitis which has implications in determining the true disease burden and cost effectiveness of vaccine.

The study showed the importance of developmental assessment in the follow-up of meningitis cases to reveal the actual extent of disability caused the disease episode.

All in all, pneumococcal meningitis in children, in addition to death, may cause severe disability that impacts the family members and community.

## HUMAN FACE OF PNEUMOCOCCAL DISEASE IN ASIA

Nayeem was admitted to the Dhaka Shishu Hospital when he was five months old. He had had a high fever for eight continuous days, and seizures for two days. On the day of his admission he was diagnosed with pneumococcal meningitis and the doctors explained exactly what had happened. They also mentioned that it would have been better if they came in earlier. The parents quickly responded by saying that they had sought help immediately, however it was from an unqualified doctor and he had misdiagnosed their sons illness. This is a typical care-seeking scenario in Bangladesh.

Over next few days Nayeem's fever did go down. However, there was little pleasure taken from that improvement as it was traded for life-long complete paralysis. Over the next few days in the hospital the family desperately waited for signs of improvement. As there was no sign of improvement and they could no longer keep up with the rising medical expenses, Nayeem's parents decided to take their son home firmly believing that their son's condition would improve with proper care at home. During this time, Nayeem's father had sold off the little property that he had inherited, to pay the hospital bills.

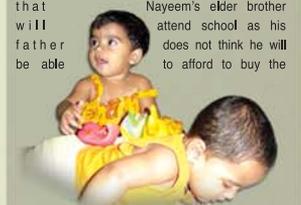
It has now been eighteen months since Nayeem's fever first broke, however he remains paralyzed in the same condition today. His twin sister, Monica, is growing quickly into a beautiful little girl.

While she spends her day crawling around the house, learning to speak and fighting with their



older brother, Nayeem, is lying on the bed, staring at the ceiling. He cannot see, hear, talk, sit or do anything that his twin sister can do.

The entire family has been impacted by this illness: Nayeem's mother is not able to fully cook or care for the family as she must attend all of Nayeem's needs; Nayeem's father is working many jobs just to support medical expenses, earning just enough extra to feed the family; and it is unlikely that Nayeem's older brother will attend school as his father does not think he will be able to afford to buy the



necessary school supplies and his mother will not have enough time to pick and drop him off at school, with Nayeem's medical requirements.

Imagine what it would have been like for everyone of the family if this episode of disease had been prevented.



## Invasive Pneumococcal Disease Burden and Implications for Vaccine Policy in Urban Bangladesh

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**Abstract.** We undertook active population-based surveillance in 5,000 urban households among children < 5 years old to determine invasive pneumococcal disease (IPD) incidence, serotype distribution, clinical presentation, and antimicrobial resistance, which have not been previously described in population-based studies from the region. IPD was documented by blood culture isolation. From 01 April 2004 to 31 March 2006, 5,903 blood cultures were collected from 6,167 eligible children. *Streptococcus pneumoniae* was isolated from 34 pneumococcal patients; IPD was clinically associated with pneumonia (24%), upper respiratory infection (62%), and febrile syndromes (14%). Overall, IPD and 13-valent serotype-related IPD incidences were 447 and 276 episodes/100,000 child-years, respectively. Peak IPD incidence occurred during the cool dry seasons. Penicillin, cotrimoxazole, chloramphenicol, and ciprofloxacin resistances were 2.9%, 82.4%, 14.7%, and 24.1%, respectively. Current conjugate vaccines should substantially reduce IPD, childhood pneumonia, and antimicrobial resistance in Bangladesh.

### INTRODUCTION

Pneumonia is the primary cause of child mortality, causing 19% of 10.6 million deaths among children < 5 years of age or 2 million deaths per year in 2000–2003<sup>1</sup>; *Streptococcus pneumoniae* (pneumococcus) contributes substantially to this disease burden.<sup>2</sup> Pneumonia is also the primary cause of childhood death in Bangladesh.<sup>3,4</sup> Published comparisons of incidence between developing and developed countries indicate that 90%<sup>5</sup> to 95%<sup>6</sup> of clinical pneumonia occurs in developing countries. Estimated pneumonia incidence among preschool children in Western countries<sup>6,7</sup> is strikingly lower than in urban Bangladesh.<sup>8</sup> In sub-Saharan Africa and North America, protein-conjugate pneumococcal vaccines showed high degrees of efficacy against pneumococcal disease burden and pneumonia.<sup>9–11</sup> However, concerns persist that, despite high pneumonia burden in Bangladesh, current protein-conjugate vaccines offer poor coverage against disease-causing serotypes.<sup>12</sup> There are no published incidence data from Bangladesh for invasive pneumococcal disease (IPD). We undertook this study to determine the IPD incidence, clinical presentation, serotype distribution, seasonality, and antimicrobial resistance (AMR) patterns associated with community-acquired disease.

