Molecular epidemiology of invasive *Streptococcus pneumoniae* serotype 19A in Thailand, 2008-2018

Unchalee Puangprasart[1], Witchuda Kamolvit[1], Chanwit Tribuddharat[1]
Wanatpreeya Phongsamart[2], and Piriyaporn Chongtrakool[1]
[1]Department of Microbiology, and [2] Department of Pediatrics,
Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Abstract

After introduction of PCV7, the incidence of Invasive Pneumococcal Disease (IPD) caused by penicillin non-susceptible *Streptococcus pneumoniae* serotype 19A dramatically increased worldwide. In Thailand, molecular epidemiology of invasive *S. pneumoniae* serotype 19A are limited. This work aimed to characterize this particular serotype, isolated during 2008-2018 based on MLST and correlate molecular types to their antimicrobial resistance from previous study (1, 2, 3). Among 62 strains of serotype 19A, 3 clonal complexes (CC) and 9 STs have been found. The most predominant was CC320 (n=26) followed by ST2930 (n=9), CC230 (n=8), CC63 (n=4), ST95 and ST8346 (n=2). Interestingly, we found 7 isolates with no establishing or new STs. Among all isolates with penicillin non-susceptible, 31% and 93% were interpreted according to non-meningitis and meningitis criteria, respectively. Moreover, 67.7% showed erythromycin mono-resistant while 33.9% were resistant to erythromycin, trimethoprim/sulfamethoxazole, and meropenem. The predominant ST; ST320 and ST2930 were resistant to at least three antimicrobial classes. Theses STs appeared to disseminate in our region throughout the study period. Prevention and infection control of highly antimicrobial-resistant clonal dissemination should be of concern. The inclusion of PCV13 in the Expanded Program on Immunization should be recommended.

Introduction

Before the introduction of PCV7, *S. pneumoniae* serotype 19A were common inhabitants found in nasopharynx and rarely cause diseases (4). After introduction of PCV7, the emergence of serotype 19A causing IPD has been noted worldwide. The data from German (5), Alaska (6), and United states (7) revealed MDR ST320 has been observed after post-PCV7 era and the strains still predominated whereas serotype 19A causing IPD decreased following PCV13 implementation in US (7). Meanwhile, in Norway where the antibiotic usage is limited, ST199 with penicillin-susceptible were predominant clones in post-PCV7 era (8).

Thus, many countries have characterized serotype 19A in their own region. Study from ANSORP in 2011 reported a limited number of 10 isolates from Thailand belonged to CC172 (n=4), ST4826 (n=2), and 1 isolate each of ST878, ST2636, ST4458 and ST6002 (9). More study is required to understand genetic characteristics and genetic variations of invasive *S. pneumoniae* serotype 19A in many countries including Thailand.

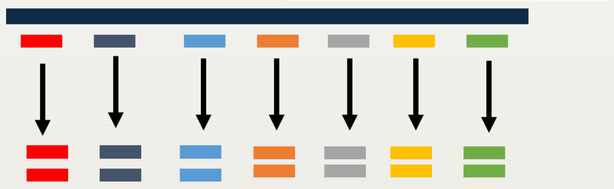
Objective

To study genetic characterization of *S. pneumoniae* serotype 19A causing invasive pneumococcal disease during 2008-2018 and correlate them to their antimicrobial resistance.

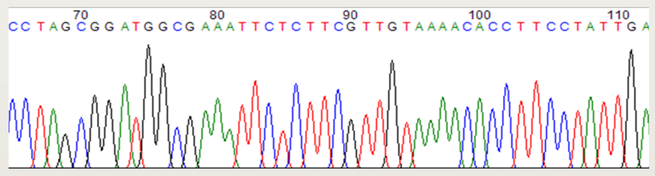
Materials and Methods

DNA extraction

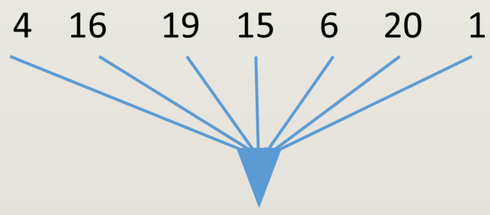
PCR of 7 house keeping genes



Sequencing



Allele assignment



ST type

Clonal complex assignment using eBURST algorithm

Result

The sequence types distribution was shown in figure 1, demonstrating 20 distinct STs. A total of 55 (88.7%) isolates belonged to 13 previously described STs whereas the remaining 7 STs had not been established in pneumococcal MLST database (http://spneumoniae.mlst.net).

Among 20 STs, the most predominant was ST320 (40.3%), followed by ST2930 (14.5%), ST230 (12.9%), ST63 (6.5%), ST95 and ST8346 (3.2%) . When categorized to clonal complex (CC), three were found in this study. They are CC320 (41.9%), CC230 (12.9%) and CC63 (6.5%) respectively.

