Original Article

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Bacterial Etiology and Serotype Distribution of Streptococcus Pneumoniae in Acute Respiratory Infections among Displaced Rohingya Population Settled in Humanitarian Refugee Camps of Cox's Bazar, Bangladesh

Md. Shakeel Ahmed[1*] Zahirul Islam[2] Md. Zakir Hossain[3] Mamunur Rashid[4] Rumana Rashid[5] Kuldeep Sharma[6]

ABSTRACT

Background: *Morbidity and mortality are a common cause of Acute Respiratory Infections (ARIs)in refugee populations. This study was carried out to examine the bacterial etiology and track the distribution patterns of Streptococcus pneumoniae serotype among the Displaced Rohingya Population (DRP) in Cox's Bazar, Bangladesh.*

Materials and methods: *It was a prospective case-control study of ARI conducted among the DRP. A total of 538 nasopharyngeal samples (NPS) of ARI cases were enrolled according to WHO criteria and also enrolled age-sex matched 514 controls in the Displaced Rohingya Population (DRP) settled in refugee camps in Cox'sBazar, Bangladesh, between June 2018 to March 2020.*

Results: *The study data showed that in both the ARI cases and control group, children ≤ 5 years of age had a higher bacterial positivity (90%) than those >5 years of age.S. pneumonia was found predominantly in both cases and controls (77% and 78%). In the serotype distribution pattern of S.pneumoniae, 6AB (12.8%), 23F (9.3%), 15B/C (8.3%), 19A (8.07%), 11A (7.3%), 35B (7.1%), 13 (6.08%), 34 (5.3%) serotypes were found in greater proportions which covered 64.25% of all isolates and 1, 21, 19F, 2, 4, 33F, 39, 8 serotypes were found in less proportions.*

Conclusion: *Overall, these comprehensive data on the specific causes and findings related to the health situation, especially in crisis contexts such as the Rohingya refugees in Cox's Bazar, Bangladesh are vital for effective disease management, prevention control and vaccination strategies in humanitarian crisis.*

Key words : Acute respiratory infection; Bacterial etiology; Pneumonia.

Introduction

Pneumonia, a severe type of lower acute respiratory infection, remains to contribute to child mortality. In 2022, global data showed 20% of deaths among children under five years of age.[1] Pneumonia is caused by several microbes, like bacteria, viruses, fungi and parasites. The most commonly known bacteria related to Acute Respiratory Infections (ARI) particularly pneumonia are Streptococcus pneumoniae, Haemophilus influenzae type b and Staphylococcus aureus. Other atypical bacteria such as Mycoplasma pneumoniae, Chlamydophila pneumoniae and Klebsiella pneumaniae are less frequently reported.[2,3] Overcrowded spaces, fires indoors, cold weather and insufficient shelter and blankets all generate perfect circumstances for the spread of respiratory droplets. Besides, when people cough or sneeze in huge gatherings, the risk of acute respiratory infections further increases due to the aerosol transmission of respiratory secretions.

Malnutrition, inadequate sanitation and stress can play a role in disease progression in complex emergencies.[1,4] Bacterial pneumonia presents largely uniform clinical symptoms, regardless of the specific causative agents. This likeness poses a diagnostic encounter in the patient's management of Acute Respiratory Infections (ARIs) making it tough for clinicians to find the most applicable way of treatment. Relying solely on clinical presentation to determine treatment can result in the overuse or improper use of medications.[5,6,7] In daily primary health care, particularly within refugee settings, there is limited understanding of the causes of Acute Respiratory Infections (ARIs) due to data scarcity.[8] S. pneumoniae is one of the main bacterial pathogens that cause ARIs and pneumonia-related deaths globally.[2,9,10] So far, 98-100 pneumococcal capsular serotypes have

1. Professor of Microbiology & Immunology
Bangladesh Institute of Tropical and Infectious Diseases (BITID) Chattogram.
2. Senior Research Officer
Institute for Developing Science and Health initiatives, Dhaka.
3. Assistant Professor of Microbiology & Immunology
Bangladesh Institute of Tropical and Infectious Diseases (BITID) Chattogram.
4. Professor of Clinical Tropical Medicine
Bangladesh Institute of Tropical and Infectious Diseases (BITID) Chattogram..
5. Assistant Professor of Epidemiology & Community Medicine
Bangladesh Institute of Tropical and Infectious Diseases (BITID) Chattogram.
6. Medical Technologist of Laboratory
Bangladesh Institute of Tropical and Infectious Diseases (BITID) Chattogram.

