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Population-based Assessment of Invasive Disease and Macrolide Resistance from *Streptococcus pneumoniae* in Atlanta from 2007 to 2010

By

Wan-Hsuan Kuo

Master of Science in Public Health

Epidemiology

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Dr. David S. Stephens

Committee Chair

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An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science in Public Health

in Epidemiology

2012

Abstract

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The incidence of macrolide resistance in *Streptococcus pneumoniae* increased in the last twenty years due to the widespread clinical use of macrolides. The seven-valent pneumococcal conjugate vaccine (PCV7) and the 13-valent pneumococcal conjugate (PCV13) were approved in February 2000 and in February 2010. We assessed the changes in macrolide-resistance invasive *S. pneumoniae* disease and molecular basis of resistance in Atlanta, GA, USA from 2007-2010. Pneumococcal isolates and demographic data were obtained from a prospective population-based surveillance under the active bacterial core surveillance (ABCs) of the Georgia Emerging Infections Program. The *mef*(E) and *erm*(B) macrolide resistance genes were detected by PCR. Incidence was calculated using population estimates and census data from US Census Bureau.

The overall incidence of invasive pneumococcal disease (ranging from 13.3-11.7/100,000) and macrolide resistance (ranging from 3.4 to 4.2/100,000) in Atlanta appeared stable from 2007 to 2010. The incidence of disease and macrolide resistance due to PCV7 vaccine serotypes however has reached all-time lows in all age groups. But the incidence of isolates carrying both *mef*(E) and *erm*(B) increased especially among children younger than 2 years old and in serotype 19A, 23.5% (94/400) of available macrolide resistant isolates and 47% (24/51) of available macrolide resistant 19A contained both genes. The continued emergence of 19A and the presence of dual macrolide resistance genes in this serotype clearly support the need for the new PCV13 vaccine containing this serotype.

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Acknowledgements

I would like to acknowledge all who helped me to accomplish this thesis.

I would like to thank my thesis advisor, Dr. Stephens. With his guidance, enthusiasm, and his great efforts to explain everything clearly and to edit my thesis carefully, I learned a lot from him.

I would like to thank Susu Zughaier who contributes to pneumococcal surveillance from 2002 to 2006.

I would like to thank all lab members, Scott Chancey, Dorathea Zahner, Xianhe Bai and Yih-Ling Tzeng, to teach me important technologic skill, help me in trouble shooting, and for giving me advises for writing the thesis.

I would like to thank Georgia Emerging Infection Program for conducting surveillance, collecting and providing this dataset. I am especially grateful to Amy Holst, Wendy Baughman to provide me further detail of data and Emily Crispell to streak trains out for me.

Finally, I would like to thank my parents, my family and friends who have supported and encouraged me during my “master’s” life.

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Introduction

Streptococcus pneumoniae remains a leading cause of sepsis, bacterial meningitis, pneumonia, bronchitis, sinusitis, and otitis media.[1] Commonly recommended first-line treatments for upper respiratory bacterial infections, including pneumococcal infections and community acquired pneumonia, include macrolide antibiotics. Studies indicate that resistance to macrolides has increased in *S. pneumoniae* over the last two decades due to the widespread clinical use and often inappropriate prescription of macrolides, such as azithromycin and clarithromycin in the United States.[2, 3] Surveillance conducted by PROTEKT US (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin in the US) reported that the percentage of pneumococcal isolates resistant to erythromycin in the United States was 29.3% from 2000 to 2004.[4]

Two major mechanisms of macrolide resistance in *S. pneumoniae* are target modification and drug efflux.[5] Methylation of adenine residues in the 23S ribosomal RNA (rRNA) peptidyl transferase center, domain 5, results from an *ermB* encoded erythromycin-ribosomal methylase. The methyl groups inhibit the binding site of macrolides (eg. erythromycin), lincosamides (eg. clindamycin), and streptogramin B.[6] *S. pneumoniae* containing *erm*(B) is thus designated as an MLS_B phenotype and this is associated with high level resistance to macrolides (minimum inhibition concentration >32 ug/ml). The other major macrolide resistance mechanism is due to a macrolide efflux pump encoded by *mef*-containing elements and is designated the M phenotype.[7] Pneumococcal strains containing *mef*(E) are resistance to many 14- and 15- member macrolide antibiotics including

erythromycin. The *mef*(E) gene is carried on a 5.5kb macrolide efflux genetic assembly (mega) which can insert into different chromosomal sites or is carried on larger transposons.[8, 9] The nucleotide sequence of mega revealed that a 119-bp intergenic region between *mef*(E) and *mel* contained a 99-bp deletion.[10] The sequence with a 99-bp insertion is designated a mega class I, and with a 99-bp deletion is a mega class II.[9]

Since children under two years old have been at the highest risk group of pneumococcal disease, a seven-valent pneumococcal glyco-conjugate vaccine (PCV7) containing serotype 4, 6B, 14, 23F, 19F 9V and 18C was approved by the US Food and Drug Administration for use in infants and young children in February 2000. This vaccine, due to both a direct effect in young children and a strong herd immunity effect in all populations including the elderly, has reduced the overall incidence of invasive pneumococcal disease in the United States by over 50%.[11] However, “serotype replacement” has altered the decline in the overall incidence of disease and also the decline in macrolide resistance resulting from the introduction of the PCV7. [11] The increase in the incidence of non-vaccine serotypes has been reported in several studies especially serotype 19A.[11-13] Therefore, a 13-valent pneumococcal conjugate vaccine (PCV13) that contained the 7 serotypes in PCV7 plus the six additional types including serotypes 1, 3, 5, 6A, 7F, 19A was developed, approved and introduced in the United States in February 2010.

This report extends and updates the 2005 report by Stephens et al.[11] on invasive *S. pneumoniae* and macrolide resistance in the Atlanta metropolitan area from 1994 to 2002, and unpublished data by Chancey S.T., Stephens D.S. et al. from

2003 to 2006. Prior to the introduction of the vaccine in Atlanta, GA, USA, from 1994 to 1999, the mean incidence of invasive pneumococcal disease was 30.2 per 100,000 population. After the introduction of PCV7, the incidence dropped to 22.3 per 100,000 in 2000 and continued to decrease to 13.1 per 100,000 by 2002[11]. This decrease in incidence in Atlanta was the result of an 82% decrease in disease in children under two years old who are recommended to receive PCV7 but also resulted from the very significant herd immunity effect reducing disease in older children and adults. In addition to the decline in overall incidence, macrolide resistance in invasive pneumococci in Atlanta decreased by 69% from 77 to 2.9 per 100,000 from 1999 to 2002 after a steady increase from 1994 to 1999. The reduction was due to the decrease in *mef*(E)-mediated and *erm*(B)-mediated macrolide-resistant isolates of PCV7 vaccine types[11]. We assessed between 2007 and 2010 the continuing effects of the 7-valent pneumococcal conjugate vaccine on overall disease and on macrolide resistance of invasive *S. pneumoniae* using a well-established population-based active surveillance system and by determining the molecular mechanisms of macrolide resistance in pneumococcal isolates.

