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Research Article

Serotype Distribution and Antimicrobial Susceptibility Profile of *Streptococcus pneumoniae* Among Myanmar Children with Acute Respiratory Infection

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Abstract

Background: Acute respiratory infection (ARI) is the leading infectious cause of death in children younger than 5 years of age worldwide. *Streptococcus pneumoniae* is the most frequent cause of pneumonia in children aged less than 5 years worldwide.

Objective: The aim of this research was to determine the capsular serotypes and antimicrobial susceptibility profile of *Streptococcus pneumoniae* strains isolated from children with ARI admitted to Yangon Children Hospital (YCH).

Methods: A cross-sectional, laboratory-based descriptive study was carried out on 231 ARI patients who were aged 1 month to less than 5 years attending YCH from 2013 to 2015. Nasopharyngeal swab specimens were taken after obtaining informed consent from the parents or guardians of the patients. Antimicrobial susceptibility profile was determined by modified Kirby-Bauer method. In addition, molecular serotyping of *S. pneumoniae* was done by Sequential Multiplex Polymerase Chain Reaction.

Results: The isolation rate of *S. pneumoniae* was 7.4% (17/231). A total of nine different serotypes were detected. The most common serotype was found to be 6A/6B/6C/6D (52.9%) followed by non-typeable serotypes (29.4%), 19F, 23F and 35F/47F serotypes (11.8% each) and 23A, 11A/11D, 34 and 20 serotypes (5.9% each). Carriage of multiple serotypes was observed in 29.4% of *S. pneumoniae* isolates. 70.6% of *S. pneumoniae* isolates were found to be penicillin non-susceptible and multi-drug resistance was observed in 41.2% of strains.

Conclusion: This study is among the first to identify existing serotypes of *S. pneumoniae* from ARI children before introduction of Pneumococcal Conjugate Vaccine (PCV) in National Immunization Programme in Myanmar. These findings have highlighted the circulating serotypes of *S. pneumoniae* strains and high occurrence of drug resistant *S. pneumoniae* isolates in Myanmar. Further larger studies on prevalent pneumococcal serotypes after introduction of PCV is necessary to assess the impact of immunization on preventing pneumococcal infection in Myanmar.

Keywords: Acute Respiratory Infection; Antimicrobial Susceptibility; Children; Serotypes; *Streptococcus pneumoniae*

Introduction

Acute Respiratory Tract Infections (ARIs) are among the leading causes of childhood mortality [1]. ARI mainly pneumonia is one of the six causes of death accounting for 17% of deaths among children younger than 5 years of age worldwide [2]. Myanmar is among 15 countries with the highest estimated absolute number of new cases of clinical pneumonia [1].

According to health statistics, the under-five mortality rate of Myanmar children is 72 deaths per 1,000 live births [3]. Pneumonia is caused by a number of infectious agents including bacteria, viruses and fungi. Among them, the most common infectious agents of bacterial origin of clinical pneumonia in developing countries include *S. pneumoniae* and *Haemophilus influenzae* type b [1].

Despite the introduction of Heptavalent Pneumococcal Conjugate Vaccine (PCV7), antimicrobial resistance in *S. pneumoniae* is still a serious concern worldwide especially in Asian countries. The estimated worldwide prevalence of penicillin-resistant *S. pneumoniae* (PRSP) was 14.1% and it was found to be much higher (30-40%) in southern Europe and Southeast Asia (exceeding 70%) [4]. In February 2017, World Health Organization has published its first list of 12 families of antibiotic-resistant 'priority pathogens' and penicillin non-susceptible *S. pneumoniae* is included in the medium priority list [5]. Multidrug Resistant *S. pneumoniae* (MRSP) has now become a public health problem in both developing and developed countries. A large multi-center study in Asian countries reported that the overall rate of Multidrug Resistance (MDR) in *S. pneumoniae* isolates was 59.3% and the highest MDR rate is found in China, followed by Vietnam, South Korea, Hong Kong, and Taiwan [6].