*Correspondence : **Dr. Md. Shakeel Ahmed**
Cell : +88 01715 02 30
Email : shakeelcmc@gmail.com

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been recognized.[11,12] For the prevention of S. pneumoniae infections, the current use of Pneumococcal Conjugate Vaccines (PCV) are PCV10 and PCV13. However, it's key to note that these vaccines give protection against a limited number of pneumococcal serotypes and do not defend against non-vaccine serotypes. To mitigate this limitation, innovative and cost-effective pneumococcal vaccines are urgently needed that can provide comprehensive serotype coverage. To decrease morbidity and mortality related to respiratory infections, it is crucial to carry out ongoing surveillance, improve diagnostic capabilities, and create complete profiles of serotype distribution. In the circumstance of existing knowledge, no studies have been carried out to examine the bacterial reasons for ARIs and the patterns of S. pneumoniae serotype distribution in refugee settings, focusing on the Displaced Rohingya Population (DRP). In today's context, conventional culture methods are still used in resource-limited settings. But there are significant limitations such asp oor sensitivity, the risk of inhibiting the growth of pathogens due to recent antibiotic therapy and slow turn around times.[13,14] Recently, the development of multiplexed molecular tests has offered laboratories an ample diagnostic solution. This solution provides the required turn around time, sensitivity, specificity and coverage of respiratory pathogens to meet the decision-making needs of physicians.[14] In the context of the management of ARI cases, it is essential to have country-specific etiological data. However, in the circumstances of the DRP camp in Bangladesh, there is a notable absence of research focusing on the etiology of pneumonia. To overcome this gap, the current case-control study was carried out to collect data on the predominant bacterial agents responsible for ARI and also to see the distribution patterns of S. pneumoniae serotypes.

Materials and methods

It was a prospective case-control study of Acute Respiratory Infections (ARI) among the Displaced Rohingya Population (DRP) carried out from June 2018 to March 2020 in Cox's Bazar, Bangladesh.The study got ethical approval from both the National Research Ethics Committee (NREC) of the Bangladesh Medical Research Council (BMRC) and the Directorate General of Health Services (DGHS) in Bangladesh. Informed written consent was received from all participants. For child participants, written consent was obtained from their parents or legal guardians. Flow chart for selection criteria of cases and control group illustrated (Figure 1).

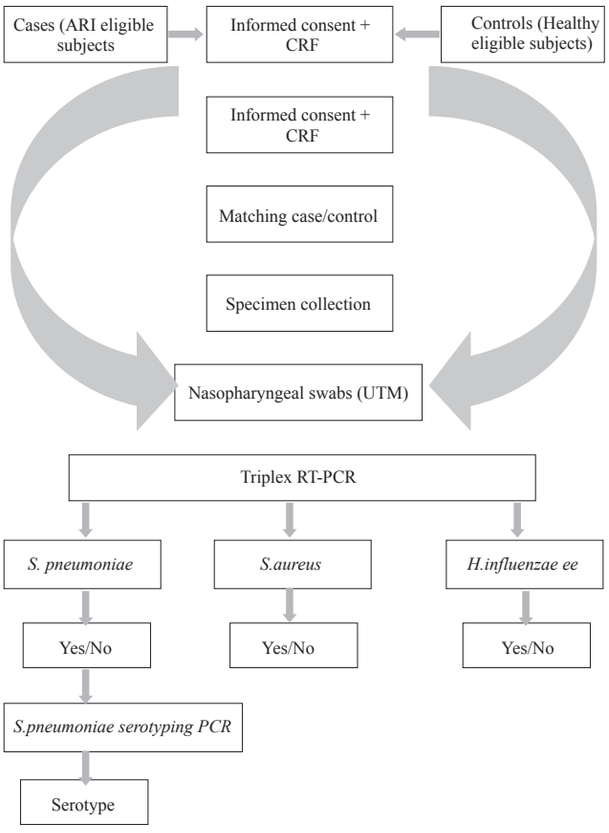

Figure 1 Flow chart for selection cases and controls group of ARI

Inclusion criteria

A total of 538 cases of ARI were registered and the inclusion criteria were those Rohingya individuals, who had presented at least one of the clinical signs and symptoms by the WHO criteria: cough or dyspnea, onset within the last 10 days,history of fever for ≤7 days or current fever measured axillary of ≥37.5 C, lower chest wall indrawing (Expected in children ≤3 years old only) or tachypnea. In this study, we included a control group (n=514) and the inclusion criteria who were carefully matched in the catchment area, age and time the previous case enrolled. These control group participants were Rohingya individuals aged ≥3 months and exhibited no symptoms of acute or chronic infections, including respiratory, Chronic Obstructive Bronchopneumonia (COBP) digestive and cutaneous conditions.

Exclusion criteria

The exclusion criteria were wheezing suggestive of asthma at auscultation and that resolves after bronchodilator therapy, a 30 days exclusion period following the date of discharge from the hospital, on previously prescribed asthma or corticosteroids treatment, received recent intravenous antibiotic therapy and lack of informed consent.