Materials and Methods

Surveillance

Active prospective population-based surveillance for invasive pneumococcal disease has been conducted in metropolitan Atlanta since 1994 under the active bacterial core surveillance (ABCs) activity of the Georgia Emerging Infections Program. Pneumococcal isolates and demographic data from patients with invasive pneumococcal disease were obtained from all hospitals and laboratories in the 20-county metropolitan Atlanta region. For this report, the cases in Georgia Health District 3(HD3) - the core eight-county Atlanta region were included. The population was 3.6 million in HD3 in 2010 according to the US census bureau. The population was 3.7 million, 3.8 million and 3.9 million in 2007, 2008 and 2009, respectively. The populations from 2007 to 2009 were obtained by Vintage pos-census population estimates. All incidences from 1994 to 2002 were obtained from Stephens et al [11] and that from 2003 to 2006 were based on unpublished data from Chancey, S.T. and Stephens D.S.

The population-based surveillance methods are described elsewhere.[5, 14] Isolates from patients with invasive pneumococcal disease are collected from normally sterile site (e.g. blood or cerebrospinal fluid). Demographic data includes county of residence, age, gender, race and ethnicity. Laboratory data include the site of isolation, serotype, and antibiotic minimum inhibition concentration (MIC) level. Clindamycin (*CLI*) and erythromycin (*ERY*) MICs are two indicators of “macrolide” resistance. Isolates that were intermediate to erythromycin were designated as resistant when it was either *mef*(E) or *erm*(B) positive.

Culture condition and antimicrobial susceptibility testing

The MICs of clindamycin and erythromycin were determined by the broth microdilution methods. The concentrations of clindamycin were 0.03-2 and for erythromycin 0.5-32 ug/ml. The MIC was determined as the lowest concentration of the antibiotic that inhibited growth. For clindamycin, if the MIC of the isolate was higher than 2 ug/ml, they were defined as resistant. For erythromycin, the break point of MIC was 0.5 ug/ml for intermediate and >0.5 ug/ml for resistant. "High-level resistance" of a pneumococcal isolate was designated with an erythromycin MIC level higher than 32 ug/ml.

Molecular typing

Both *erm*(B) and *mef*(E) were identified in isolates by the polymerase chain reaction (PCR). Erythromycin resistant isolates grown on sheep's blood agar were used for crude DNA preparations. Crude DNA preparation of genomic DNA were obtained by boiling bacterial pallets for 10 min in a lysis buffer which consisted of 100mM NaCl, 10mM Tris-HCl (pH=8.3), 1mM EDTA, and 1% Triton X-100. Primer sets KG1F (5-TTGGAACAGGTAAAGGGCATT-3) and KG1R2(5-GTTCGTTACTTTGTGCGGTTT-3)[14], KG8(5- GTATCATGTCACTTGCTATGCC-3) and KG10 (5-ACA CCT AGC TTG CCT ACA AGT G-3)[15] were used to detected *erm*(B) and *mef*(E). A 555-bp DNA fragment indicated *erm*(B). A 555 bp and 456 bp fragments indicates mega-1 or mega-2.

PCR amplification consisted of 30 cycles with a 30-s denaturation at 94℃ , a 30-s anneal at 52℃, and a 30-s extension at 72℃ in a thermal cycler (Eppendorf).

Each reaction contained 1.5mM MgCl2, 1.5uM each of the two primers, 200uM deoxyribonucleoside triphosphate, 2.5U Taq DNA polymerase and 5uL 10 fold dilute of crude DNA sample. PCR products were visualized through 1% agarose gels and ethidium-bromide staining.

Statistical analysis

The x^2 test was used for comparisons of proportions. The incidence (cases per 100,000 population) of total invasive pneumococcal cases, macrolide resistant cases, and *mef*(E), *erm*(B) mechanism of macrolide resistance or both present in metropolitan Atlanta surveillance area were calculated by the number of identified cases divided by the census population. We assumed the distribution for isolates with unknown serotype, unknown antibiotic-sensitivity data and isolates not available was the same as that of isolates with information available. The isolates with discrepancy between molecular types and antibiotic-sensitivity phenotype were excluded. All statistics were calculated using SAS v9.3.

Results

In Atlanta, 1859 invasive pneumococcal infections were identified from 2007 to 2010 (499 in 2007, 460 in 2008, 467 in 2009 and 433 in 2010). Of the cases, 55 percent were in males. The number of cases was similar from 2007 to 2010 in the 8 county surveillance areas. The mean age of disease onset was 47 years. The mean age of onset increased from 46 years to 50 years from 2007 to 2010; 70% of isolates were from blood; 2.5% were from cerebrospinal fluid; 1.3% were from other sites (e.g. bone, internal body site, joint, peritoneal fluid, pleural fluid and tissue); and 27% were not available. Ten percent of cases died. Of the isolates, 499 (27%) did not have available serotype and antimicrobial-susceptibility data [109 (22%) in 2007, 87 (19%) in 2008, 66 (14%) in 2009 and 237 (55%) in 2010].

The annual incidence per 100,000 population of invasive pneumococcal disease was 13.3 cases, 11.9 cases, and 12 cases in 2007, 2008, 2009 respectively and 11.7 cases in 2010. (Table 1, Figure 1A) The incidence of disease from 2007 to 2010 was highest, generally >30 per 100,000, among children younger than 2 years old and adults aged 65 and older. Among children younger than 2 years old, the incidence ranged between 32.3 and 36.6 per 100,000 from 2007 to 2009 but decreased to 23.7 per 100,000 in 2010 (year of the introduction of PCV13). (Table 2, Figure 1B) Among adults aged 65 years and older, the incidence fluctuated between 37.7 and 40.9 per 100,000 from 2007 to 2010. (Table 2, Figure 1C) The incidence was between 9.9 - 17.2, 1.4 - 2.1, 4.4 - 7.6, and 14.3– 18.4 per 100,000 in those 2-4 years, 5-19 years, 20-39 years, and 40-64 years respectively. Fifty-two percent of cases occurred in African-Americans, and 41% of cases were in whites. The incidence

among African-Americans was 21.5 per 100,000 in 2007 and 15.2 per 100,000 in 2010. ($p < 0.0001$) The incidence among whites was 8.7 per 100,000 in 2007 and 10.3 per 100,000 in 2010. ($P=0.11$)

