ORIGINAL RESEARCH

Serotype Distribution and Antimicrobial Resistance Profiles of Streptococcus pneumoniae in Hospitalized Patients Over 50 Years with Invasive Pneumococcal Diseases in Vicente Sotto Memorial Medical Center for the Year 2014-2015*

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Introduction: More than 90 serotypes of *Streptococcus pneumoniae* have been identified. Since the introduction of pneumococcal vaccines, the epidemiology of *Streptococcus pneumoniae* has changed specifically the serotype distribution and antimicrobial resistance of this organism.

Objective: The objective was to determine the serotype distribution according to certain clinical factors and antimicrobial resistance profiles of *Streptococcus pneumoniae* in hospitalized patients with invasive pneumococcal diseases in Vicente Sotto Memorial Medical Center for the year 2014-2015.

Methods: The study design is a retrospective, hospital-based descriptive cross-sectional study. Included in this study are all 71 patients 50 years and older with invasive pneumococcal disease. A case report form was used to collect data from the charts of previously admitted patients. Serotypes and the antimicrobial resistance profiles of the isolates were obtained from the RITM database.

Results: The three most common serotypes isolated from patients were serotypes 3, 4 and 18, comprising 29.58% of the isolates. Only serotype 7 was found in a patient with septic shock. Serotypes 4, 7, 18 and 34 were present among those who died within 72 hours of admission. Among the antibiotics tested, tetracycline has a resistance rate of 11.29%.

Conclusion: There were a total of 21 different serotypes found in this study. Most of the serotypes were covered by the current pneumococcal vaccines that are commonly given in family practice. Most of the serotypes present were found among those who improved and were eventually discharged. Tetracycline was found to have the highest resistance rate among the antibiotics tested.

Key words: *Streptococcus pneumoniae*, serotypes, antimicrobial resistance

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Introduction

Streptococcus pneumoniae is one of the most common cause of potentially life-threatening community-acquired diseases and is associated with an estimated global mortality rate that is in the same order of magnitude as that of tuberculosis (3-5 million deaths per year).[1]

Most, if not all, serotypes are capable of causing serious disease in humans.[2] More than 90 serotypes of Streptococcus pneumoniae have been identified, based on varying polysaccharides that are found in the bacterial cell wall.[2] The serotypes responsible for disease vary with age and geographic location. The most prevalent serogroups in Asia are serotypes 6, 9, 14, 19, 23.[2]

Due to its implication on health, vaccines have been developed to protect those who are susceptible from developing pneumococcal disease. Pneumococcal vaccines are classified into two categories, namely, pneumococcal polysaccharide vaccine (PPV) and pneumococcal conjugate vaccine (PCV).[3]

During this PCV era, the serotypes of the Streptococcus pneumoniae could have changed. Those serotypes that have not caused serious diseases could have already emerged as the ones causing diseases in this present time due to the fact that people are already being protected from the previously common serotypes.

Vaccines can reduce pneumococcal resistance in vaccinated and unvaccinated populations by reducing the carriage of antibiotic-resistant serotypes, which protects the vaccinated population and prevents spread of disease to others, and by decreasing antibiotic resistance through overall reduction in antibiotic use.[4] However, while reducing the level of vaccine serotypes and drug-resistant serotypes in the nasopharynx, PCV7 also causes non-vaccine pneumococci replacement. According to ANSORP study in 2008-2009, there were significant changes in the distribution of serotypes in Asian countries after the introduction of PCV7 vaccination.[5] However, further studies are needed to fully understand the current epidemiology.

Aside from the possible change in the distribution of the serotypes due to the use of pneumococcal vaccine, there could also be an emergence of resistant strains either from inherent adaptation of the organism or from irrational antibiotic use.

Before 1967, *Streptococcus pneumoniae* was uniformly susceptible to penicillin.[6] In contrast to the early 1990s, pneumococcal isolates have already exhibited a high level of resistance to penicillin and other β-lactam antibiotics. Increasing resistance to penicillin among pneumococcal isolates has been widely reported in most countries during the last decade. As a consequence, there has been a shift in the use of antibiotics.

The serotype distribution and antimicrobial resistance of *S. pneumoniae* would greatly contribute to the epidemiology of the said organism most especially characterizing its invasiveness and the associated clinical manifestations. There are many factors that affect the severity of invasive pneumococcal disease some are host related, others are organism-related.[7] Presence of certain serotype could affect the clinical presentation of a certain individual. Not only the serotype, presence of a certain co-morbidity could also affect the said presentation.