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Universal Transport Media (UTM) was used to collect the nasopharyngeal swab samples. The collected samples were placed instantly at 2–8°C for transportation and further identification of bacterial pathogens, the samples were preserved at -70°C following nucleic acid extraction and were also subsequently analyzed using Real-Time PCR (qPCR).

Assay Technique

DNA Extraction

QIAamp DNA Mini kit (Qiagen, Germany) was used to extract bacterial DNA from nasopharyngeal swab samples. The extraction was carried out as per the manufacturer's guidelines.With a final elution volume of 100µL, the DNA was preserved in 1.5mL Eppendorf tubes at -20°C until PCR amplification.

DNA Detection via real time PCR

Bacterial DNA is amplified by real-time triplex PCR assay which detects three bacteria including *S. pneumoniae, S. aureus* and H. influenza type B. Briefly, 5µl cell lysate was added to 13µl PCR master mix (9µl Takyon master mix + 4µl primers and probes) to carry out amplification on Bio-Rad CFX96 touch real-time PCR (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The manufacturer's guidelines were carried out for conducting the whole process .qPCR result was reported as positive when the Cycle threshold (Ct) value for targets was ≤40. If the Ct was >40 or undetectable, the result was considered negative.

Primers and Probes design for *S. pneumoniae* serotyping

This study incorporates a multiplex PCR assay that consists of an internal positive control. This control specifically targets the lytA gene that is found in various strains of pneumococci. The lytA gene-specific primers and probes are unique to *S. pneumoniae* and do not complement other closely linked bacteria. The primer sets and probes designed for each of the 40 serotypes were organized into 11 multiplex reactions (Table I). The probes were labeled at the 5' end with four distinct Fluorophores: Carboxy-fluorescein (FAM) Hexachloro-6-carboxy-fluorescein (HEX) Texas red (Sulforhodamine 101 acid chloride) or Cyanine 5 and at the 3' end with two distinct black hole quenchers: BHQ1 and BHQ2. For the design of the probe, Locked Nucleic Acid (LNA) chemistry was employed.[15] Using a maximum of four probes and primer pairs per multiplex reaction, the best multiplex PCR combinations were created using Beacon Designer TM software (PREMIER Biosoft International, Palo Alto, USA). The primers and probe sequences of the pools used in the molecular quantitative typing assay are detailed in Table II.

Table I Distribution of the 40 S. pneumoniae serotypes

Pool	Serotype name	Fluorophore name
1	19A	Cy5
	22F	HEX
	3	FAM
	6AB	Texas Red
2	14	Cy5
	4	HEX
	12F	FAM
	9V	Texas Red
3	23F	Cy5
	11A	HEX
	33F	FAM
	7F	Texas Red
4	16F	Cy5
	35B	HEX
	Sg18	FAM
	19F	Texas Red
5	15B/C	Cy5
	31	HEX
	38	FAM
	8	Texas Red
6	10A	Cy5
	35F	HEX
	1	FAM
	34	Texas Red
7	15A	Cy5
	17F	HEX
	20	FAM
	7C	Texas Red
8	LytA	FAM
	5	Texas Red
9	23B	Cy5
	35A	HEX
	6C	FAM
	9N/L	Texas Red
10	10F	Cy5
	2	HEX
	23A	FAM
	Sg24	Texas Red
11	13	Cy5
	21	HEX
	39	FAM