### MATERIALS AND METHODS

The study was conducted in Kamalapur, an urban slum in southeast Dhaka, Bangladesh, that the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B), has used as a field site since 1998. It is an impoverished area comprised of seven communities in four municipal wards with 200,000 residents in an area of 4 km<sup>2</sup>, with a median household income of US \$50.72 (95% CI = 26.09, 144.93) and mean education of 4 years for men and 3 years for women. Pneumonia surveillance in Kamalapur has been described.<sup>8</sup>

Briefly, ICDDR,B has established a field clinic onsite staffed by project physicians, nurses, and health assistants. The clinic services all studies conducted onsite. Furthermore, Kamalapur is divided into seven geographical strata and 450 geographical clusters, each consisting of ~100 households. Using stratified cluster sampling, we identified ~5,000 households with children < 5 years old. All children < 5 years old residing in selected clusters were kept under active morbidity surveillance, after parents provided informed written consent. Children born or newly immigrated into the surveillance area were also enrolled. Each week, 40 field research assistants (FRAs) visited each household and, using standardized calendar questionnaires, asked about specific illness signs during the previous 7 days. FRAs also measured two major signs at each visit: respiratory rate for 1 minute using a timer and axillary temperature using a digital thermometer. If the respiratory rate was elevated per age,<sup>13</sup> the measurement was repeated and the mean reported as the final rate. Clinical signs were divided into major (Category A) or minor (Category B) signs. Major signs included fever (axillary temperature  $\geq 38^{\circ}\text{C}$ ), age-specific tachypnea using WHO criteria<sup>13</sup> in breaths per minute (0–2 months  $\geq 60/\text{min}$ , 2–11 months  $\geq 50/\text{min}$ , 12–59 months  $\geq 40/\text{min}$ ), danger signs (chest indrawing, lethargy, cyanosis, inability to drink, convulsions), difficult breathing, noisy breathing, and ear pain/discharge. Minor signs included cough, rhinorrhea, sore throat, myalgia/arthritis, chills, headache, irritability/decreased activity, and vomiting. Children needed one major or, if absent, two minor signs for clinic referral. Thus, the basis of FRA referrals was identification of standardized key signs and not diagnosis.

In the clinic, children were examined as follows. Nurses removed clothing from the torso to inspect for chest-indrawing and count respirations and measured every child's vital signs (axillary temperature by digital thermometer, respiratory rate for 1 minute, pulse rate, blood pressure). Project physicians used standardized diagnoses to determine whether to collect blood cultures. They diagnosed pneumonia if a child had age-specific tachypnea<sup>13</sup> and crepitations on auscultation (fine crackles on inspiration). If the child had pneumonia and chest-indrawing, they diagnosed severe pneumonia. If, be-

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sides chest-indrawing, other danger signs<sup>13</sup> were present, they diagnosed very severe pneumonia. Children were diagnosed with meningitis if they had fever and nuchal rigidity or a bulging fontanelle. Children were diagnosed with otitis media if the tympanic membrane was inflamed and/or bulging (otoscopes were not equipped to perform insufflation). Children with ear discharge were diagnosed with suppurative otitis media. Children with fever, cough, and rhinorrhea were diagnosed with upper respiratory infection (URI), including those with age-specific tachypnea but no crepitations on auscultation.

All standardized diagnoses were classified as suspected IPD, and a blood culture was obtained. Spinal taps were not done in the field clinic, but in the hospital after referral. We confirmed IPD by culture. Cultures that were negative for *S. pneumoniae* were defined as non-IPD. Blood cultures required 3 mL of blood, which was injected into pediatric isolator bottles. Blood culture specimens were sent twice daily (within 4 hours of collection) to the Clinical Microbiology Laboratory at ICDDR,B for culture by BactAlert 3D (BioMeriux, France).