1,360 of 1,859 isolates to date have had serotyping and antimicrobial-susceptibility testing performed; of these, 415 isolates were resistant to erythromycin, 941 were susceptible, and 4 were intermediate. The incidence of macrolide-resistant invasive *S pneumoniae* was 3.4 per 100,000 in 2007, 3.8 in 2008, 3.7 in 2009 and 4.2 in 2010. (Table 1, Figure 1A) Among children under 2 years of age, the incidence decreased from 20.1 per 100,000 to 18.6 per 100,000. (Table 2, Figure 1B) Among children aged 2 to 4, the incidence fluctuated between 6.3 per 100,000 and 11 per 100,000. (Figure 1D) Among adults aged 65 years and older, the incidence of macrolide resistance increased from 8.5 per 100,000 to 18.7 per 100,000. ($p=0.002$)

Among 419 isolates resistant or intermediate to erythromycin, 406 isolates were available for typing *mef*(E) and *erm*(B) by PCR(two isolates were contaminated and 11 isolates were not recovered). Of three classified as intermediate isolates, two contained neither *mef*(E) or *erm*(B), and one contained *mef*(E) only. Among the remaining 403 available erythromycin resistant isolates, 373 (92.6%) isolates contained either *mef*(E) or *erm*(B) and were associated with the M or MLS_B phenotype. There were 4 isolates that whose molecular types were not consistent

with the MICs to erythromycin and clindamycin.* All MLS_B (*erm*(B))-associated macrolide resistant isolates with MIC >32 ug/ml to erythromycin contained *erm*(B).

Excluding the 4 inconsistent isolates, from 2007 to 2010, the incidence of *mef*(E) was between 1.8 and 2.1 per 100,000, *erm*(B) was between 0.7 and 0.9 per 100,000, and isolates containing both *mef*(E) and *erm*(B) increased from 0.6 to 1.3 per 100,000. (p =0.006) (Table 1) Changes in the incidence of *mef*(E) or *erm*(B) from 2007 to 2010 were observed in children younger than 2 years old and in adults aged 65 years and older. (Table 2, Figure 1B) Among children younger less than 2 years old, the incidence of *mef*(E)-mediated resistance fluctuated from 11.1 per 100,000 to 8.5 per 100,000 (p =0.52) and that of *erm*(B)-mediated resistance from 2.2 per 100,000 to 0 per 100,000. (p =0.99) The incidence of isolates containing both *mef*(E) and *erm*(B) steadily increased in children < 2 years from 4.5 per 100,000 to 10.2 per 100,000. (p =0.12) Among children aged 2 to 4 years, the incidence of *mef*(E)-mediated resistance was between 1.4 per 100,000 to 5.0 per 100,000 and that of *erm*(B)-mediated resistance ranged from 0.7 per 100,000 to 1.2 per 100,000. Similarly, the incidence of isolates containing both *mef*(E) and *erm*(B) fluctuated between 1.2 per 100,000 and 2.8 per 100,000 from 2007 to 2009 and increased to 6.1 per 100,000 in 2010. (p =0.037)

In adults aged 65 years and above the incidence of *mef*(E)-mediated resistance fluctuated between 4.5 per 100,000 and 5.7 per 100,000 from 2007 to 2009 and

* In 2008, isolate GA54281 was serotype 23A, positive to *mef*(E), negative to *erm*(B) and resistant to both clindamycin and erythromycin. In 2008, isolate GA54686 was serotype 19A, positive to both *mef*(E) and *erm*(B), and resistant to erythromycin and susceptible to clindamycin. In 2010, two isolates were serotype 3, resistant to

increased to 12.2 per 100,000 in 2010. (p =0.002) The incidence of *erm*(B)-mediated resistance fluctuated between 2.0 per 100,000 and 3.3 per 100,000. The incidence in the elderly of isolates containing both *mef*(E) and *erm*(B) was ~1.3 per 100,000 in 2007 and 2008 and increased to 3.6 per 100,000 in 2009 and 2010. (p =0.060) To investigate the relationship between the macrolide resistant antibiotic phenotype and molecular genotype, all M (*mef(E)*)-associated macrolide resistant isolates that had erythromycin MICs less than 32 ug/ml and which were susceptible to clindamycin contained *mef(E)* only. Among the 181 MLS_B type isolates, all isolates contained to *erm*(B) but more than 50% (92/181) were positive for *mef(E)* as well.

During this surveillance period (2007 to 2010), the presence of *mef*(E) and *erm*(B) were investigated in relation to capsular serotype. The incidence of the serotypes found in the 7-valent conjugate vaccine (serotype 4, 6B, 14, 23F, 19F 9V and 18C) was 1.0 per 100,000 in 2007 and decreased to 0.3 per 100,000 in 2010 (Table 3). Between 2007 and 2010, serotype 19A was the most prevalent serotype among all invasive pneumococcal isolates and macrolide resistant isolates. (Figure 2) The incidence of serotype 19A ranged from 4.7 per 100,000 and 5.9 per 100,000 and the macrolide resistant incidence ranged from 1.9 per 100,000 to 2.3 per 100,000. The incidence of *mef*(E) only-positive 19A isolates was 0.7 per 100,000 , that of *erm*(B) only-positive isolates was 0.1 to 0.4 per 100,000, and that of both *mef*(E) and *erm*(B) positive isolates increased from 0.6 per 100,000 in 2007 to 1.2 per

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erythromycin and susceptible to clindamycin. Isolate GA58629 and isolate GA59972 was negative and positive to *mef*(E) and were both positive to *erm*(B).

100,000 in 2010. (p =0.010) The second most prevalent serotype was 7F but only one of 159 isolates was macrolide resistant isolate.

Serotype 19A accounted for more than 20% of isolates from 2007 to 2010. (Figure 3) Among all available macrolide resistant isolates, serotype 19A accounted for more than 45%. Among *mef*(E)-positive isolates, the percent of serotype 19A fluctuated between 35.3% and 46%. Among both *mef*(E) and *erm*(B) positive isolates, more than 85% of isolates were serotype 19A. Among *erm*(B)-positive isolates, the percent of serotype 15A fluctuated from 70% to 18% from 2007 to 2009 and increased to 58% in 2010. The percent of serotype 19A increased from 15% to 48% from 2007 to 2009 and decreased to 8% in 2010. Thus, serotype 19A was the most prevalent serotype in 2007-2010. Also, serotype 19A was the serotype with the majority of macrolide resistant isolates.