Identification of the capsular serotypes of *S. pneumoniae* is important not only for surveillance programs but also for evaluation of the effect of vaccination on nasopharyngeal carriage [7]. Accurate determination of pneumococcal serotypes is critically important since the vaccine development presently relies on serotype prevalence data [8].

In most developing countries, PCVs are not routinely used because of their high costs and lack of data regarding the burden of the disease. Despite the importance of the *S. pneumoniae*, there have not been any previous published studies regarding detailed epidemiological information on pneumococcal serotypes in Myanmar.

The information on circulating capsular serotypes of *S. pneumoniae* among children aged less than 5 years is important since it provides baseline information about disease epidemiology before introduction of PCV in Myanmar. The aim of this study was to characterize *S. pneumoniae* strains isolated from nasopharyngeal swab specimens of children with acute respiratory infection admitted to Yangon Children Hospital from 2013 to 2015.

Material and Methods

A cross-sectional, laboratory-based descriptive study was carried out on 231 ARI patients (135 males, 58.4% and 96 females, 41.6%) who were aged 1 month to less than 5 years (Median age = 12.5 months) from 2013 to 2015. All patients aged 1month to <5 years with clinically diagnosed ARI and admitted to Yangon Children Hospital were selected. Severely ill ARI patients and ARI patients who have received parental antibiotics beyond first 24 hours after admission were excluded from the study.

Out of 231 ARI patients, 135 cases (58.4%) were male children and 96 cases (41.6%) were females. Majority of the ARI patients (195/231, 84%) were less than 2 years of age. The youngest patient was 1 month old and the oldest one was 4 years and 10 months.

Concerning the different clinical conditions of ARI patients, severe pneumonia was the most commonly diagnosed clinical condition (101/231, 43.7%) followed by bronchiolitis (86/231, 37.2%) and severe bronchiolitis (28/231, 12.1%). Pneumonia was diagnosed in 15 patients (6.5%) and very severe pneumonia was noted in only one patient (0.4%).

Nasopharyngeal swab specimens were taken using a sterile Dacron swab after obtaining informed consent from the parents or guardians of the patients and were placed in normal saline and transported to the laboratory at Department of Medical Research, Yangon.

Each swab was placed in Skim-Milk Tryptone Glucose Glycerol (STGG) medium and was inoculated onto blood agar and incubated in a CO_2-enriched atmosphere at 37˚C overnight. *S. pneumoniae* was identified by characteristic alpha-hemolytic colonial morphology and Gram stained smear showing lancet-shaped gram-positive diplococci. Confirmation of *S. pneumoniae* was made by biochemical tests such as optochin susceptibility test and bile solubility test [9].

Identification of the serotypes of *S. pneumoniae*

Nasopharyngeal samples in normal saline were boiled at 100˚C for 10 minutes and stored at-20˚C until PCR was performed. These samples were transported to Department of Oral and Molecular Microbiology, Osaka University Graduate School of Dentistry, Osaka, Japan where Sequential Multiplex Polymerase Chain Reaction (PCR) was performed. Boiled nasopharyngeal samples in normal saline were used as DNA template. Each multiplex PCR reaction was designed to sequentially include primer pairs targeting serotype-specific regions of the most frequently occurring serotypes [10].

Forty-one serotype-specific primer pairs were grouped into eight sets for sequential testing. Forty-one primer pairs were used to target serotypes 1, 2, 3, 4, 5, 6A/6B/6C/6D, 7C/7B/40, 7F/7A, 8, 9N/9L, 9V/9A, 10A, 10F/10C/33C, 11A/11D, 12F/12A/12B/44/46, 13, 14, 15A/15F, 15B/15C, 16F, 17F, 18C/18F/18B/18A, 19A, 19F, 19Fvar, 20, 21, 22F/22A, 23A, 23B, 23F, 24F/24A/24B, 31, 33F/33A/37, 34, 35A/35C/42, 35B, 35F/47F, 38/25F/25A, and 39. A primer pair pneumococcal capsular polysaccharide synthesis gene (primers cpsA-f and cpsA-r) was also included as the positive control targeting the cpsA locus found in all 93 known serotypes of *S. pneumoniae* [11].