Hence, the general objective of the study was to determine the serotype distribution according to certain clinical factors and antimicrobial resistance profiles of *Streptococcus pneumoniae* in hospitalized patients over 50 years old with invasive pneumococcal disease or Community Acquired Pneumonia in Vicente Sotto Memorial Medical Center (VSMMC) for the year 2014-2015. Specific objectives included the underlying medical conditions, clinical manifestations, laboratory data, and the serotype distribution according to the clinical factors mentioned. The results of the study could also help in the scope of family practice since family physicians are experts in the field of primary prevention that includes giving of vaccination. This would give them knowledge if the current vaccines cover for the present serotypes in VSMMC.

Materials and Methods

Study Design

The study design is a retrospective, hospital-based descriptive cross-sectional.

Study Setting

The study was conducted at Vicente Sotto Memorial Medical Center.

Study Population

Inclusion Criteria

Included in this study were 71 patients 50 years and older who were hospitalized in Vicente Sotto Memorial Medical Center from 2014-2015 and diagnosed with invasive pneumococcal disease or Community Acquired Pneumonia with *S. pneumoniae* isolates grown from normally sterile body sites or from lower respiratory tract.

The data gathered from this study were based on the study by Asian Network Surveillance of Resistant Pathogens (ANSORP) and Research Institute for Tropical Medicine (RITM)8 study wherein the isolates were already submitted to them by VSMMC Laboratory Department. Criteria of which isolates from respiratory specimens to include were already determined by the criteria set by ANSORP which can be found on the case report form to be used in this study. Thus, all of the patients included in this study were based on the list of qualified patients set by RITM.

Exclusion Criteria

Adults who were < 50 years old, Adults > 50 years old but have not fulfilled the criteria for the diagnosis of Community Acquired Pneumonia or pneumococcal disease, duplicate isolates of the same patient or inclusion of the same patient in multiple times.

Manuevers

Data collection technique/data collecton tool-equipment

A case report form was used to collect data from the charts of previously admitted adult patients > 50 years old of Vicente Sotto Memorial Medical Center for the year 2014-1015. The data collection form was adapted from the case report form of Asian Network for Surveillance of Resistant Pathogens (ANSORP) study group.[8]

Demographic and clinical data were collected based on chart review of previously admitted patients which included the following information:

- Sociodemographic characteristics (age, gender)
- Specimen source, types of infection
- Underlying conditions
- History of antibiotic use before admission
- Clinical manifestations
- Laboratory results
- Radiologic findings
- Antimicrobial therapy
- Clinical course
- Infection-related mortality

For the serotypes of pneumococcal isolates, information were gathered from the database of RITM, the institution in charge of the ANSORP study in the Philippines. The resistance profiles of the isolates submitted to RITM were obtained from the database of both the RITM and VSMMC Laboratory.

Data Analysis

The following were the study variables and outcome measures:

1. Serotype distribution of S. pneumoniae clinical isolates from adult patients in VSMMC to determine whether the current PCV vaccines provide sufficient coverage for the prevailing serotypes

2. The serotype distribution according to certain clinical factors
3. Current status in antimicrobial resistance of *S. pneumoniae* in VSMMC

Ethical Considerations

Participation in the study posed no risks to the patients and there was no payment for participating in the study.

All the *S. pneumoniae* isolates from the enrolled cases were collected and sent by VSMMMC Laboratory Department to RITM. The investigator of this study is the one in charge of collecting the demographic and clinical data in collaboration with RITM. A letter was sent to the said institution asking permission and requesting the data for the serotypes of the submitted isolates.

Confidentiality of all the data gathered from the charts and the database of both the VSMMC Laboratory Department and RITM were implemented. To ensure confidentiality of information, patient data were assigned codes. No active intervention in diagnosis and treatment of the cases were performed by the investigator on the patients.

Results

More than half of the admitted patients with pneumococcal disease are males comprising 76.05% of the total study population (Table 1). Only 23.95% were females. Most of the admitted patients were in the age range of 61-70 years old (45.07%).