Based on the surveillance from 1994 to 2006, the total incidence of serotype 19A was 0.7 per 100,000 in 2000, increased to 2.9 per 100,000 in 2004 and fluctuated between 2.6 and 3.0 per 100,000 from 2004 to 2010. (Figure 4A) The incidence of macrolide resistant 19A isolates was less than 0.5 per 100,000 before 2000 and increased to 1.4 per 100,000 in 2004 and fluctuated between 1.4 and 2.2 per 100,000 from 2005 to 2010. The incidence of *mef*(E)-positive and *erm*(B)-positive 19A isolates increased from 2000 to 2010. The incidence of *mef*(E) and *erm*(B) both positive isolates increased from 0.6 to 1.1 per 100,000 from 2007 to 2010. The incidence of *mef*(E) and *erm*(B) dual isolates was higher than that of *mef*(E)-positive and *erm*(B)-containing isolates starting in 2009.

Among children less than 2 years old, the total incidence of 19A fluctuated between 15.3 and 17.1 per 100,000 from 2007 to 2010. (Figure 4B) The incidence of macrolide resistant 19A in this age group fluctuated between 13.5 and 15.3 per 100,000. The incidence of *mef*(E)-positive varied from 8.9 to 5.1 per 100,000 from 2007 to 2010. The incidence of *erm*(B)-positive fluctuated between 1.1 and 2.0 per 100,000 from 2007 to 2009 and was 0 in 2010; but the incidence of *mef*(E)-positive and *erm*(B) dual positive isolates increased from 4.5 to 8.5 from 2007 to 2010.

Discussion

The overall incidence of invasive pneumococcal disease (ranging from 13.3-11.7/100,000) and macrolide resistance (ranging from 3.4 to 4.2/100,000) in Atlanta appeared stable from 2007 to 2010, seven-ten years after the introduction of PCV7, the pneumococcal conjugate vaccine, into the city.[11] Prior to the introduction of PCV 7 the incidence of invasive pneumococcal disease was ~30/100,000 and between 2000 and 2002 fell dramatically to 13.1/100,000 while macrolide resistance fell from 9.3 to 2.9/100,000 in those same years [5, 11]. The decline in 2000-2002 was due to the rapid reduction of vaccine serotypes that were associated with increasing levels of macrolide resistance. A modest reduction of total incidence and especially in children younger than 2 years old in 2010 may reflect the new 13-valent pneumococcal conjugate vaccine introduced in February 2010.

However, several important trends or currents were observed when the incidence data were carefully analyzed. One trend was the continued disappearance of invasive disease due to PCV7 serotypes. The suppression of 7-valent conjugate vaccine associated serotype 4, 14, 6B, 9V, 18C, 19F, and 23F resulting from the introduction of the 7-valent conjugate pneumococcal vaccine PCV7 in Atlanta in February 2000 continued through 2010. The overall incidence of disease and of macrolide resistance due to PCV7 vaccine serotypes has reached all-time lows in all age groups.

In contrast, the incidence of invasive pneumococcal disease in adults aged 65 and above fluctuated between 37 and 44/100,000 (approximately half of the pre PCV7 rates) but a trend toward an increase in the incidence of macrolide resistance

was observed in this population. The incidence of macrolide resistance in 2010 in those >64 years was 18.7/100,000 similar to rate (18.3/100,000) in 1999 prior to the introduction of PCV7. This was largely a result of the emergence of resistance and incidence of serotype 19A in this population.

Another current was that the incidence of isolates carrying both *mef*(E) and *erm*(B) increased significantly (P=0.006), especially among children younger than 2 years old, and this phenotype was largely found in serotype 19A; 23.5% (94/400) of available macrolide resistant isolates and 47% (24/51) of available macrolide resistant 19A contained both genes in this study. Interestingly, in Russia from 2003 to 2005, 30.3% of isolates contained both genes.[16] The primary mechanism associated with dual resistance is Tn2010, a transposon of tetracycline resistance gene with both the *erm*(B) element and the *mef*(E)-containing mega element. Recent study indicates that most of dual strains belong to clonal complex 320 (CC320). These isolates have evolved from multidrug resistant clones Taiwan^{19F}-14 by acquisition of *erm*(B). The emergence of this clone is a major result of the selective pressures of an immunized population resulting from introduction of PCV7. [17-19] Clonal complex 320 (CC320), now predominantly 19A, has been reported to often have dual macrolide resistance gene *mef*(E) and *erm*(B). [18] It appears that the presence of dual genes is an indicator of CC320 especially in serotype 19A. The other mechanism is associated with Tn2017, a transposon consisting of *erm*(B)-carrying Tn917 and the mega element[18]. In this report, the types of transposon associated with both *erm*(B) and mega elements were not investigated.

The two emerging non-PCV7 vaccine serotypes accounting for the increased incidence of invasive pneumococcal disease were 19A and 7F. However, serotype 7F did not contributed to the increase in incidence of macrolide resistance since only 1 macrolide resistant isolate was observed and that in 2007. The most prevalent emerging serotype was 19A. The emergence of serotype 19A began in 2000 and increased 3.7 fold from 2000 to 2004 and fluctuated at ~3/100,000 from 2004 to 2010. The overall incidence of macrolide resistance also increased in 19A isolates to ~2/100,000. Before 2002, most of the macrolide resistance in 19A isolates was *mef*(E)-mediated. However, *erm*(B)-mediated macrolide resistant isolates were observed in 2002, and increased from 2002 to 2006. The emergence of *mef*(E) and *erm*(B) positive isolates was first identified in 2001 and increased from 2001 to 2010. Overall, if we include the incidence of *erm*(B)-only and *mef*(E) and *erm*(B) dual positive isolates, the overall incidence of the *erm*(B)-mediated macrolide resistance increased from 2002 to 2006 to ~2/100,000 and remained high between 2006 and 2010.

Serotype 6A was a prevalent serotype from 1994 to 2000. [11] The incidence of serotype 6A was 1.4-2.1 per 100,000 prior to 2000 and decreased to between 0.7 and 1.0 per 100,000 from 2002 to 2006. In this report, the incidence continued to decrease to less than 0.5 per 100,000 from 2007 to 2009. There may be some cross-protection between the serotype 6B glyco-conjugate found in PCV7 and subgroup 6A.[20] Also, the introduction of PCV13, which included serotype 6A, may further explain the decrease of the incidence to 0 in 2010.