Serotyping was done at Shishu Hospital Microbiology Laboratory by the capsular swelling procedure (quellung reaction) with type-specific anti-pneumococcal omni, pool, type, or group, and factor sera (Statens Seruminstitut, Copenhagen, Denmark).<sup>14</sup> ATCC strains 6314, 6301, and 10341 and Johns Hopkins University strains 9, 23, and 4 were used as known control strains. Non-typable *S. pneumoniae* strains were screened out, using omni sera, at the first step of serotyping.

Vaccine serotypes were categorized based on the following vaccine preparations: 7 valent [Wyeth]—(4, 6b, 9v, 14, 18c, 19f, and 23f); 9 valent [Wyeth]—(1, 4, 5, 6b, 9, 14, 18c, 19f, and 23f); 10 valent—[GlaxoSmithKline] (1, 4, 5, 6b, 7f, 9v, 14, 18c, 19f, and 23f); 13 valent—[Wyeth] (1, 3, 4, 5, 6a, 6b, 7f, 9v, 14, 18c, 19a, 19f, and 23f).

Drug susceptibility testing was done by screening *S. pneumoniae* strains for resistance to oxacillin, co-trimoxazole, chloramphenicol, erythromycin, ampicillin, and ceftriaxone by disk diffusion method.<sup>15</sup> Resistant and intermediate strains, based on Clinical and Laboratory Standards Institute guidelines, were subjected to an E-test (AB Biodisk, Solna, Sweden) for determination of minimum inhibitory concentration (MIC). E-tests were performed on Muller-Hinton agar (Oxoid, UK) supplemented with 5% defibrinated sheep blood. Inocula were prepared in Mueller-Hinton broth by direct suspension of pneumococcal colonies grown overnight on sheep blood agar to a density that matched a 0.5 McFarland opacity standard tube.<sup>16</sup> Results were interpreted as susceptible, intermediate, or resistant according to National Committee for Clinical Laboratory Standards—defined break points. Only the penicillin-resistant strains were tested for susceptibility to ampicillin and cephalosporins.<sup>15</sup>

Children diagnosed with pneumonia (any severity) or otitis media were placed on antibiotics. Those with very severe pneumonia, meningitis, or suspected sepsis were referred to the hospital after an initial antibiotic dose in the field. Outpatient pneumonia was treated with amoxicillin 50 mg/kg ÷ 12 hourly (twice daily) as first-line therapy. If children failed to improve after 72 hours, they were placed on Augmentin (amoxicillin clavulanate) at 50 mg/kg ÷ 12 hourly (twice daily) as second-line therapy. Children failing both first- and sec-

ond-line antibiotics (after 72 hours) or who were neonates (defined for this study as < 2 months) were referred to hospital and treated with parenteral ampicillin and gentamicin (neonates) or ceftriaxone. Children with URI were provided supportive care and daily home observation.

FRAs followed all suspected IPD patients daily at home until illness resolved. End of illness was defined by a consecutive 7-day disease-free interval, requiring the absence of elevated respiratory rate, danger signs, and fever throughout the interval. Project staff visited hospitalized children daily and continued home follow-up as described above after discharge.

After 7 disease-free days, FRAs referred children to clinic for an exit interview with the physician to document illness resolution, clinical course, and final disposition. Thus, project physicians determined all clinical assessments and outcomes.

The surveillance sample size was based on an expected rate of 0.5 episodes of clinical pneumonia per year among a cohort of 4,400 children under surveillance or 2,200 episodes/yr. Pneumonia was chosen, because it was felt to be an identifiable surrogate for IPD. We predicted a 5.0% isolation rate.<sup>17</sup> If pneumococcus caused 10% of pneumonia and had a 5.0% isolation rate, we would need 200 cases of pneumonia to find one isolate.

Statistical analysis was performed using StataSE Release 9.2.<sup>18</sup> A child's observation period began at consent and continued until the child matriculated from the age group or left the cluster. Incidence was calculated as the number of isolates over the person-years of observation. Seasonality was plotted as mean incidence per month.

The Research Review and Ethical Review Committees of ICDDR,B approved the study.

## RESULTS

Between 01 April 2004 and 31 March 2006, 6,167 children met our criteria for suspected IPD and were blood culture eligible. Of these, 5,949 submitted blood cultures; however, 3 were not tested because of insufficient volume. Thus, 5,946 blood cultures were successfully obtained (96.4%).