The three most common serotypes isolated from the admitted patients with pneumococcal diseases were serotypes 3, 4 and 18, comprising 29.58% of the isolates (Figure 1). Other serotypes that were also common were serotypes 7, 19 and 29 with a total percentage of 16.89%. Other serotypes isolated were serotypes 1, 6, 10, 11, 12, 16, 17, 21, 23, 31, 32, 33 and 40. Fifteen of the isolates were non-serotypable which comprised 21.12% of the isolates.

A total of 14 antibiotics were tested. Among these antibiotics, tetracycline has the highest resistance at

Table 1. Sociodemographic data of admitted patients over 50 years old with pneumococcal disease.

Sociodemographic Characteristics	No.	%
Gender		
Male	54	76.05%
Female	17	23.95%
Age		
51-60	19	26.76%
61-70	32	45.07%
71-80	15	21.13%
81-90	5	7.04%

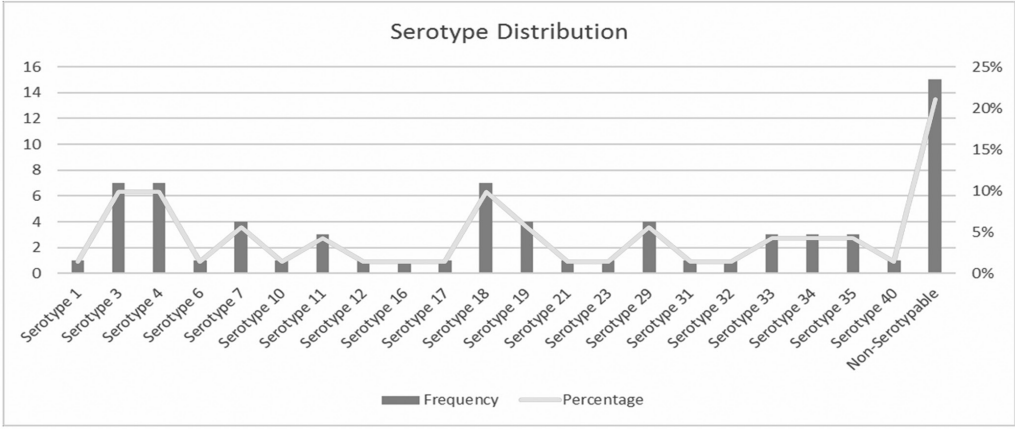

Figure 1. Serotype distribution of pneumococcal isolates.

11.29%, followed by erythromycin at 5.88% and ofloxacin at 4.55% (Figure 2). All of the other isolates are susceptible to ceftriaxone, ciprofloxacin, imipenem, linezolid, and vancomycin.

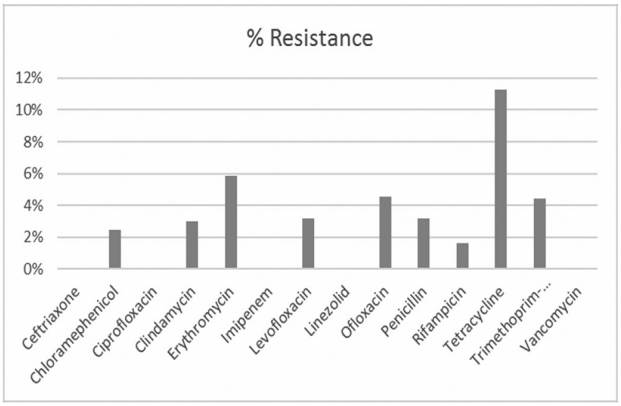

Figure 2. Antimicrobial susceptibility profiles of antibiotics

Serotypes 4, 11, 21, 29, 31, 33, 34 and 35 were commonly isolated from patients with bronchopulmonary diseases such as Chronic Obstructive Pulmonary Diseases and Tuberculosis (Table 2). Serotypes 7, 10 and 18 were isolated from patients with cerebrovascular diseases. Serotypes 18 and 40 were isolated from patients with congestive heart failure and serotypes 3, 4 and 7 were isolated from those who smoke.

Table 2. Serotype distribution according to a particular underlying medical condition or risk factor.

Medical Condition	Serotypes
Bronchopulmonary Disease	4, 11, 21, 29, 31, 33, 34, 35
Cerebrovascular Disease	7, 10, 18
Congestive Heart Failure	18, 40
Smoker	3, 4, 7

Serotypes 7, 10, 18, 21, 33 and 34 were found to be present in those with altered mentality or with confusion of new onset (Table 3). Serotypes 6, 7, 10, 11, 18, 19, 21, 29, 31, 33, 34, 35, 40 were present in patients with unstable vital signs. Patients with CURB 65 score > 1 have isolates with serotypes 7, 10, 28, 32, 33, 34, and 40. In patients with severe sepsis, serotypes 6, 7, 18, 31, 33, 34 and 35 were present. Only serotype 7 was found in a patient with septic shock. This was the only serotype which was found in all clinical manifestations.