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Table II

Primer and Probe sequences used for S.pneumoniae serotyping detection by qPCR

Pool	Primer' name	Sequence (5'-3')
1	19A -F1[a]	ACTACTGTTCAAGAGGATTATACAC
	19A -R1[b]	CATTCAATACTCGTTTAACTGCTC
	19A-P1[c]	TGTCTCATCGGTTCGCC
	22F-F1	ATTTACCCATCATCACAACTATTTG
	22F-R1	TACCGTTTATACCATCTTGAATCAG
	22F-P1	CTCTCCTCCAGCACTTGC
	3-F1	GAAAACAAGGAAAGATGGAACTTC
	3-R1	ATTACAACAAAGGGATCGTCAC
	3-P1	AGAACAGCGTCACCAAGA
	6AB-F1	AGATGGTTCCTTCAGTTGATATTG
	6AB-R1	ATTATGTCCATGTCTTCGATACAAG
	6AB-P1	CTCAGGGCAGAACAACAC
2	14-F1	CGTCTTTTTGTATGGTGCTATG
	14-R1	GCCAATTAAATGCTTTACTCAAATC
	14-P1	ACACTTGAACAGCCAATCC
	4-F1	GGGGAAGTATTTTCAGAGTCG
	4-R1	AATCACCAACTAACCATCCAATAG
	4-P1	TGACCAGCCTAACAGTAGC
	12F-F1	TGATATGCGTGAATCTCCAAC
	12F-R1	ACTGATTCGCCACAACATC
	12F-P1	AGAATCTATGAGCCGCCA
	9V-F1	TCCTCAGTCAATTTTAACAAGAAAC
	9V-R1	AGAGAATATACCCCGAAATCATG
	9V-P1	CCAGCACAACACCAATAAC
3	23F-F1	TAGTGACAGCAACGACAATAG
	23F-R1	AAAACAAATGAAACCTATCTGATTC
	23F-P1	TCACAACACCTAACACTCG
	11A-F1	CATTGTGTATGCTACCATTCTTC
	11A-R1	GTGCTAACTGTAAAACTTGATTATG
	11A-P1	TCTCCCAATTTCTGCCACG
	33F-F1	AGATTAGATGGTTTGCTGGAC
	33F-R1	CAGAATAACTGATACCACAAGTAAC
	33F-P1	AGTCGCCCATTTCCCT
	7F-F1	TTGATGAAGGCTTTGGTTTG
	7F-R1	CCATCAATTGCATATTCAAATACAC
	7F-P1	ACTAACGCACAGCCACAA
4	16F-F1	TTCGCAAGGAGAGATTACTG
	16F-R1	TTGGGAAGGATATCCTATTTTAATC
	16F-P1	TTTAACACCCACGCCTGAA
	35B-F1	ATTGCAAATATTCGACTACCTAG
	35B-R1	ATATGAACTTTTTCCTTTTGCTAG
	35B-P1	CCGCTATAACCACTCCATC
	Sg18-F1	TGGCTAGAACAGATTTATGGAAG
	Sg18-R1	AAAGTCATCCAATTATTATCCATCC
	Sg18-P1	CAAACCCTATCCCTCTCCC
	19F-F1	CTTTACAGGAAGGAAATATACAACC
	19F-R1	CGTCAATAACACGAATGAGAAC
	19F-P1	TCGCACTGTCAATTCACC
5	15B/C-F1	TTTGCTACAGGTTTTAGTATTGAG
	15B/C-R1	AAAGCAATATAAGAGGTATAGTTGG
	15B/C-P1	CGCTACAATCATCCGCT
	31-F1	TTAAGAATCTTGTTTCGAATGAGAC
	31-R1	TGTTAAAAGAGTACTCTGATACCAG
	31-P1	AACACACTCGCCGATAAA
	38-F1	GAAGCCTACCTACAATAACAGTG
	38-R1	CAGCCATTATACTCCAACCTAAG
	38-P1	TGCTCCAAGTTTCCTCAG
	8-F1	AGGAGCCTATGATTTAATAGATGC
	8-R1	TTGATATCTTTTGACGTATGTCTTC
	8-P1	TTCTCCATCTCCAGCCATT
6	10A-F1	GGGAGTCTACAATTACTTCGC
	10A-R1	GCATAACGAAATCCTAACATCTC
	10A-P1	TCCAGTGCAATACATTCCA
	35F-F1	ATTAGGTGGTCGTATATACTTGATG
	35F-R1	ACATACAGATAGGTCTGATAGTTTC
	35F-P1	TCAGACCATTTCCATTCCA
	1-F1	TACGGGGTATTCTAATCAAACTTG
	1-R1	AGCCTGTTAGCATAAAAATCATTAC
	1-P1	TCACATATCCCTCTCCCAC
	34-F1	CTTATTGTTGTAGTGGCAGTTG
	34-R1	CTTGAATAGTTCTCGATTAAGAGC
	34-P1	CCATCTTGACCTACTCCA
7	15A-F1	CGTTATTTAGTGAATTGCTATACTC
	15A-R1	TCCCTGCAGAATAAGAATCTAC
	15A-P1	TACTGCTGCTGCCAACA
	17F-F1	TGATTCGTGATGATAATTCCAATG
	17F-R1	TTTGTCTAACATCGTTTAATAACCC
	17F-P1	AGGCTCCATGATATTCCGA
	20-F1	GAGGATGAACTTATTTCTAAAGGAG
	20-R1	TCTTTGAAGAATCTATACATTTCCC
	20-P1	TTACTTCTCGCTGTCAGGT
	7C-F1	TTTCAACAACGGAAGGTTTTG
	7C-R1	CTTCTGTTATTAATTCTGGACTAGC
	7C-P1	CACAAAGACCGTTCGCT
8	lytA-F1	GCGGAAAGACCCAGAATTAG
	lytA-R1	GAATGGCTTTCAATCAGTTCAAC
	lytA-P1	TCTCAGCATTCCAACCGC
	5-F1	AGTAGCGGATTGCTGATTTAC
	5-R1	ACGTCTCCTGAAACCATATATAAAC
	5-P1	CTAACAGAACTGCTCGTGA
9	23B -F1	AATCTGCTTTGGTTGGAATG
	23B -R1	AACTAGCTATCATAGTTGAGATTG
	23B -P1	ATCCCCTGTAGTCCCAT
	35A-F1	ATCATAAGGTAGTCAATAAGATGC
	35A -R1	TGAACAAAATCATCATTCACAATC
	35A -P1	ATCCCTGCCATAATCGG
	6C/D-F1	TTTTGTTATTGTCGGCGATG
	6C/D -R1	ATTGAACTGAGCTAAATAATCCTC