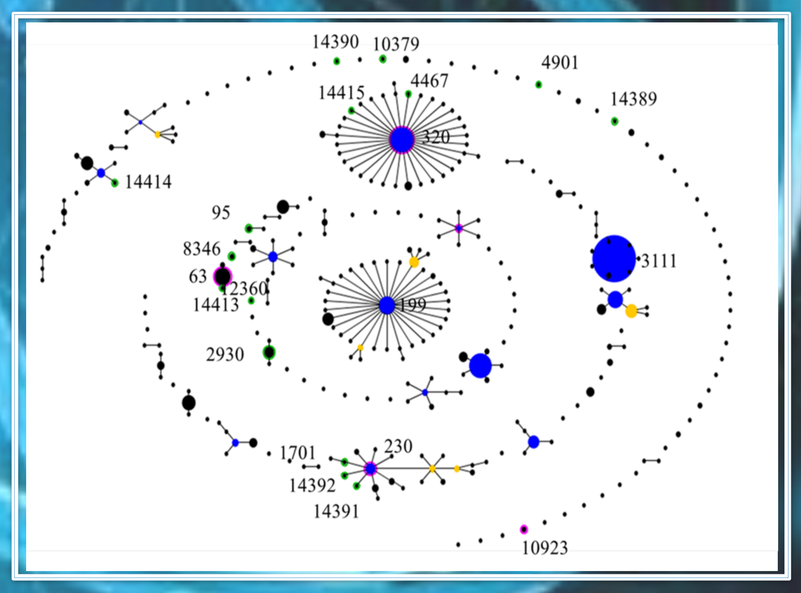


Figure1: eBURST snapshot of serotype19A isolates from all IPD isolates all over the world comparing to this study. ST labels were found in this study. The size of circle corresponds to amount of isolates. ST founder highlighted with blue circles. The pink circle represent ST found in both dataset whereas green circle was found only in this study.

The most predominant ST320 showed percentage of non-susceptible to the following agents; penicillin : 64.7%, meropenem : 46.5%, and erythromycin : 48.8%, whereas the second predominant ST2930 showed less percentage of non-susceptible; penicillin : 17.6%, meropenem : 20.9%, erythromycin : 20.9%. Moreover, all five isolates that were non-susceptible to either cefotaxime or ceftriaxone (MIC ≥2 µg/ml) belonged to ST320. In this study, there were 33 isolates (53.2%) that were resistant to at least 3 antimicrobial classes, categorized as Multiple Drug Resistant *S. pneumoniae* (MDR-*S.pneumoniae*). Among them, 18 (54.5%) belonged to ST320, and 9 (27.3%) belonged to ST2930.

Discussion and Conclusion

The predominant highly antimicrobial non-susceptible ST320 and ST2930 appeared to disseminate in our region throughout the study period. Prevention and infection control of highly antimicrobial non-susceptible clonal dissemination should be of concern. The inclusion of PCV13 in the Expanded Program on Immunization should be recommended.

References

1. Srifeungfung S, Tribuddharat C, Comerungsee S, Chatsuwan T, Treerauthanaweeraphong V, Rungnobhakhun P, et al. Serotype coverage of pneumococcal conjugate vaccine and drug susceptibility of Streptococcus pneumoniae isolated from invasive or non-invasive diseases in central Thailand, 2006-2009. Vaccine 2010; 28: 3440-4.
2. Phongsamart W, Srifeungfung S, Chatsuwan T, Nunthapisud P, Treerauthaweeraphong V, Rungnobhakhun P, et al. Changing trends in serotype distribution and antimicrobial susceptibility of Streptococcus pneumoniae causing invasive diseases in Central Thailand, 2009-2012. Hum Vaccin Immunother. 2014; 10: 1866-73.
3. Nitayanon p. Determination of serotypes and drug susceptibility of S. pneumoniae causing invasive pneumococcal disease, 2012-2015, and prevalence of m protein gene in group C and group G streptococci: Mahidol University; 2016.
4. Hanage WP. Serotype replacement in invasive pneumococcal disease: where do we go from here? J Infect Dis. 2007;196(9):1282-4.
5. Van der Linden M, Reinert RR, Kern WV, Imöhl M. Epidemiology of serotype 19A isolates from invasive pneumococcal disease in German children. BMC Infect Dis. 2013;13(1):70.
6. Rudolph K, Bruce MG, Bulkow L, Zulz T, Reasonover A, Harker-Jones M, et al. Molecular epidemiology of serotype 19A *Streptococcus pneumoniae* among invasive isolates from Alaska, 1986-2010. Int J Circumpolar Health. 2013;72.
7. Hulten KG, Kaplan SL, Lamberth LB, Barson WJ, Romero JR, Lin PL, et al. Changes in *Streptococcus pneumoniae* serotype 19A invasive infections in children from 1993 to 2011. J Clin Microbiol. 2013;51(4):1294-7.
8. Vestrheim DF, Steinbakk M, Aaberge IS, Caugant DA. Postvaccination increase in serotype 19A pneumococcal disease in Norway is driven by expansion of penicillin-susceptible strains of the ST199 complex. Clin Vaccine Immunol. 2012;19(3):443-5.
9. Shin J, Baek JY, Kim SH, Song JH, Ko KS. Predominance of ST320 among *Streptococcus pneumoniae* serotype 19A isolates from 10 Asian countries. J Antimicrob Chemother. 2011;66(5):1001-4.