The serotype distribution according to the overall outcomes of the patient is shown in Table 4. Serotypes 4, 7, 18 and 34 were present among those who died within 72 hours of admission. Death after 72 hours of admission had one non-serotypable *Streptoccocus pneumoniae*. Most of the serotypes present were found among those who improved and were eventually discharged.

Table 3. Serotype distribution according to clinical manifestations and laboratory data of the study population.

Clinical Manifestation	Serotype
Altered Mentality	7, 10, 18, 21, 33, 34
Unstable Vital Signs	6, 7, 10, 11, 18, 19, 21, 29, 31, 33, 34, 35, 40
Curb 65 Score >1	7,10, 28, 32, 33, 34, 40
Patients with SIRS	Non-serotypable
Patients with Severe Sepsis	6, 7, 18, 31, 33, 34, 35
Patients with Septic Shock	7
Use of Vasopressor	7

Table 4. Serotype distribution according to overall outcomes.

Outcomes	Serotypes
Death within 72 Hours of Admission	4,7,18, 34
Death after 72 hours of Admission	Non-serotypable
Survival	1, 3, 6,7,10,11,12, 16, 17, 18,19, 21, 23, 29, 31,32, 33, 34, 35, 40

Discussion

It was shown in the previous section that there were more males who were admitted due to pneumococcal diseases. Sex is a significant epidemiological factor for several diseases. Males are more commonly affected with otitis media, croup, and most importantly, lower respiratory infections such as pneumonia.[9] Several studies were performed regarding the potential risk factors for development of Community Acquired Pneumonia (CAP)[10,11,12], most of them enrolled hospitalized patients. In most of these studies, men were statistically more frequently enrolled and in some of them male sex was a risk factor for hospitalization of CAP. Most of the admitted patients were also in the age range of 61-70 years old. One study has shown that those patients who are 65 years and older are more prone to develop pneumonia.[13] The increased susceptibility can be attributed to the declining immune function of the elderly population.

A total of 21 different serotypes were isolated from 71 patients. These serotypes were 1, 3, 4, 6, 7, 10, 11, 12, 16, 17, 18, 19, 21, 23, 29, 31, 32, 33, 34, 35, and 40. The most prevalent serogroups in Asia are serotypes 6, 9, 14, 19, 232 some of which are present in the isolates of VSMMC. Moreover, analysis of data from six ASEAN countries, from information on pneumococcal serotypes showed that the most common disease causing serotypes (in rank order) were 19F, 23F, 14, 6B, 1, 19A and 3. Serotype distribution of pneumococcal isolates was similar across the ASEAN region.[14]

The serotypes found among these patients have a major impact on the epidemiology of *Streptococcus pneumoniae*. Current pneumococcal vaccine covers many of the pneumococcal serotypes found in the hospital. Pneumococcal polysaccharide vaccines particularly cover a total of 23 serotypes[11] while conjugate vaccines cover a total of 7 or 13 serotypes. However, the results of this study also showed a few serotypes that are not covered by these vaccines are serotypes 16, 21, 29, 31, 32, 34, 35 and 40. However, these are not part of the most common serotypes that are causing an invasive pneumococcal disease. Several factors have to be taken into account before an inference can be made with regards to these non-vaccine serotypes. A longer study with a larger population size is needed to further characterize the epidemiology of *S. pneumoniae* in Vicente Sotto. Administration of vaccines could prevent infection with pneumonia and could lessen the use of antibiotics thereby reducing chances of the emergence of resistance.

Fifteen samples were identified as non-serotypable which comprised most of the samples. It is a specific group of natural atypical pneumococci whose identification is often difficult, and which has remained insufficiently studied. Some pneumococci do not react with commercially available antisera used for serotyping and are thus regarded as non-serotypeable (NT). These pneumococci are commonly isolated during carriage studies and very rarely cause invasive disease.[14]

In this study, tetracycline has the highest resistance among the antibiotics at 11.29%. Some studies have showed resistance profiles of 8% - 16.6% of all the isolates tested for tetracycline.[15,16] The mechanism by which S. pneumoniae develops resistance to tetracycline is through alteration in the tetM gene.