The reaction mixtures were amplified in a DNA thermal cycler and run under the following amplification conditions for all eight reactions: initial denaturation at 95°C for 15 min followed by 35 amplification cycles of 94°C for 30 secs, 54°C for 90 secs, 72°C for 60 secs and a final extension step at 72°C for 10 min. The amplified products were analyzed by electrophoresis in 3% agarose gel using ethidium bromide and the DNA bands were visualized in ultraviolet transilluminator.

Primer pair	Primer sequence (5' →3')	Product size (bp)
1-f 1-r	CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA CCA AAG AAA ATA CTA ACA TTA TCA CAA TAT TGG C	280
2-f 2-r	TAT CCC AGT TGA ATA TTT CTC CAC TAC ACC ACA CAA AAT ATA GGC AGA GAG AGA CTA CT	290
3-f 3-r	ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G	371
4-f 4-r	CTG TTA CTT GTT CTG GAC TCT CGA TAA TTG G GCC CAC TCC TGT TAA AAT CCT ACC CGC ATT G	430
5-f 5-r	ATA CCT ACA CAA CTT CTG ATT ATG CCT TTG TG GCT CGA TAA ACA TAA TCA ATA TTT GAA AAA GTA TG	362
6A/6B/6C/6D*-f 6A/6B/6C/6D-r	AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG TTA GCG GAG ATA ATT TAA AAT GAT GAC TA	250
6C/6D-f 6C/6D-r	CAT TTT AGT GAA GTT GGC GGT GGA GTT AGC TTC GAA GCC CAT ACT CTT CAA TTA	727
7C/7B/40-f 7C/7B/40-r	CTA TCT CAG TCA TCT ATT GTT AAA GTT TAC GAC GGG A GAA CAT AGA TGT TGA GAC ATC TTT TGT AAT TTC	260
7F/7A-f 7F/7A-r	TCC AAA CTA TTA CAG TGG GAA TTA CGG ATA GGA ATT GAG ATT GCC AAA GCG AC	599
8-f 8-r	GAA GAA ACG AAA CTG TCA GAG CAT TTA CAT CTA TAG ATA CTA GTA GAG CTG TTC TAG TCT	201
9N/9L-f 9N/9L-r	GAA CTG AAT AAG TCA GAT TTA ATC AGC ACC AAG ATC TGA CGG GCT AAT CAA T	516
9V/9A-f 9V/9A-r	GGT GTA GAT TTA CCA TTA GTG TCG GCA GAC CCA TGA ATG AAA TCA ACA TTG TCA GTA GC	816
10A-f 10A-r	GGT GTA GAT TTA CCA TTA GTG TCG GCA GAC GAA TTT CTT CTT TAA GAT TCG GAT ATT TCT C	628