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Pool	Primer' name	Sequence (5'-3')
	6C/D -P1	TTATCCACCCACCCTGT
	9N/L-F1	AATTGTACCGCAAGCTATTC
	9N/L-R1	TTAGAGAATAGACACCAAAATGTG
	9N/L-P1	AATAACGCCCACCAAG
10	10F-F1	ATCCTTCTGATTTTGGTAACTTCC
	10F-R1	TGAGGAAGGACATCTCATGC
	10F-P1	CCGCTTTGTGTTCACCT
	2-F1	CTGCCTATATTTTGATGTGTTTGC
	2-R1	TTCTAAGAGTTCCAATACGTTGAC
	2-P1	AACCATCAGCCAGTCCA
	23A-F1	CTATTCTAGCAAGTGACGAAGATG
	23A-R1	GCAGAACTTGTAGTGTGACAG
	23A-P1	ATCCGCTCCAAATCCCA
	Sg24-F1	TGTGGTTTTCAGGACTTATTGC
	Sg24-R1	TTGACTTTATCATAGGTCGGAAAG
11	13-F1	AGGTGTAATCTCTATCTTCC
	13-R1	AAATGATCTCTACCAATAAATTC
	13-P1	AGGCAACCACATACTTA
	21-F1	AAGTGATTATAAGTCTGTGAAG
	21-R1	AAAAGAATTGAACAAAGTCG
	21-P1	ATCAACATCCCAGCAA
	39-F1	CAGTCTTATTACTCCCAATAG
	39-R1	AAATAAATAAGCTGCTTTATAATG
	39-P1	AGGCTCCATCATCAGTA

MultiplexPneumoniae Serotyping Real-time PCR

Eleven multiplex tubes were used for the *S. pneumoniae* serotype real-time PCR typing experiment (40-PCR) one of which contained the lytA positive control.[16] A CFX96 thermal cycler (BioRad, Hercules, CA, USA) was used for multiplex PCR reactions. The reaction mixtures contained 5 µl of template DNA, 9µl Takyon master mix and 4µl each primer and probe. One sample is tested with the 11 pools. So 55µl of each sample is required for typing. The reaction conditions were: 95°C for 5 min, then 40 cycles of 95°C for 15 sec and 60°C for 60 sec. The S. pneumoniae serotype identification and curve analyses were carried out with the aid of CFX Manager Software version 3.1 (Bio-Rad). The Cycle Threshold (CT) was used to express the results.

The study participants were defined using descriptive statistics according to several attributes and the frequency of each attribute was reported as a percentage. The chi-square test was used to compare characteristics and pathogen detection. A significant level of P value was considered at <0.05. Statistical Package for Social Science (SPSS) Version 23.0 from IBM Corporation was used for statistical analysis.

Results

A total of 538 cases were examined, of which 54% (291) were male and 46% (247) were female. According to age group, it was found 59% (320) were ≤5 years of age and 41% (218) were above 5 years among these cases. Furthermore, 514 control participants were included in the study (Table III). Cough, especially dry cough, was the most common symptom in enrolled cases and according to case definition criteria by WHO, about 97% of cases were classified as pneumonia.[1] Other symptoms were dyspnea, tachypnea and the lower chest wall in drawing (Table III).

Table III The demographic and clinical characteristics of ARI cases (n = 538) and the controls (n = 514) group

Gender	Cases (n/%)	Controls (n/%)	Total
Male	291 (54%)	254 (49%)	545 (51.8%)
Female	247 (46%)	260 (51%)	507 (48.2%)
Age group			
≤5 years	320 (59%)	299 (58%)	619 (58.8%)
<5 years	218 (41%)	215 (42%)	433 (41.2%)
Symptoms			
Cough	538 (100%)		
Productive	52 (9%)		
Dry	486 (90%)		
Dyspnea	534 (99%)		
Fever (temperature)			
≥102.2	20 (3%)		
<102.2	518 (96%)		
Tachypnea			
<1 year (>50 breaths per minute)	17 (94%)		
1 to 5 years (>40 breaths per minute)	229 (87%)		
>5to 18 years (>30 breaths per minute)	215 (89%)		
Lower chest in drawing (<3 years of age)	135 (25%)		
Oxygen saturation			
<95	15 (2%)		
≥95	523 (97%)		

Overall, the cases and controls showed a similar percentage of bacterial pathogens, accounting for 85% and 88% respectively. This study found no significant variances between the two groups in the existence of at least three tested bacteria. Similar trends also observed in terms of gender and age distribution. However, children aged ≤5 years were more inclined to have bacterial pathogens compared to those greater than 5 years with (90%, 289/320) compared to (78%, 170/218) for cases, p<0.05, and control (93%, 280/299) compared to (80%, 172/215), p<0.05 (Table IV).