There were 315 bacterial isolates from blood during the period for an isolation rate of 5.3%. Of these, 34 (10.9%) were *S. pneumoniae*. Other isolations included 144 *Salmonella typhi* (45.7%), 24 *Moraxella catarrhalis* (7.6%), and 13 *Salmonella paratyphi* (4.3%). *Haemophilus influenzae* was not isolated during the study period. The contamination rate, defined as *Staphylococcus epidermidis* (21), bacillus spp. (27), and coagulase-negative staphylococci (50), was 1.6% and comparable to published outpatient rates.<sup>19</sup> There were no CSF isolates of eight CSF specimens cultured. No additional organisms were identified from specimens from which pneumococci were isolated. One child had more than one pneumococcal infection; the first at 14.7 months on 06 January 2005 (serotype 4) and the second at 17.7 months on 05 April 2005 (serotype 18F). Both final diagnoses were febrile bacteremia.

The mean age of children with culture-confirmed IPD was 14.8 ± 9.5 (SD) months compared with 24.4 ± 15.1 months for non-IPD patients ( $P < 0.001$ ). There were 14 male (41.2%) and 20 female (58.8%) IPD cases compared with 3,041 men (51.4%) and 2,871 women (48.6%) in the non-IPD group,

making IPD patients less likely to be male, although not statistically significant (OR = 0.66, 95% CI = 0.31, 1.38). Of the 5,946 blood cultured patients, 1,871 (31.5%) admitted prior medicine exposure, including 407 (6.8%) with prior antibiotic exposure. Of 34 patients with pneumococcal blood isolation, 13 (38.2%) admitted exposure to medications. These were as follows: antibiotics, one (2.9%); antihistamine, two (5.9%); homeopathic remedy, one (2.9%); paracetamol, nine (26.5%).

There were 7,600 observation years for the surveillance period. There were 3,840 clinical pneumonia cases, of which 315 (8.2%) were severe and 65 (1.7%) very severe pneumonia. The overall pneumonia incidence for the period was 50,526 episodes/100,000 child-years (0.51 episodes/child-year). There were eight confirmed meningitis cases during the period for a meningitis incidence of 105 episodes/100,000 child-years.

Ninety-six (2.6%) of all pneumonias had bacteria isolated from blood (excluding contaminants). Bacteremia incidence was 4,118 episodes/100,000 child-years. Adjusting for only focal bacteremias, incidence was  $113/7,600 \times 100,000 = 1,486$  episodes/100,000 child-years.

Overall IPD incidence was 447 episodes/100,000 child-years; vaccine type-specific incidences were 263 episodes/100,000 child-years (10-valent) and 276 episodes/100,000 (13-valent). Table 1 summarizes the vaccine serotype-specific incidence.

IPD incidence ranged from 0 in August–September to 1,150 episodes/100,000 child-years in February (Figure 1); it

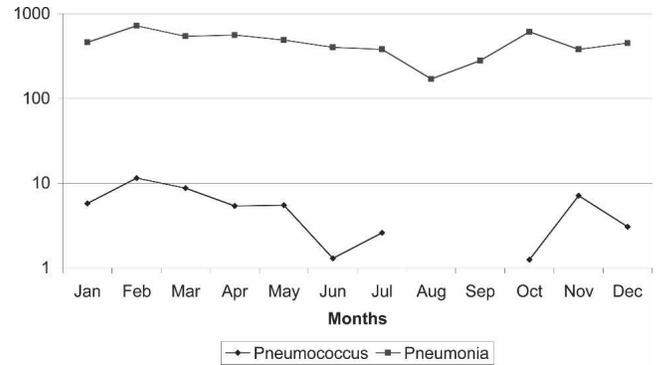


FIGURE 1. Seasonality invasive pneumococcal disease and pneumonia incidence: Kamalapur April 2004 to March 2006.

peaked during the drier, cooler months and reached a nadir during the monsoon season.

Among children with pneumococcal bacteremia, there was a 2-fold increase in the diagnosis of pneumonia between preliminary and final diagnosis (Table 2). Of patients with final pneumonia diagnoses, one was initially diagnosed as fever without localizing signs, two as URI, and one as bronchiolitis. Of eight pneumonia cases, four were severe or very severe. Four of five “other” final diagnoses were febrile bacteremia, and the fifth was otitis media; another was respiratory tract disease. Thus, 29/34 IPD cases (85.3%) had respiratory tract infections.