There are limitations of this study. The population data in 2010 were obtained from Census Bureau in 2010 and data from 2007 to 2009 was estimated. There was a small drop in estimated population in 2009 versus the actual population in 2010. Therefore, the incidence determinations from 2007 to 2009 may be slightly underestimated. The erythromycin MIC and serotype data in 2010 was incomplete. 55% (237/433) of the isolates in 2010 were yet to be reported for susceptibility and serotype. The incidences by serotype and antibiotic resistance in the entire population were imputed based on the available data. The proportion of macrolide resistant isolates that consisted of neither *mef*(E) nor *erm*(B) was high in this study (7.5%) than from 1995 to 2002 (<1%).[11] This could be due to an increase in ribosomal mutations but southern blots or other approaches are needed to completely exclude *mef*(E) nor *erm*(B).

In conclusion, the incidence of invasive pneumococcal disease and macrolide resistance in Atlanta appeared stable from 2007 to 2010, seven-ten years after the introduction of PCV7. However, the continued disappearance of PCV7 vaccine serotypes was countered by an increase of macrolide resistant isolates especially 19A carrying both *mef*(E) and *erm*(B) (p=0.010); 19A became the most prevalent serotype in overall disease and macrolide resistance isolates especially in children under 2 years. The continued emergence of 19A and the presence of dual macrolide resistance genes in this serotype clearly support the need for the new PCV13 vaccine containing this serotype.

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Tables

Table1. Incidence of invasive pneumococcal disease in metropolitan Atlanta by year, macrolide resistance, and mechanism of resistance

Year	1994	1999	2000	2001	2002	2004	2006	2007	2008	2009	2010[#]
Total number of cases	717	855	699	578	413	416	497	499	458	467	431
Total incidence[*]	27.6	29.4	22.3	18.4	13.1	12.3	13.6	13.3	11.9	12.0	11.7
Macrolide resistant	4.0	9.4	7.7	4.6	2.9	3.3	4.0	3.4	3.8	3.7	4.2
mef(E) only	3.0	7.7	6.2	3.5	2.1	2.4	2.5	1.8	1.8	1.5	2.1
erm(B) only	1.5	1.6	1.5	1.1	0.8	0.9	1.5	0.7	1.0	0.9	0.8
mef(E) and *erm*(B)	-	-	-	-	-	-	-	0.6	0.6	1.1	1.3

[#] The incidence of disease, macrolide resistance, and mechanism of resistance in 2010 were incomplete.
[*] Total incidence was uncorrected.

Table2. Incidence of invasive pneumococcal disease in metropolitan Atlanta by year, age-group, macrolide resistance, and mechanism of resistance[*]

Year	1994	1999	2000	2001	2002	2004	2006	2007	2008	2009	2010[+]
<2 years											
Total number of cases	178	235	177	70	45	41	38	40	49	44	25
Total incidence	219	260	191	78.6	50	36.2	32.7	32.3	38.9	36.6	23.7
Macrolide resistant	55.2	134.0	98.4	24.2	20.2	14.7	16.9	20.1	21.8	21.4	18.6
mef(E) only	43.6	115.4	89.6	15.7	17.5	11.3	105	11.1	11.4	11.2	8.5
erm(B) only	12.0	18.3	8.8	8.5	2.7	3.4	6.3	2.2	3.1	2.0	0.0
mef(E) and *erm*(B)	-	-	-	-	-	-	-	4.5	6.2	7.1	10.2
2-4 years											
Total number of cases	61	56	38	39	18	22	16	29	18	24	28
Total incidence	49.8	44.2	28.4	30.2	13.3	13.4	9.1	16.2	9.9	13.0	17.2
Macrolide resistant	12.9	17.5	10.3	9.3	2.9	4.7	4.2	6.3	4.9	8.1	11.0
mef(E) only	7.0	14.7	8.6	7.6	2.8	2.4	2.8	3.5	1.4	5.0	3.7
erm(B) only	6.0	2.8	1.7	1.7	0.0	2.4	1.4	0.7	0.7	1.2	1.2
mef(E) and *erm*(B)	-	-	-	-	-	-	-	1.4	2.8	1.2	6.1
5-19 years											
Total number of cases	23	28	16	24	21	18	17	13	17	14	11
Total incidence	4.3	4.7	2.5	3.9	3.5	2.5	2.2	1.6	2.1	1.7	1.4
Macrolide resistant	0.2	0.8	1.1	0.3	0.6	0.4	0.5	0.6	0.6	0.4	0.0
mef(E) only	0.2	0.8	1.1	0.3	0.4	0.2	0.3	0.5	0.4	0.1	0.0
erm(B) only	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.1	0.3	0.0
mef(E) and *erm*(B)	-	-	-	-	-	-	-	0.0	0.0	0.1	0.0
20-39 years											
Total number of cases	160	139	135	104	76	72	73	89	52	75	60
Total incidence	16.6	13.9	12.6	9.8	7.4	6.7	6.6	7.6	4.4	6.3	5.4
Macrolide resistant	1.6	3.3	2.8	2.3	2.1	1.1	2.2	1.4	1.2	2.0	0.5
mef(E) only	1.1	2.5	1.9	1.9	1.7	0.8	1.5	0.6	0.5	0.6	0.2
erm(B) only	0.5	0.8	1.0	0.4	0.5	0.2	0.7	0.6	0.1	0.5	0.2
mef(E) and *erm(B)*	-	-	-	-	-	-	-	0.1	0.1	0.9	0.0

Table2. (Continued)

Year	1994	1999	2000	2001	2002	2004	2006	2007	2008	2009	2010+
40-64 years											
Total number of cases	156	245	207	205	158	174	200	219	221	178	182
Total incidence	21.9	28.4	22.9	22.9	18.7	16.5	16.3	18.4	18.2	14.3	15.2
Macrolide resistant	2.5	5.8	7.3	5.2	3.4	4.2	4.6	4.1	4.6	2.8	4.1
mef(E) only	1.7	4.2	5.2	4.2	2.6	3.1	2.4	2.0	2.2	0.9	1.6
erm(B) only	0.9	1.6	2.1	1.0	0.9	1.1	2.3	0.7	1.6	0.8	1.4
mef(E) and *erm*(B)	-	-	-	-	-	-	-	0.9	0.3	0.8	0.8
>65 years											
Total number of cases	139	152	126	136	95	89	116	109	102	132	125
Total incidence	69.9	68.9	55.7	60.6	44.6	35.6	41.6	37.7	34.0	41.7	40.9
Macrolide resistant	11.5	18.1	15.8	15.5	7.36	11.7	12.1	8.5	11.3	13.4	18.7
mef(E) only	7.3	16.7	12.6	11.5	4.5	9.1	8.7	4.5	5.7	5.4	12.2
erm(B) only	4.3	1.6	3.8	4.0	2.8	2.5	3.5	2.0	3.0	3.3	2.9
mef(E) and *erm*(B)	-	-	-	-	-	-	-	1.6	1.1	3.6	3.6

* Total incidence was uncorrected.
+ The incidence of disease, macrolide resistance, and mechanism of resistance in 2010 were incomplete.