10F/10C/33C-f 10F/10C/33C-r	GGA GTT TAT CGG TAG TGC TCA TTT TAG CA CTA ACA AAT TCG CAA CAC GAG GCA ACA	248
11A/11D-f 11A/11D-r	GGA CAT GTT CAG GTG ATT TCC CAA TAT AGT G GAT TAT GAG TGT AAT TTA TTC CAA CTT CTC CC	463
12F/12A/12B/44/46-f 12F/12A/12B/44/46-r	GCA ACA AAC GGC GTG AAA GTA GTT G CAA GAT GAA TAT CAC TAC CAA TAA CAA AAC	376
13-f 13-r	TAC TAA GGT AAT CTC TGG AAA TCG AAA GG CTC ATG CAT TTT ATT AAC CGC TTT TTG TTC	655
14-f 14-r	GAA ATG TTA CTT GGC GCA GGT GTC AGA ATT GCC AAT ACT TCT TAG TCT CTC AGA TGA AT	189
15A/15F-f 15A/15F-r	ATT AGT ACA GCT GCT GGA ATA TCT CTT C GAT CTA GTG AAC GTA CTA TTC CAA AC	434
15B/15C-f 15B/15C-r	TTG GAA TTT TTT AAT TAG TGG CTT ACC TA CAT CCG CTT ATT AAT TGA AGT AAT CTG AAC C	496
16F-f 16F-r	GAA TTT TTC AGG CGT GGG TGT TAA AAG CAG CAT ATA GCA CCG CTA AGC AAA TA	717
17F-f 17F-r	TTC GTG ATG ATA ATT CCA ATG ATC AAA CAA GAG GAT GTA ACA AAT TTG TAG CGA CTA AGG TCT GC	693
18C/18F/18B/18A-f 18C/18F/18B/18A -r	CTT AAT AGC TCT CAT TAT TCT TTT TTT AAG CC TCT GTA AAC CAT ATC AGC ATC TGA AAC	573
19A-f 19A-r	GAG AGA TTC ATA ATC TTG CAC TTA GCC A CAT AAT AGC TAC AAA TGA CTC ATC GCC	566
19F-f 19F-r	GTT AAG ATT GCT GAT CGA TTA ATT GAT ATC C GTA ATA TGT CTT TAG GGC GTT TAT GGC GAT AG	304
19Fvar-f 19Fvar-r	GAC AAT TCT GGT TGA CTT GTT GAT TTT G CTA CCA AAT ACC TCA CCA GCT TCC	585
20-f 20-r	GAG CAA GAG TTT TTC ACC TGA CAG CGA GAA G CTA AAT TCC TGT AAT TTA GCT AAA ACT CTT ATC	514
21F-f 21F-r	CTA TGG TTA TTT CAA CTC AAT CGT CAC C GGC AAA CTC AGA CAT AGT ATA GCA TAG	192
22F/22A-f 22F/22A-r	GAG TAT AGC CAG ATT ATG GCA GTT TTA TTG TC CTC CAG CAC TTG CGC TGG AAA CAA CAG ACA AC	643
23A-f 23A-r	TAT TCT AGC AAG TGA CGA AGA TGC G CCA ACA TGC TTA AAA ACG CTG CTT TAC	722

23B-f 23B-r	CCA CAA TTA GCG CTA TAT TCA TTC AAT CG GTC CAC GCT GAA TAA AAT GAA GCT CCG	199
23F-f 23F-r	GTA ACA GTT GCT GTA GAG GGA ATT GGC TTT TC CAC AAC ACC TAA CAC ACG ATG GCT ATA TGA TTC	384
24F/24A/24B-f 24F/24A/24B -r	GCT CCC TGC TAT TGT AAT CTT TAA AGA G GTG TCT TTT ATT GAC TTT ATC ATA GGT CGG	99
31-f 31-r	GGA AGT TTT CAA GGA TAT GAT AGT GGT GGT GC CCG AAT AAT ATA TTC AAT ATA TTC CTA CTC	701
33F/33A/37-f 33F/33A/37-r	GAA GGC AAT CAA TGT GAT TGT GTC GCG CTT CAA AAT GAA GAT TAT AGT ACC CTT CTA C	338
34-f 34-r	GCT TTT GTA AGA GGA GAT TAT TTT CAC CCA AC CAA TCC GAC TAA GTC TTC AGT AAA AAA CTT TAC	408
35A/35C/42-f 35A/35C/42-r	ATT ACG ACT CCT TAT GTG ACG CGC ATA CCA ATC CCA AGA TAT ATG CAA CTA GGT T	280
35B-f 35B-r	GAT AAG TCT GTT GTG GAG ACT TAA AAA GAA TG CTT TCC AGA TAA TTA CAG GTA TTC CTG AAG CAA G	677
35F/47F-f 35F/47F-r	GAA CAT AGT CGC TAT TGT ATT TTA TTT AAA GCA A GAC TAG GAG CAT TAT TCC TAG AGC GAG TAA ACC	517
38/25F/25A-f 38/25F/25A -r	CGT TCT TTT ATC TCA CTG TAT AGT ATC TTT ATG ATG TTT GAA TTA AAG CTA ACG TAA CAA TCC	574
39F-f 39F-r	TCA TTG TAT TAA CCC TAT GCT TTA TTG GTG GAG TAT CTC CAT TGT ATT GAA ATC TAC CAA	98
cpsA-f cpsA-r	GCA GTA CAG CAG TTT GTT GGA CTG ACC GAA TAT TTT CAT TAT CAG TCC CAG TC	160
*All serotypes that are co-detected are listed		