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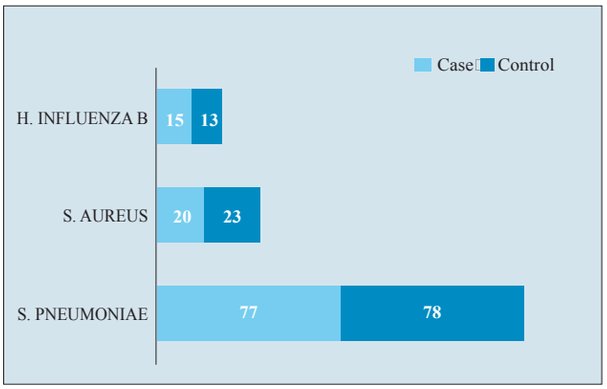
Table IV The positivity of bacterial pathogens in both the ARI cases and the control group

	Case (n = 538)	Control (n = 514)	p value
All	459(85.32)	452(87.94)	0.7382
Gender			
Male	253(86.94)	225(88.58)	0.5166
Female	206(83.40)	227(87.31)	0.2118
Age			
≤5 years	289(90.31)	280(93.65)	0.8931
>5 years	170(77.98)	172(80)	0.6453
p value	< 0.0001	< 0.0001	

A diverse range of bacteria was detected in both the case and control groups using Real-TimePCR.

In this study, the most commonly detected bacterial pathogen was *S. pneumoniae* in both the case (77%, 414/538) and the control group (78%, 402/514). Subsequently, *S. aureus* was identified (20%, 110/538)of cases and (23%, 120/514) of controls while *H. influenzatype* b was found in 15% of cases and 13% of controls. Interestingly in both the case and control groups for *S. pneumonia,* a higher detection rate was observed in ≤5 years compared to those >5 years. S. pneumoniae was detected(85%, 272/320) in those aged ≤5 years and (65%, 142/218) in those aged >5 years in the case group, p<0.05. Similarly, in the control group, S. pneumoniae was detected (88%, 264/299) in individuals aged ≤5 years and (64%, 138/215) of those aged >5 years, p<0.05. For H. influenzatype b, the detection rates were 15% vs 13% in cases and 13% vs 11% in controls, with no significant age-based differences. Remarkably for S.aureus we witnesseda higher detection ratein the control group. We found 15% vs 27%for cases, p<0.05 and 16% vs 33%for controls, p<0.05 (Figure 1).

(a)



(b)

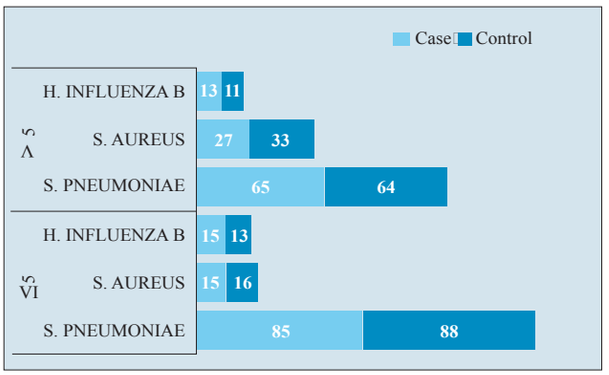


Figure 2 Detection of pathogens from Nasopharyngeal Swab (NPS) samples via Real-Time PCR in cases and control groups

(a) Percentage of three bacteria detected in cases and control group (b) Age-specific percentage of three bacteria in cases and control group

A total of 816 positive for *S. pneumoniae,* 722 numbers of serotypes have been identified where 30.6% (221) belong to PCV10, 41.1% (297) and 60% (427) for PCV13 and non-PCV13 respectively. In PCV-13, themost predominant serotypes were 6AB (11.9%, 64/538 in the case and 13.8%, (71/514 in control) followed by 23F (9.7% & 8.9%) and 19A (8.3% & 7.8%) in caseand control respectively. On the other hand, the least predominant serotypes in the case were 1(1.9%), 4 (0.7%), 19F (0.6%) and in control were 19F (0.9%). 1(0.8%) and 4 (0.4%) (Figure 3).
Innon-PCV13 group, highest proportion of serotypes for cases were as follows 15B/C (8.5%), 35B (7.6%), 20(5.9%), 11A (5.8%), 16F (5.6%), 10F (5.4%), 15A (5.2%), and 13(4.3%) where in control 11A (8.9%), 15B/C (7.8%), 13(7.7%), 34(7.4%), 35B (6.7%), 10A (5.8%), 16F (5.4%), and 20(4.7%). On the other hand, the lowest proportion of serotypes for cases were as follows 35A (1.1%), 33F (0.9%), 35(0.9%), 21(0.9%), 2(0.9%), 8(0.5%) and 39(0.3%) where in control 23B,9N/L and 21(all 1.2%), followed by 38(1%), 2(0.8%), 39(0.6%), 33F (0.2%) and 8(0.2%) (Figure 3). It was found that 402 isolates were positive for l ytA out of 414 (97.1%) in the case and 94.27% in controls, indicating the presence of non-encapsulated non-typeable S. pneumoniae. Furthermore, 9.9% (41/414) in case and 9.3% in control could not identify serotypes, implying that those patients likely carried serotypes that were not included in the serotyping panel (Figure 4). There were no notable distinctions observed in terms of the occurrence of multiple serotypes between the two groups (Figure 4).