Table3. Incidence of invasive pneumococcal disease in metropolitan Atlanta by year, serotype, macrolide resistance, and mechanism of resistance

Year	2007	2008	2009	2010*
PCV 7 vaccine (14, 8C, 19F, 23F, 4, 6B, 9V)				
Total number of cases	29	14	10	5
Total incidence	1.0	0.5	0.3	0.3
Macrolide resistant	0.1	0.1	0.1	0.1
mef(E) only	0.1	0.0	0.1	0.0
erm(B) only	0.0	0.1	0.0	0.0
mef(E) and *erm*(B)	0.0	0.0	0.0	0.0
Additional 6 types (19A, 3, 6A, 7F, 1, 5)				
Total number of cases	155	146	196	82
Total incidence	5.2	4.7	5.9	5.0
Macrolide resistant	1.9	1.9	2.3	2.1
mef(E) only	1.0	0.8	0.8	0.7
erm(B) only	0.1	0.3	0.5	0.1
mef(E) and *erm*(B)	0.6	0.6	0.9	1.2
Serotype 19A				
Total number of cases	90	79	100	45
Total incidence	3.0	2.5	3.0	2.7
Macrolide resistant	1.7	1.8	2.2	2.0
mef(E) only	0.8	0.7	0.7	0.7
erm(B) only	0.1	0.3	0.4	0.1
mef(E) and *erm*(B)	0.6	0.6	0.9	1.2
Serotype 7F				
Total number of cases	28	42	69	20
Total incidence	1.0	1.4	2.1	1.2
Macrolide resistant	0.03	0.0	0.0	0.0
mef(E) only	0.0	0.0	0.0	0.0
erm(B) only	0.0	0.0	0.0	0.0
mef(E) and *erm*(B)	0.0	0.0	0.0	0.0
Serotype 6A				
Total number of cases	14	6	4	0
Total incidence	0.5	0.2	0.1	0.0
Macrolide resistant	0.1	0.1	0.1	0.0
mef(E) only	0.1	0.1	0.1	0.0
erm(B) only	0.0	0.0	0.0	0.0
mef(E) and *erm*(B)	0.0	0.0	0.0	0.0
Serotype 3				
Total number of cases	23	19	22	17
Total incidence	0.8	0.6	0.7	1.0
Macrolide resistant	0.03	0.1	0.0	0.1
mef(E) only	0.0	0.0	0.0	0.0
erm(B) only	0.0	0.0	0.0	0.1
mef(E) and erm(B)	0.0	0.0	0.0	0.0

Table3. (Continued)

Year	2007	2008	2009	2010*
All other types				
Total number of cases	208	212	195	107
Total incidence	7.0	6.8	5.8	6.5
Macrolide resistant	1.5	1.8	1.3	2.1
mef(E) only	0.7	0.9	0.8	1.3
erm(B) only	0.6	0.6	0.4	0.6
mef(E) and *erm*(B)	0.0	0.1	0.1	0.0
Serotype 22F				
Total number of cases	29	33	27	14
Total incidence	1.0	1.1	0.8	0.8
Macrolide resistant	0.0	0.1	0.1	0.1
mef(E) only	0.0	0.1	0.1	0.0
erm(B) only	0.0	0.1	0.0	0.1
mef(E) and *erm*(B)	0.0	0.0	0.0	0.1
Serotype 6C				
Total number of cases	17	20	18	14
Total incidence	0.6	0.6	0.5	0.8
Macrolide resistant	0.2	0.2	0.2	0.4
mef(E) only	0.2	0.2	0.2	0.3
erm(B) only	0.0	0.0	0.0	0.0
mef(E) and *erm*(B)	0.0	0.0	0.0	0.0
Serotype 15A				
Total number of cases	15	14	16	7
Total incidence	0.5	0.5	0.5	0.4
Macrolide resistant	0.5	0.4	0.4	0.4
mef(E) only	0.0	0.0	0.0	0.0
erm(B) only	0.5	0.4	0.3	0.4
mef(E) and *erm*(B)	0.0	0.0	0.03	0.0
Serotype 33F				
Total number of cases	14	15	14	5
Total incidence	0.5	0.5	0.4	0.3
Macrolide resistant	0.4	0.5	0.3	0.3
mef(E) only	0.4	0.4	0.3	0.3
erm(B) only	0.0	0.0	0.0	0.0
mef(E) and *erm*(B)	0.0	0.0	0.03	0.0

* The incidence of disease, macrolide resistance, and mechanism of resistance in 2010 were incomplete.