List of oligonucleotide primers used for pneumococcal serotype deduction by conventional multiplex PCR (SIGMA-ALDRICH).

Antimicrobial Susceptibility Testing of *S. pneumoniae*

Antimicrobial susceptibility profile of culture-confirmed *S. pneumoniae* isolates was determined by modified Kirby-Bauer method on Mueller-Hinton agar medium supplemented with 5% horse blood according to the performance standards of Clinical and Laboratory Standards Institute (CLSI) [12]. Fourteen antibiotic discs (HiMedia) containing oxacillin (1µg), ampicillin (10µg), amoxicillin-clavulanic acid (30µg), cloxacillin (1µg), flucloxacillin (5µg), cefotaxime (30µg), ceftriaxone (30µg), erythromycin (15µg), azithromycin (15µg), ciprofloxacin (5µg), levofloxacin (5µg), vancomycin (30µg), co-trimoxazole (25µg) and gentamicin (10 µg) were used. Then, CLSI zone size interpretation chart was used to identify as resistant, intermediate or susceptible *S. pneumoniae* isolates.

Data Analysis

After collection of data, data entry, data editing, data cleansing, data compilation, data processing and data analysis were done using appropriate statistical software, SPSS version 16.0.

Ethical Consideration

Approval to conduct this study was obtained from the Research and Ethical Committee of University of Medicine 1, Yangon on 10.10.2013.

Results

Streptococcus pneumoniae was isolated from 7.4 % of total cases (17/231). A total of nine different serotypes were detected. The most common serotype was found to be 6A/6B/6C/6D (9 isolates, 52.9%) followed by non-typeable serotype (5 isolates, 29.4%), 19F, 23F and 35F/47F serotypes (2 isolates, 11.8% each) and 23A, 11A/11D, 34 and 20 serotypes (1 isolate, 5.9% each). (Figure 1).

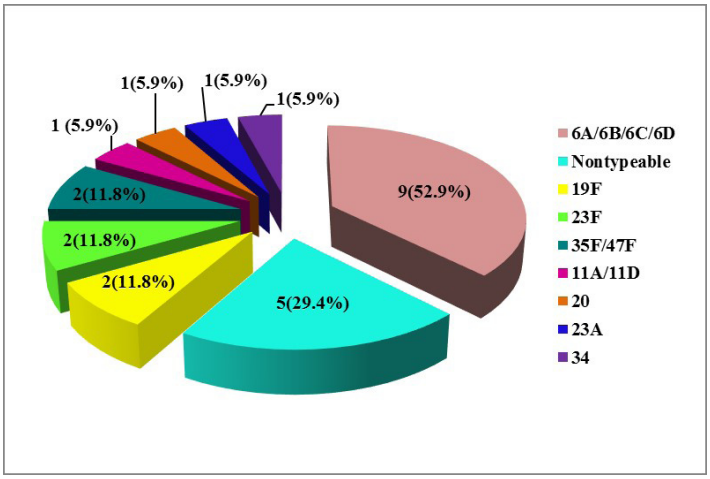

Figure 1: Proportion of capsular serotypes of isolated *S. pneumoniae*.