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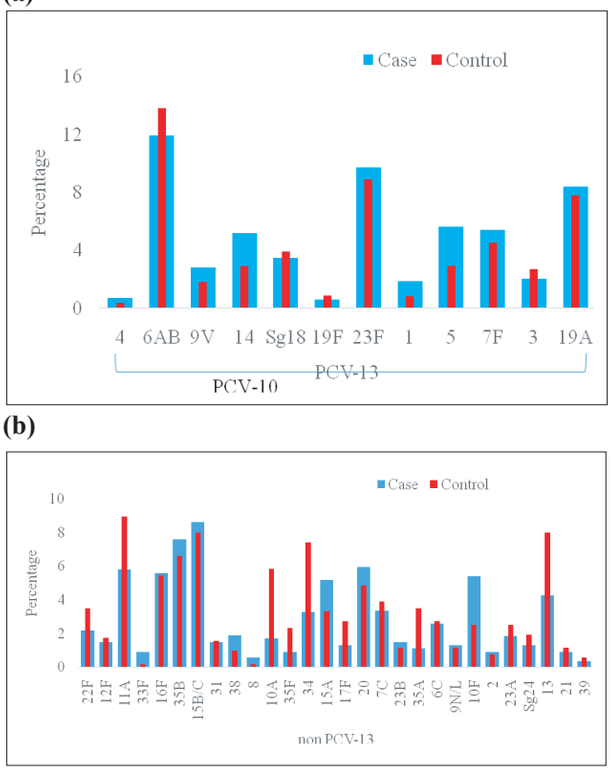

Figure 3 *S. pneumoniae* serotype distribution in case and control

(a) The percentage distribution of *S. pneumoniae* serotypes covered by PCV-10 (Pneumococcal conjugate vaccine 10) and PCV-13 (Pneumococcal conjugate vaccine 13) in both cases and the control group. (b) The percentage distribution of *S. pneumoniae* serotypes in cases and control group were covered other than PCV-13.

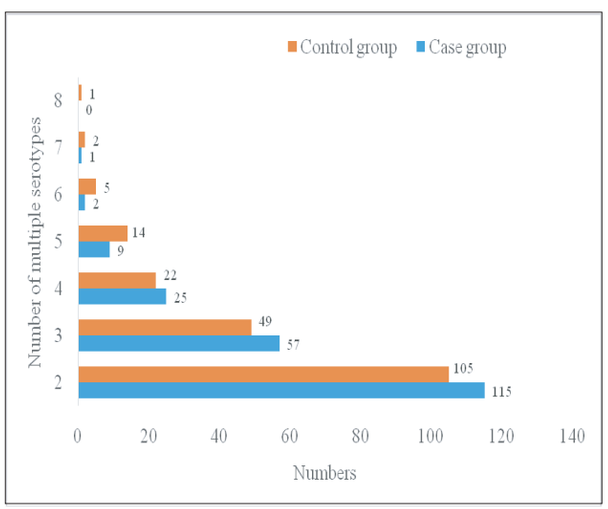

Figure 4 Presence of multiple serotypes in cases and control group.