Figures and Figure Legends

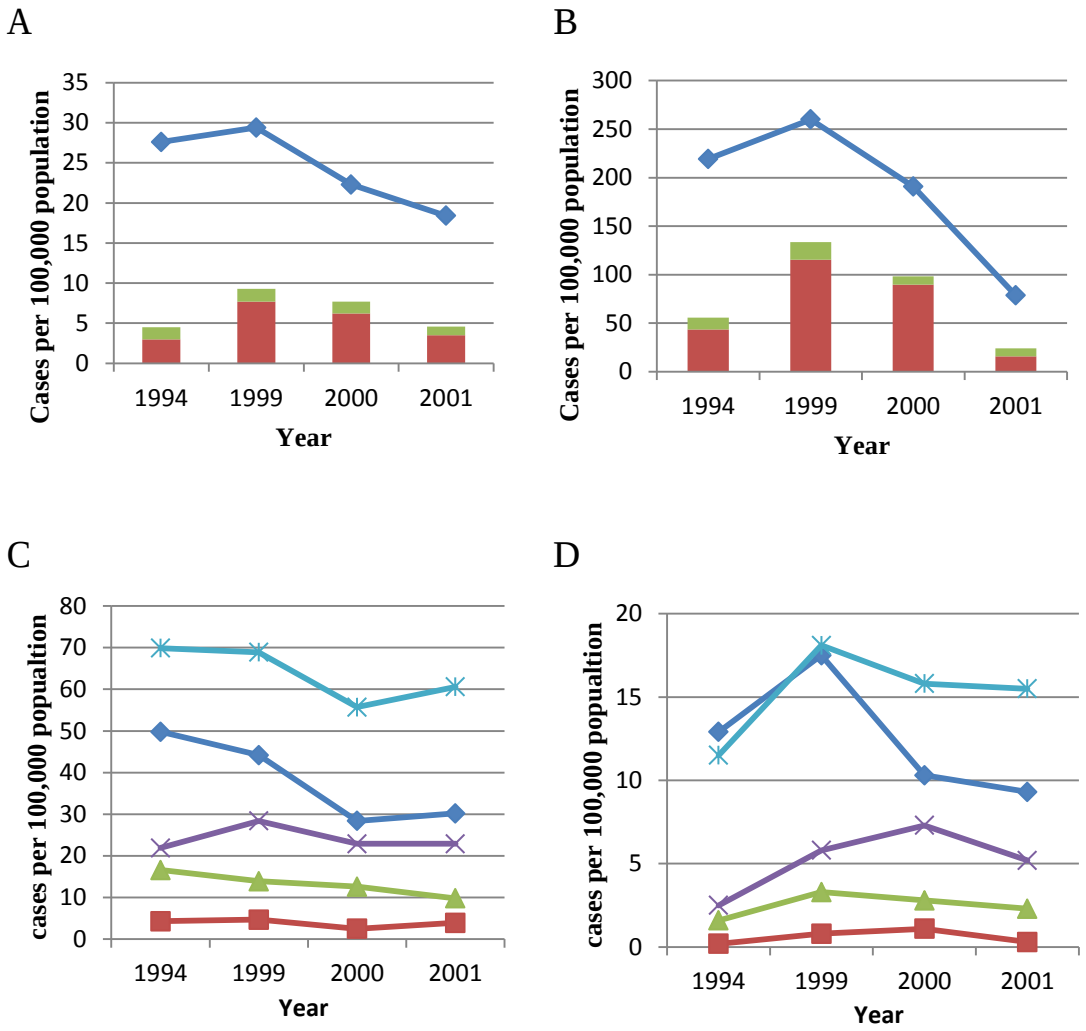

Figure 1. Incidence of *S. pneumoniae* disease in metropolitan Atlanta 2007-2010 A) All isolates, total incidence (), *mef*(E) (), *erm*(B) (), *mef*(E)+*erm*(B) () **B) in children <2 years**, total incidence (), *mef*(E) (), *erm*(B) (), *mef*(E)+*erm*(B) () **C) total incidence by age-groups**, 2-4 years(), 5-19 years(), 20-39 years(), 40-64 years(), >65 years () **D) macrolide resistant by age-groups**, 2-4 years(), 5-19 years(), 20-39 years (), 40-64 years( ), >65 years ()

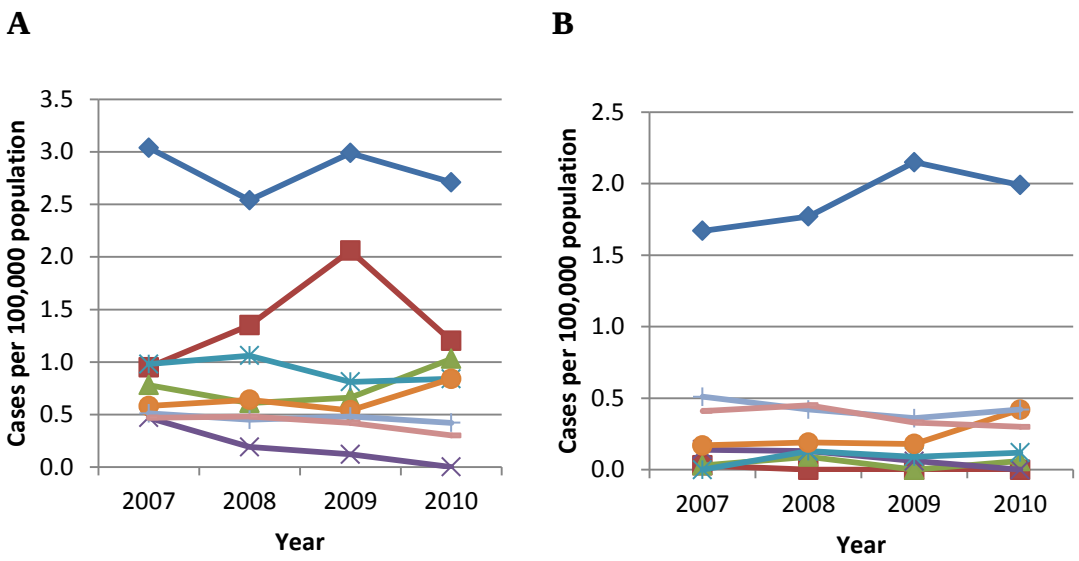

Figure 2. Incidence of *S. pneumoniae* disease by serotype in metropolitan Atlanta 2007-2010 A) Total incidence of disease B) Incidence of macrolide resistance, 19A(), 7F(), 3(), 6A(), 22F (), 6C(), 15A(), 33F ()