To compare these findings with other published reports, outcomes were re-categorized using WHO/IMCI criteria.<sup>13</sup> Using respiratory rate cut-offs and history of cough without auscultation findings, 17 (81.0%) of the URI cases and 2 (40.0%) of the “other” cases were recategorized as pneumonia, increasing the pneumonia fraction to 27 (79.4%) of all IPDs. However, for the remainder of this analysis, the term “pneumonia” will be applied to the more restrictive clinical definition, unless specified otherwise.

Table 2 shows the relationship between diagnoses and serotype distribution. Table 3 summarizes the same findings using the IMCI definitions. No meningitis cases had pneumococci isolated from their blood or CSF. There were no deaths, although five patients recovered with disability (recurrent wheezing/night-time cough).

Antimicrobial resistance revealed only modest intermediate penicillin resistance (Figure 2), because of serotype 14

TABLE 1  
Total and vaccine-related serotype distribution and incidence

Serotypes*	Count†	7-valent‡	9-valent§	10-valent¶	13-valent
1	5		**	**	**
10F	2				
12A	5				
12F	1				
14	2	**	**	**	**
18A	1	††	††	††	††
18C	2	**	**	**	**
18F	1	††	††	††	††
19A	1				**
19F	1	**	**	**	**
2	1				
23F	2	**	**	**	**
25	1				
38	1				
4	2	**	**	**	**
45	2				
5	1		**	**	**
6B	1	**	**	**	**
9V	2	††	††	††	††
Total serotype-specific	34				
Number of vaccine serotypes		14	20	20	21
Proportion of vaccine serotypes		41.2%	58.8%	58.8%	61.8%
Incidence/1,000 child-years		1.8	2.6	2.6	2.8

\* All serotypes isolated between 01 April 2004 and 31 March 2006.  
 † The number of isolates in each serotype.  
 ‡ The Wyeth 7-valent vaccine.  
 § The Wyeth 9-valent vaccine.  
 ¶ The GSK 10-valent vaccine.  
 || The Wyeth 13-valent vaccine.  
 \*\* Full coverage.  
 †† Partial coverage.

TABLE 2  
Distribution of preliminary and final diagnoses among children with pneumococcal blood isolates

Diagnostic category*	Preliminary diagnosis, † N (%)	Final diagnosis‡, N (%)	Serotype§
Pneumonia	4 (11.8)	8 (23.5)	<b>1, 4, 12A, 12F, 18A, 18F, 23F, 25</b>
URI	26 (76.4)	21 (61.8)	<b>1, 4, 5, 10F, 12A, 14, 18F, 19F, 23F, 38, 45, 6B, 9V</b>
Other	4 (11.8)	5 (14.7)	2, 12F, <b>14, 18C, 19A</b>

\* Provided by project medical officers.  
 † Diagnosis at time of clinical presentation before blood culture result.  
 ‡ Diagnosis provided at exit interview after blood culture results and adjusted using modified WHO classification for pneumonia with auscultation findings.  
 § All serotypes isolated between April 2004 and March 2006. Numbers in bold are vaccine serotypes.

TABLE 3  
Distribution of IMCI diagnoses among children with pneumococcal blood isolates

IMCI clinical syndrome*	N (%)	Serotypes
Very severe pneumonia	1 (3)	12A
Severe pneumonia	9 (26)	<b>1, 23F, 14</b> , 10F, <b>5, 18F</b> , 12A, <b>9V</b>
Pneumonia	17 (50)	<b>1, 25, 12F, 4, 18A, 9V</b> , 12A, <b>23F, 19A, 6B, 38, 19F, 45</b>
Non-pneumonia (bacteremia)	1 (3)	<b>18C</b>
Cough or cold	5 (15)	12A, 10F, <b>14, 45, 1</b>
Acute ear infection	1 (3)	2
Total	34 (100)	

\* World Health Organization, Dept. of Child and Adolescent Health and Development, and UNICEF. Management of the child with a serious infection or severe malnutrition: guidelines for care at the first-referral level in developing countries. 2000, Geneva: Dept. of Child and Adolescent Health and Development UNICEF. 162.  
Bold indicates vaccine serotypes.

isolated from an 11.6-month-old girl with febrile bacteremia. Cotrimoxazole resistance was high, involving many vaccine serotypes (1, 4, 5, 6A, 9V, 18C, 19F, and 23F) and seven of eight (including all severe/very severe) pneumonia cases. Chloramphenicol resistance was low. Fluoroquinolone resistance was present, involving vaccine serotypes 1, 14, and 9V. One fluoroquinolone-resistant isolate was also resistant to cotrimoxazole and chloramphenicol.