In this study, 70.6% of *S. pneumoniae* isolates (n=12) were detected as carrying single serotype (non-typeable serotypes-5 isolates, 6A/B/C/D- 4 isolates, 19F, 23F and 23A- 1 isolate each).

Carriage of multiple serotypes was observed in 5 *S. pneumoniae* isolates. Four isolates (23.5%) were found carrying two serotypes in different combination (6A/B/C/D + 11A/11D, 6A/B/C/D + 19F, 6A/B/C/D + 34 and 6A/B/C/D + 35F/47F). Carriage of four serotypes (6A/B/C/D + 20 + 23F + 35F/47F) was found in only one isolate (5.9%) (Table 1).

Number of detected serotypes	Number of isolates	Percentage (%)
1 serotype	12/7	70.6%
2 serotypes	4/17	23.5%
4 serotypes	1/17	5.9%

Table 1: Detection of different number of serotypes of isolated *S. pneumoniae*.

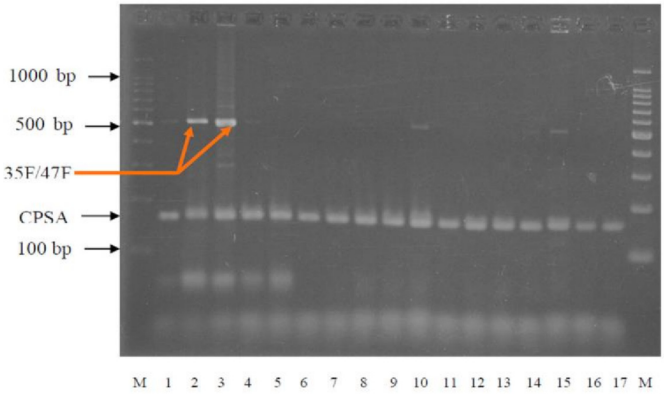

Plate (1) Detection of Serotype 35F/47F (517bp) by Sequential Multiplex PCR (6). Lane 1 and 19 (M): showing 100 base pair DNA ladder marker. Lane 2 to 18 - Sample 1 to Sample 17. Lane 3 and 4 - (Sample 2 and 3) showing 517 bp bands indicating serotype 35F/47F. CPSA - showing 160bp bands indicating internal positive control

Antibiotic Susceptibility Determined by Modified Kirby-Bauer Method

Most of the *S. pneumoniae* isolates showed high sensitivity to injection form antibiotics like cefotaxime and ceftriaxone and also to oral antibiotics like levofloxacin, vancomycin, cloxacillin, flucloxacillin and azithromycin. It was also observed that these isolates were highly resistant to oral antibiotics like co-trimoxazole, oxacillin and ampicillin. Oral and parenteral penicillin are commonly used antibiotics in the current treatment of ARI and Penicillin Resistant *S. pneumoniae* (PRSP) was found in 12 isolates (70.6%) (Figure 2).