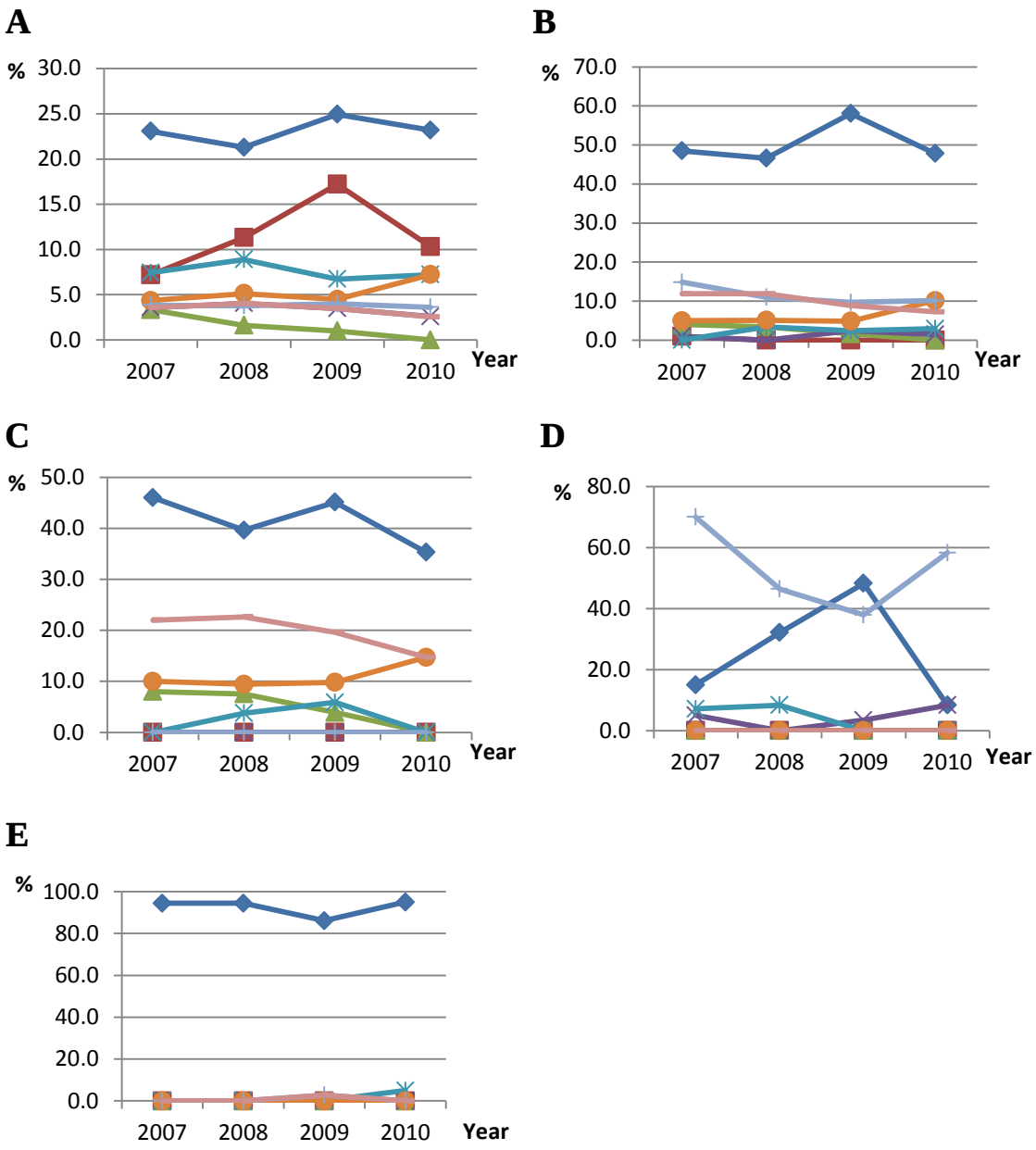

Figure 3 Distribution of invasive *S pneumoniae* by serotypes by macrolide resistant and mechanism of resistance* A) In total isolates B) In macrolide resistant isolates C) In *mef*(E) only isolates D) *erm*(B) only isolates E) In both *mef*(E) and *erm*(B) isolates, 19A(◆), 7F(■), 3(▲), 6A(×), 22F (✳), 6C(●)

* All the percent were calculated by numbers of isolates divided by numbers of isolates data available.

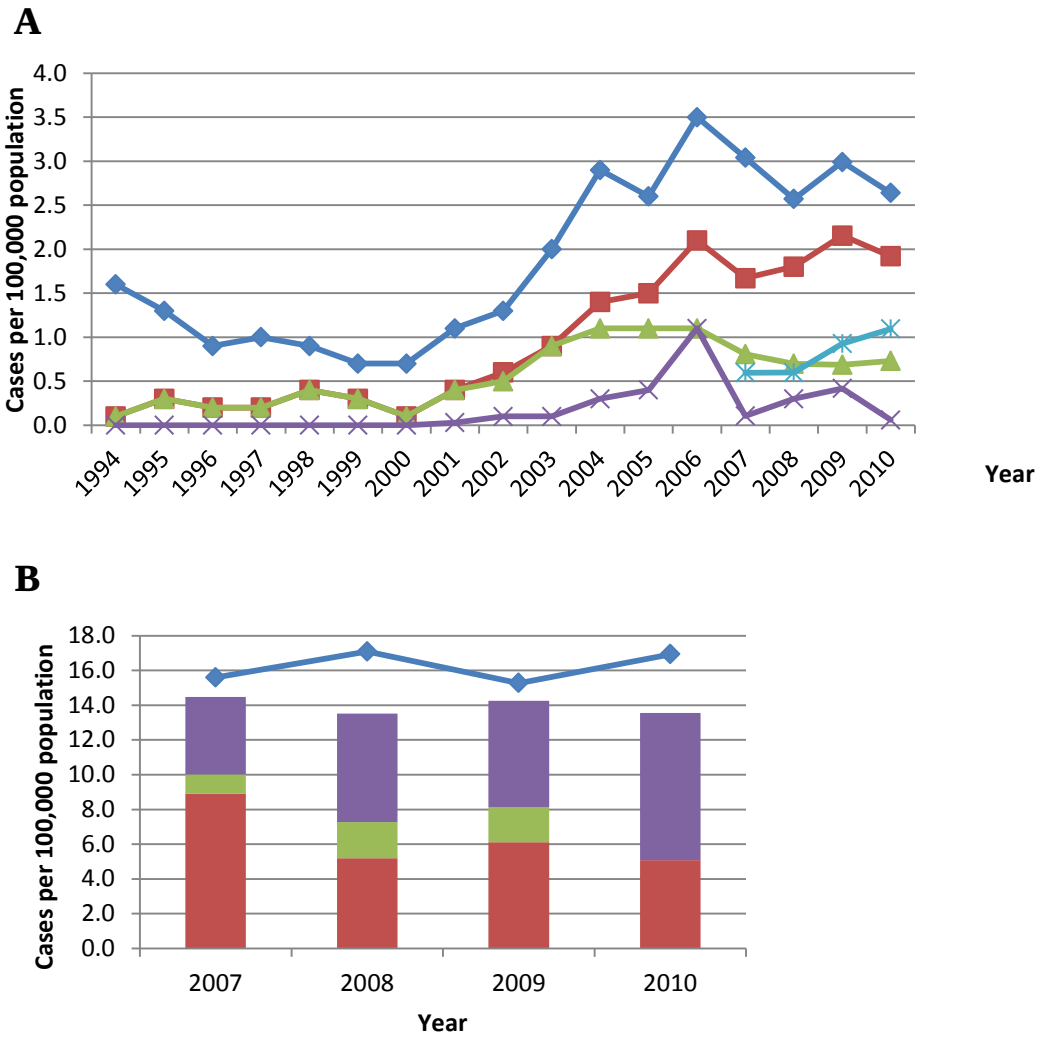

Figure 4. The overall trend of serotype 19A and the trend among children <2 years in metropolitan Atlanta A) Overall incidence 1994-2010. The incidence of dual *mef*(E) and *ermB* isolates was determined from 2007-2010 , total incidence (), macrolide resistance (), *mef*(E) (), *erm*(B) (), *mef*(E)+ *erm*(B) () B) Incidence < 2 years 2007-2010, total incidence ( ), *mef*(E) ( ), *erm*(B) ( ), *mef*(E)+*erm*(B) ()