## DISCUSSION

This study reported high rates of both bacteremia and invasive pneumococcal disease in a pediatric population with high pneumonia incidence. A distinguishing feature of this study is that its findings are based on active community- and population-based surveillance. Another important feature is that it successfully obtained blood cultures from > 96% of suspected IPD cases. The results therefore represent a comprehensive population-based burden assessment. This likely explains the broader clinical syndrome distribution and total burden estimate than would have been possible with hospital surveillance.

This study underscores how surveillance methodology affects clinical syndrome distribution estimates. First, using a standardized but strict set of clinical criteria, 24% of the IPD cases were categorized as pneumonia and 62% as URI. The proportion of pneumonia is comparable or greater than that

from recent studies of similar age groups.<sup>9,20–23</sup> Using standard WHO definitions,<sup>24</sup> nearly 80% of IPD cases in this study were classified as pneumonia, and 29% were classified as severe or very severe pneumonia. The rationale for using the *sensu stricto* pneumonia definition is that fast-breathing with or without chest indrawing may represent non-pneumonia illness,<sup>25</sup> whereas ascertainment of fluid in the lungs (as identified by crepitations during auscultation) may reflect vaccine-preventable pneumonia that will present to a health facility. The more generous definition may overestimate potential vaccine pneumonia impact and underestimate empirical efficacy against clinical pneumonia through misclassification.

Second, as shown by the different definitions of pneumonia and URI, disease severity is subject to interpretation, which influences health-seeking behavior,<sup>26</sup> including hospitalization. Many of these cases of pneumonia and URI would not have presented to hospitals. Active surveillance resulted in early case detection, in which most IPD cases with respiratory disease (including those with tachypnea) had no documented fluid in the lungs. Four URI patients subsequently progressed to clinical pneumonia. Whether this indicates that the bacteremic phase of IPD is largely pre-pneumonic is uncertain. It does indicate that surveillance of only more severe infections would underestimate IPD. This is consistent with a study in Kenya that obtained blood cultures from 10% of children presenting to an outpatient clinic.<sup>21</sup> Clinically significant bacteremia was twice as high and pneumococcal bacteremia four times as high as estimated from hospital surveillance of more severe cases.

These additional cases identified through active surveillance are important, not only because they expand burden estimates, but because these cases are exposed to outpatient antimicrobial agents. This broader base of antibiotic use is also associated with antimicrobial resistance<sup>10,27–29</sup> and should be factored into a decision to introduce vaccine.

This study showed total and vaccine serotype-specific invasive pneumococcal disease incidence nearly identical to that in the Gambian vaccine trial<sup>9</sup> and comparable to total and non-ocult bacteremia incidences in Kenya.<sup>21</sup>

Our serotype distribution data differ notably from previous IPD reports in Bangladesh, which indicated poor vaccine coverage. Earlier reported studies were either clinic-<sup>30</sup> or hospital-based,<sup>12,14,31</sup> and only one included pneumonia patients.<sup>14</sup> Thus, blood isolate data for respiratory diseases were under-represented. In contrast, 85% of the Kamalapur isolates (97% using IMCI criteria) were associated with respiratory tract infections. The difference in syndromic distribution and sample collection practices between the hospital and population-based studies likely explains the difference in serotype distribution<sup>32</sup> and vaccine coverage estimates.