Erythromycin and ofloxacin in this study had resistance percentages of 5.88% and 4.55% respectively. Although pneumococcal macrolide resistance has increased worldwide, prevalence rates vary highly from country to country. Macrolide resistance rates range from 15% to 49%. On the other hand, the worldwide prevalence of fluoroquinolone resistance in S. pneumoniae is low, although it also varies over time, geographic region, age group, and origin of isolates.[16]

It has also been shown in the previous chapter the serotypes that are present according to a particular underlying disease. Many serotypes were found in those who have bronchopulmonary diseases. Serotypes found were 4, 11, 21, 29, 31, 33, 34, 35. One of the common complications of COPD patients is pneumonia since the protective and clearing mechanisms of the lungs against pathogens are impaired that would make them susceptible to respiratory tract infections. Increased susceptibility to

infections has been attributed not only to anatomical, physiological, and/or immunological aging but also to an increase in the prevalence of chronic diseases, especially cardiovascular and pulmonary diseases.[17]

Serotype 7 was also noted to be found across all the categories of clinical presentation even to those patients with severe sepsis, septic shock and on vasopressor. Several studies have evaluated several serotypes and the risk factor of having an increased morbidity or fatal outcome. One journal article stated that the 92 capsular serotypes of *Streptococcus pneumoniae* differ greatly in nasopharyngeal carriage prevalence, invasiveness, and disease incidence. There has been some debate, though, regarding whether serotype independently affects the outcome of invasive pneumococcal disease (IPD). Published studies have shown variable results with regard to case-fatality ratios for specific serotypes and the role of host factors in affecting these relationships.[18] Overall, serotypes 1, 7F, and 8 were associated with decreased relative risks (RR), and serotypes 3, 6A, 6B, 9N, and 19F were associated with increased RR.[18] The result of the studies mentioned are in contrast to the result of this study in which serotype 7 was also found in those with severe sepsis and septic shock. However, the underlying risk factor or co-morbidity may have a role in the severity of the clinical presentation.

Serotypes 4, 7, 18 and 34 were found among those who died within 72 hours of admission. The mortality however, cannot be attributed directly to the serotype present since some of these patients had co-morbidities such as cerebrovascular accident and congestive heart failure. A meta-analysis study showed only an increased risk of death in patients with serotypes 3, 6A, 6B, 9N, and 19.[31] The serotypes mentioned were not found in those patients who died of pneumonia.

Conclusion

The three most common serotypes isolated from these patients with pneumococcal diseases were serotypes 3, 4 and 18, comprising 29.58% of the isolates. Also common were serotypes 7, 19 and 29 with a total percentage of 16.89%. Other serotypes isolated were serotypes 1, 6, 10, 11, 12, 16, 17, 21, 23, 31, 32, 33 and 40.

In the scope of family practice where preventive management is also emphasized giving of pneumococcal vaccines serve as a primary prevention. Most of the serotypes present in this study are currently covered by the pneumococcal vaccines being given that would answer questions of concern for sufficient coverage of current vaccines.

Serotypes 7, 10, 18, 21, 33 and 34 were found to be present in those with altered mentality or with confusion of new onset. Serotypes 6, 7, 10, 11, 18, 19, 21, 29, 31, 33, 34, 35 and 40 were present in patients with unstable vital signs. Only serotype 7 was found in a patient with septic shock. Serotypes 4, 7, 18 and 34 were present among those who died within 72 hours of admission. Most of the serotypes present were found among those who improved and were eventually discharged.

A total of 14 antibiotics were tested. Among these antibiotics, tetracycline has the highest resistance at 11.29%, followed by erythromycin at 5.88% and ofloxacin at 4.55%. All of the other isolates are susceptible to ceftriaxone, ciprofloxacin, imipenem, linezolid, and vancomycin.

This is only an initial study that determined the serotypes present in Vicente Sotto Memorial Medical Center and the antibiotic resistance of *Streptococcus pnuemoniae* in a span of 2 years. A further study spanning more than 2 years and a greater number of sample size is recommended since this might determine a certain association of a serotype to a clinical factor. No statistical inference can be made in this study but a future study consisting of a greater population size and a greater frequency of a certain serotype can contribute significantly to the epidemiology of *S. pneumoniae* in the hospital.

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