Discussion

This is the initial investigation carried out on a representative vulnerable group (DRPs) with a specific focus on the bacterial causes in conjunction with their corresponding control groups. Additionally, this study examined the degree to which each etiological agent played a role in ARI and the distribution of *S. pneumoniae* serotypes. The purpose of the control group was to compare the presence of bacteria in healthy individuals to those with ARI cases to see whether there were any etiological associations/ differences. This study included 538 ARI cases, the majority of which were from children under the age of five (59%). In terms of detecting bacteria using Real-Time PCR, equal proportions were found in both cases and controls (85% versus 88%). There was no age or gender difference between the case and the control group. It is in agreement with previous reviews that the detection of bacteria has a significant age difference of ≤5 years compared to those above 5 years of age of the case and the control groups ($p<0.05$).[17,18] *S. pneumoniae* was observed in the largest number of ARI cases (77%) and controls (78%) in this research, followed by *S. aureus* and *H. influenza type* B. Although *S. pneumoniae* can cause sickness, a strong immune system and a good balance of indigenous flora and invaders can assist in clearing it out. Because of the host's poor defenses, *S. pneumoniae* regularly and repeatedly colonized them, implying that children were reservoirs due to the duration of carriage and colonization.[19,20] Despite accounting for a greater percentage of carriage, Althouse et al. concluded that newborns have a significantly lower role in transmission than toddlers and older children.[20] However, these findings suggest that further research is required to gain a comprehensive understanding of the transmission's progress. Furthermore, no significant changes in bacterial detection were identified between cases and controls in this investigation, which corroborated earlier research.[21] Among the three primary bacteria, *S. pneumoniae* was found to be more prevalent for the ≤5 years of age group than >5 years (85% vs 65%, $p<0.05$) in instances where H. influenza type B was discovered in a similar proportion with no significant difference. *S. aureus* was identified in greater proportions for >5 years of age in both the case and control groups. Similarly, the PERCH multi-country case-control research discovered that these same respiratory microorganisms were commonly associated with ARI.

The exception was *S. aureus,* which was found in a larger proportion in the >5-year-old age group in both cases and controls, suggesting opportunistic infection in

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the elderly. Furthermore, no gender differences were seen in the pathogen detection in the control group or cases, indicating that gender does not affect the likelihood of contracting an ARI infection. An earlier investigation produced findings similar to these.[22] In the present study, *S. pneumonia, H. influenzae type* B and *S. aureus* were associated with the controls. *S. aureus* and *S. pneumonia* were found more frequently in control group participants and both of these pathogens are known to cause acute respiratory infections. However, even if some of the microorganisms were more frequently detected in the control group, this does not necessarily mean that they are not associated with ARI. The results of the investigation were in line with those of the PERCH study, which found that controls had much higher detection rates of *S. pneumoniae* compared to cases. *S. pneumoniae* and *H. influenzae type* B, however, were highly linked to infections in the lower respiratory tract.[23] *S. pneumonia* is the leading cause of child mortality especially for younger children of 5 years despite the vaccination program and it is responsible for 33% of deaths worldwide.[24] Following the introduction of PCV-10 vaccination by Bangladesh and various NGOs, PCV-13 which is protective against 13 serotypes replaced PCV-7 and PCV-10 in the DRP population.[25,26] In this study, identified 40 serotypes from NPS using qRT-PCR where 6AB (12.8%) 23F (9.3%) 15B/C (8.3%) 19A (8.07%) 11A (7.3%) 35B (7.1%) 13 (6.08%) 34 (5.3%) serotypes were found in higher proportions which comprised 64.25% of all isolates and 1, 21, 19F, 2, 4, 33F, 39 and 8 serotypes were found in less proportions. The higher proportion of serotypes was 6AB (11.9% and 13.8%), 23F (9.7% and 8.9%) and 19A (8.3% and 7.8%) under PCV-10 and PCV-13 in cases and control. Serotypes with higher proportions were 15B/C (8.5% and 7.8%), 35B (7.6% and 6.7%), 20 (5.9% and 4.7%) 11A (5.8% and 8.9%) 16F (5.6% and 5.4%) were found to be a non-PCV-13 both for case and control. However, this study has several limitations. For example, since the majority of the cases were children, couldn't get sputum samples. Furthermore, due to the challenging circumstances of the humanitarian crisis in which the study was conducted, X-rays were unavailable. As a result, relied solely on clinical symptoms and physical data to develop a decision tree for assessing illness presentation.

Furthermore, due to the limits of the PCR technology, we were unable to differentiate between the *S. pneumoniae* serotypes 6A and 6B, 15B and 15C, 9N and 9L or serogroups 18 and 24. Despite this drawback, it was found that the etiology of ARI was associated with factors such as age, gender, pathogen spectra and the distribution pattern of *S. pneumoniae* serotypes. These findings could be useful in public health initiatives such as determining the predominant respiratory pathogen, identifying the age group at highest risk and administering vaccines in such humanitarian settings. Crucially, information on the distribution of *S. pneumoniae* serotypes may also help identify serotypes that cause illness and in the development of future vaccines.

Conclusion

Comparison of bacterial detection between the two groups yielded similar results, with the highest detection rate attributed to *S. pneumoniae.* In particular, the infection rates of bacterial pathogens were notably higher in children aged ≤5 years. In addition, an increased prevalence of *S. pneumonia*e serotypes, such as 6AB, 23F and 19A were observed within the PCV-13 vaccine, while serotypes 11A, 15B/C, 35B and 13 were more prevalent within the non-PCV-13 category. The distribution of *S. pneumoniae* serotypes in both cases and controls provides valuable insights for public health policies, particularly concerning vaccination strategies in crisis settings.

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