IPD burden in this study is likely underestimated. Blood cultures are insensitive. In the Gambian study, which reported identical IPD rates to ours by blood culture, the 9-valent pneumococcal conjugate vaccine reduced radiographic pneumonia by 37% and radiographic severe pneumonia by 35% (per protocol analysis),<sup>9</sup> showing that the preventable pneumococcal disease burden is likely orders of magnitude greater than what is microbiologically detectable. Hib vaccine studies also show blood culture is insensitive,<sup>33–35</sup> resulting in substantial disease burden underestimation.<sup>36</sup> Although a vaccine probe study might better establish disease burden,

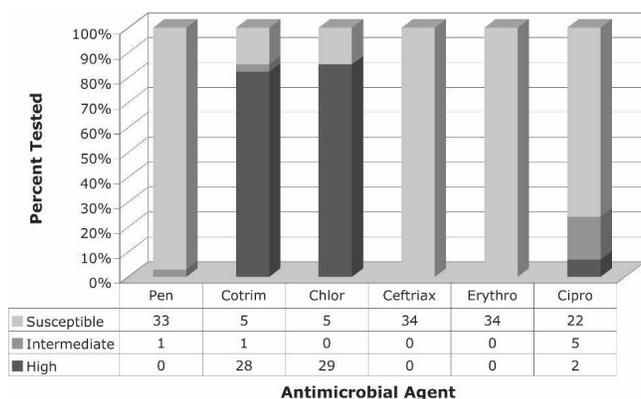


FIGURE 2. *Streptococcus pneumoniae* antimicrobial resistance pattern: Kamalapur April 2004 to March 2006. AMR pattern for all *S. pneumoniae* isolates.

factors related to pneumococcal disease incidence, such as serotype replacement, would require additional observation and cautious interpretation,<sup>37</sup> which would be facilitated by ongoing surveillance.

The absence of meningitis isolates may be caused by several factors. First, lumbar punctures are all referred to hospitals. A blood culture is drawn, and following IMCI guidelines, children are given an antibiotic dose and referred.<sup>13</sup> This prior antibiotic exposure may compromise the CSF culture.<sup>38</sup> Second, of 18 children referred to hospitals for suspected meningitis, only 8 (44%) had CSF collected. Local physicians are commonly reluctant to perform lumbar punctures on ill children and frequently diagnose them as sepsis, thus underdiagnosing childhood meningitis. Another factor is that isolation is related to the type of CSF specimen. Among hospitalized children < 5 years of age with meningitis, one study reported pneumococci associated from 94 of 412 pyogenic CSF samples (22.8%).<sup>14</sup> It is not known what proportion of the cases from Kamalapur had pyogenic CSF, but if even one in five had it, we would have required at least 22 meningitis cases to have 90% power to detect a single pneumococcal isolate. Active surveillance, with early case detection and intervention, may halt progression to meningitis in some cases. Finally, meningitis may simply be relatively uncommon in IPD for this population.

The ratio of pneumococcal pneumonia to meningitis has implications for vaccine impact on IPD: the larger the proportion of pneumonia, the larger the impact on IPD. Given the Bangladesh population of 140 million, if 11.5% are children < 5 years of age, if the mean national childhood pneumonia incidence is one half of the Kamalapur rate (i.e., 0.26 episodes/child/yr), and if vaccine efficacy of any valence is at least 25% against pneumonia, it should prevent > 1 million cases of pediatric pneumonia per year in Bangladesh. Data from regions with similar burden suggest that this is a reasonable effect size estimate.<sup>9,10</sup> Furthermore, the Gambian study reported a vaccine-related 16% reduction in all-cause mortality, despite only a 6% reduction in clinical pneumonia (intention-to-treat analysis), indicating that pneumonia and community-acquired bacteremia may contribute more to childhood mortality than previously recognized from direct assessments,<sup>9</sup> a finding that our data support, and suggesting a potentially substantial impact on overall childhood mortality in this population.

Antimicrobial resistance data indicate low penicillin resistance, similar to hospital findings,<sup>12,14,31</sup> that is seen only in vaccine serotype (14), an association previously reported from Bangladesh<sup>12,31</sup> and elsewhere.<sup>39–44</sup> Of note, fluoroquinolone resistance has risen from 3.9% and 0% high and intermediate resistance, respectively, in early 2005 to 6.9% and 17.2%, respectively, in 2006, and should be monitored. A possible explanation is that ciprofloxacin, which is manufactured locally, is often provided to young children for febrile and respiratory illnesses. The introduction of an efficacious pneumococcal vaccine should lower the overall prevalence of antimicrobial-resistant IPD.<sup>10,45</sup>

Limitations to this study include reliance on blood isolation and lack of access to non-pretreated CSF specimens, both of which combine to underestimate burden; limited 2-year duration of data collection, which may have missed important serotype distribution and AMR trends; and the number of

culture-confirmed cases, potentially limiting the power to detect serotype and syndrome-specific trends.