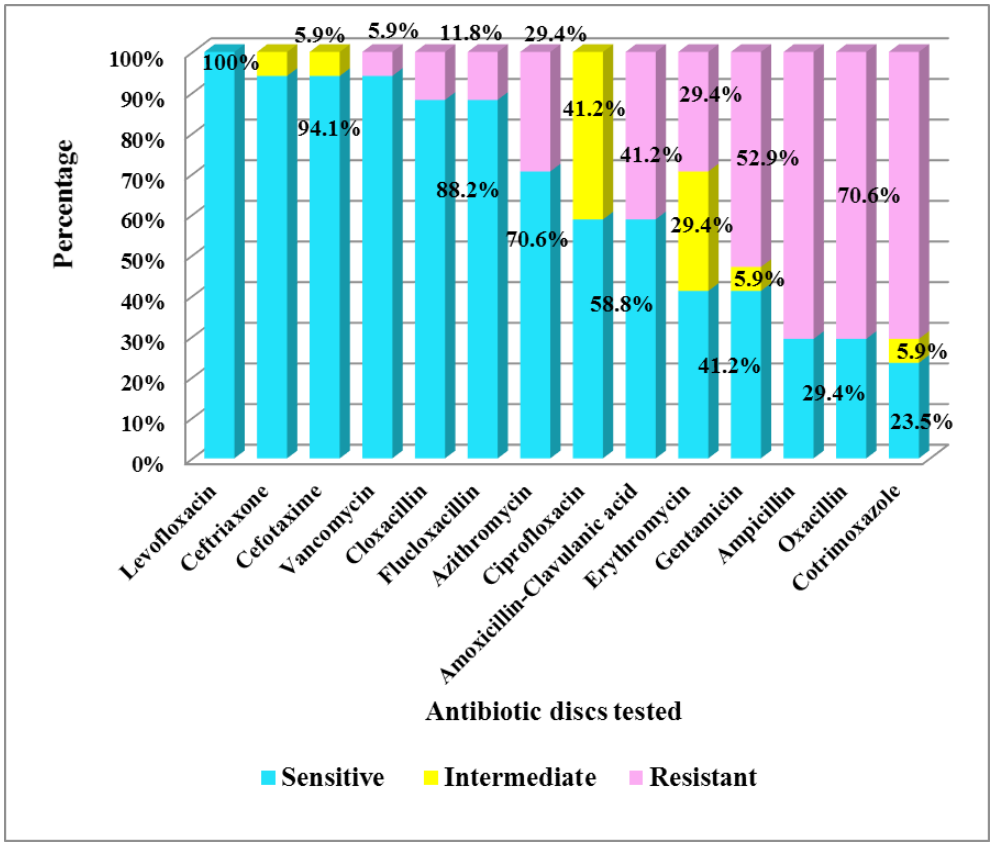

Figure 2: Antibiotic susceptibility pattern of *S. pneumoniae* isolates determined by modified Kirby-Bauer method.

S. pneumoniae strains that were intermediate resistant or resistant to at least 3 or more antibiotics of different classes prescribed in current treatment regimen namely: penicillin, cefotaxime, ceftriaxone, cotrimoxazole, erythromycin and ciprofloxacin were analyzed. A total of 7 MRSP strains were detected among the drug resistant isolates (Table 2).

Resistant antibiotics	No of S. Pneumoniae isolates	Total
P + E + Ci + Co	3	7
P + E + Ce	1	
P + E + Cef	1	
P + E + Co	2	
P= Penicillin Co= Cotrimoxazole Ce= Cefotaxime E= Erythromycin Ci= Ciprofloxacin Cef= Ceftriaxone		

Table 2: Drug resistant pattern of *S. pneumoniae* showing multiple resistance to antibiotics.

Discussion

In this study, the incidence of ARI was found to be higher among the children who were less than 2 years of age (n=195, 84%), a rate comparable with studies in Malaysia (76%) and in Bangladesh (87.9%) [13,14]. The occurrence of ARI was found to be higher in male children (58.4%) in the present study that was consistent with other recent international reports from Brazil (52.8%), Bangladesh (62.5%) and previous local study showing 56% (ranging from 52.8% to 62.5%) [14-16].

The prevalence of *S. pneumoniae* in Myanmar is closely linked to neighboring countries within South East Asia region and other Asian countries. The culture positivity rate of 7.4 % obtained in this study was nearly the same rate identified in Bangladesh (7%) [17]. However, in comparison with that of Philippines, Thailand and previous study in Myanmar (51%, 34.5% and 30%respectively), it was relatively very low [16,18,19]. This low isolation rate of *S. pneumoniae* may be due to prior use of oral antibiotics at home and prior parenteral antibiotic therapy on admission of the patients.