IPD in urban Bangladesh seems to be primarily associated with respiratory illnesses and contributes to the pneumonia disease burden in the community. The high IPD rates indicate that introduction of a protein conjugate pneumococcal vaccine would substantially reduce both childhood illness and related antimicrobial resistance.

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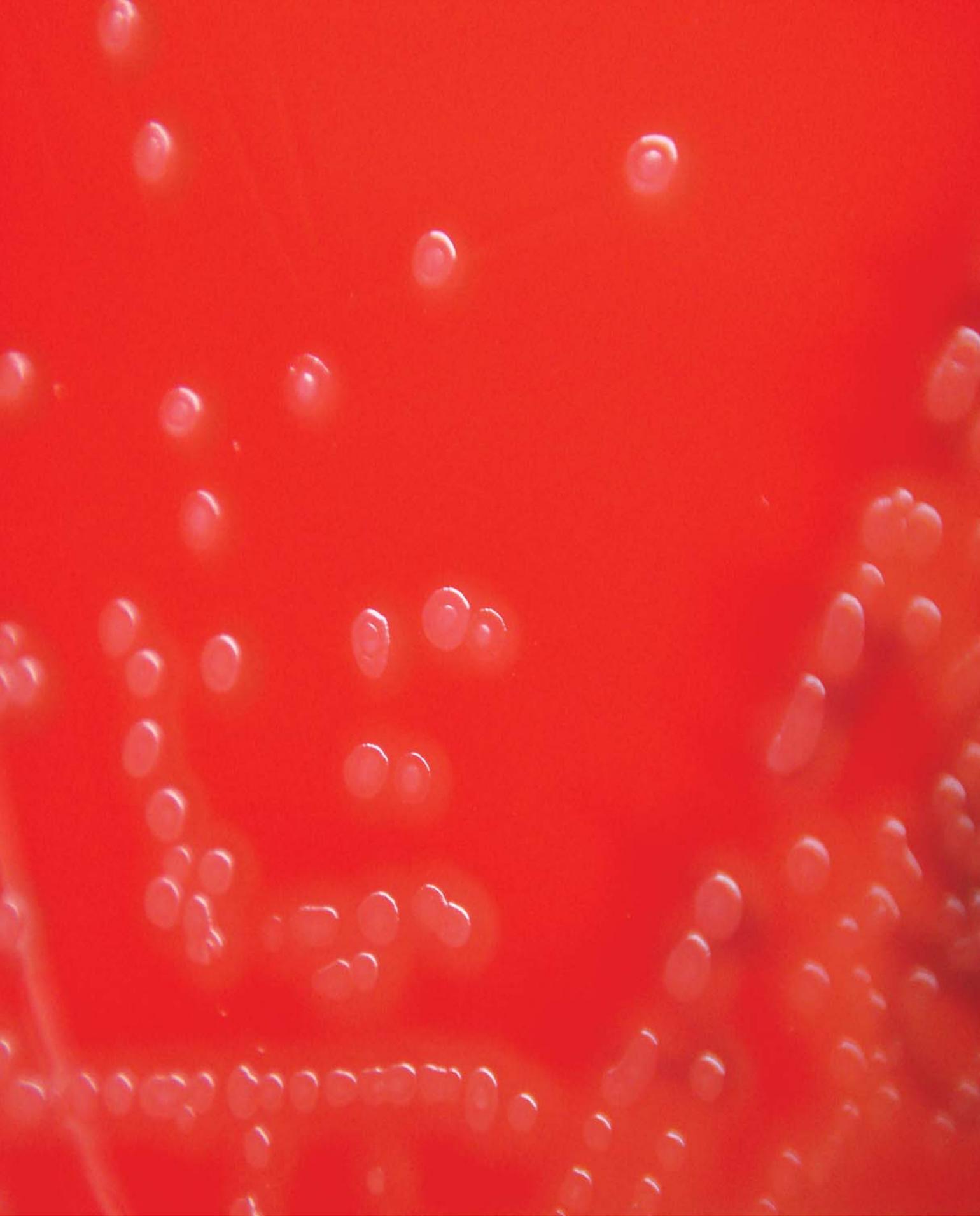
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