In this study, oral penicillin (amoxicillin) was prescribed in two *S. pneumoniae* infected patients (11.8%) while only one patient (5.9%) have received oral sulphonamides (co-trimoxazole) before the collection of nasopharyngeal swab specimens. Regarding the prior therapy with parenteral antibiotics, only four out of 17 *S. pneumoniae* infected patients (23.6%) were prescribed with

intravenous penicillin within first 24 hours after admission before the collection of specimens. It was found that isolation rate of *S. pneumoniae* was higher in patients who have not received prior therapy with oral or parenteral antibiotics before the collection of specimens.

In our collection of isolates, which was in agreement with recent reports from other South East Asian countries like Singapore, Malaysia, China and Thailand-Myanmar Border that serotype 6A/6B/6C/6D, 19F and 23F were among the most common serotypes [20-23]. Co-colonization of multiple pneumococcal serotypes is another emerging epidemiological concern as it may have an influence on the efficacy of pneumococcal vaccines [24,25]. The carriage rate of multiple serotypes in this study (n=5, 29.4%) was relatively high when compared to that of study at Thailand-Myanmar Border (5.1%) [23].

Interestingly, recently emerged serotype 19A, the frequently detected nonvaccine serotype was not identified probably because of the small size of isolated pneumococcal strains (n=17) that fails to show significant predominance of the frequency of serotypes and pneumococcal vaccines have not been introduced yet in Myanmar. Introduction of Pneumococcal Conjugate Vaccine (PCV) in National Immunization Programme was launched in July 2016 in Myanmar.

The prevalence rates of antibiotic-resistant and non-susceptible *S. pneumoniae* have increased in South East Asia over recent decades like in many other countries worldwide [26]. In India, the rate of reduced susceptibility of *S. pneumoniae* strains to penicillin was reported as only 4.9% which is very low when compared to a high rate (70.6%) of penicillin-resistant pneumococci in Myanmar [27]. Another national antimicrobial resistance survey conducted in Thailand demonstrated that country-wide penicillin resistance rate of the pneumococcal isolates was 37.2% that was lower than our data [19].

In comparison to the previous study conducted in Myanmar by Tin Nwe Oo, the rate of sensitivity to penicillin was markedly decreased from 60% in 2008 to 29.4% in 2015 [16]. Data from the present study have indicated a high rate (70.6%) of penicillin-resistant pneumococci among ARI children in Myanmar. This was probably due, in part, to the easily availability of antibiotics and lack of an antibiotic policy in the country.

Multidrug resistance was defined as intermediate resistance or resistance to penicillin plus intermediate resistance or resistance to ≥2 antimicrobial agents of different classes [28]. In this study, 41.2% of *S. pneumoniae* strains were found to be multidrug resistant (MDR) which was in accordance with data from Northern Palestine (49%), Vietnam (45%) and Korea (45.2%) [26,29,30]. However, the rate of MDR strains in the present study was relatively high when compared with data from India (5.3%) [26]. Increased rate of multi-drug resistant *S. pneumoniae* strains in this study might be due to availability of oral antibiotics without prescription of the medical doctors and indiscriminate use of antibiotics leading to emergence of drug-resistant strains in the community and hospitals.

Strategies for prevention of pneumococcal pneumonia include treatment with appropriate antimicrobial therapy and immunization with currently available pneumococcal conjugate vaccines. Although this study characterized only a relatively small number of isolates from one center, it can firstly demonstrate the presence of previously unreported information of circulating capsular serotypes of *S. pneumoniae* before introduction of pneumococcal vaccine. However, the present study was unable to show significant predominance of the frequency of serotypes because of the small sample size.

In conclusion, the findings from the present study have highlighted the high incidence of penicillin nonsusceptible and multidrug resistant *S. pneumoniae* isolates among the Myanmar population. In addition, there is an urgent need of a long-term surveillance system to monitor the distribution of capsular serotypes of *S. pneumoniae* among pediatric population of Myanmar or to detect the serotypes implicated in pneumococcal disease cases.

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