Antibiotic Resistance Patterns and Related Mobile Genetic Elements of Pneumococci and β -Hemolytic Streptococci in Thai Healthy Children

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Abstract Transmission of antibiotic resistance genes among *Streptococcus pneumoniae* and beta-hemolytic streptococcus (BHS) was generally associated with transmissible genetic elements. The objectives of this study were to investigate carriage rate, antibiotic resistance and related mobile genetic elements of pneumococci and BHS from school-children. The pneumococci and BHS were recovered from 220 Thai school-children, and then tested for antibiotic susceptibility pattern by disc diffusion. Antibiotic resistance genes and related genetic elements were detected by PCR with specific primers. A total of 77 pneumococcal isolates were resistant to erythromycin (42 %), tetracycline (44 %), clindamycin (8 %), or penicillin (3 %). Fifty-four BHS isolates were resistant to erythromycin (28 %), tetracycline (52 %), or clindamycin (13 %). All isolates tested were 100 % sensitive to penicillin and levofloxacin. Among erythromycin-resistant streptococcal isolates showed different phenotypes of clindamycin resistance. It was found that isolated pneumococci showed constitutive clindamycin resistance (19 %), and inducible clindamycin resistance (12 %). The BHS isolates exhibited constitutive clindamycin resistance (40 %), and inducible resistance (20 %) phenotypes. The predominant erythromycin resistance genes in pneumococci and BHS were *mefE* and *ermB*, while the most common tetracycline resistance gene in this population was

tetM. Furthermore, almost all erythromycin- and tetracycline-resistant streptococci (97 %) mainly contained various genetic elements, including mega elements and six different transposon types (Tn2009, Tn2017, Tn917, Tn3872, Tn6002 and Tn916). Therefore, carriages of pneumococci and BHS with multidrug resistance in children might be important reservoirs of antibiotic-resistance genes carried by transposons. Tn916-like elements could lead to dissemination of the antibiotic resistance genes among genus streptococcus in human oral cavity and nasopharynx.

Keywords Antibiotic resistance patterns · Mobile genetic elements · Carriage rate · Pneumococci · β -Hemolytic Streptococci

Introduction

Beta-hemolytic streptococci (BHS) and *Streptococcus pneumoniae* are recognized as important human pathogens that lead to global health problems. Pneumococci cause otitis media, pneumonia, bacteremia, and meningitis [1]. BHS is classified according to the Lancefield groupings of group A, B, C, F, and G. Group A beta-hemolytic streptococcus (GAS) is the most common causative agent of pharyngitis, which also causes immune-mediated post-streptococcal sequelae, such as rheumatic fever and post-streptococcal glomerulonephritis. Group B streptococcus is associated with neonatal infection. The streptococcus groups C, G, and F are usually members of normal flora colonizing in human oral cavity. Nevertheless, they are frequently found to be pathogens which cause not only pharyngitis but also serious infection, e.g., bacteremia, endocarditis, and meningitis [2]. The BHS and *S.*

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pneumoniae can colonize in the mucosal surface of a healthy individual without any clinical symptoms, called an “asymptomatic carrier”. The reports showed the association of carrier ratio with age, geography, socioeconomic status, and crowded places. Children were more often found to be carriers than adults [3, 4].

In the past few decades, many studies reported high prevalence of antibiotic-resistant streptococci worldwide [5–7]. Although rate and pattern of antibiotic resistance vary according to geographic origin. Trend of antibiotic resistance rate did not seem to reduce in Asian countries, moreover most antibiotic-resistant streptococcal isolates were found to be resistant to more than one class of antibiotic [8]. These findings revealed high proportion of strains less susceptible to commonly prescribed drugs, especially beta-lactam, macrolide and tetracycline. Beta-lactam is still the drug of choice for the treatment of streptococcal infection, macrolide and lincosamide are alternative drugs in cases of patients with beta-lactam allergy. Furthermore, tetracycline has been widely used to treat infectious disease in humans. Therefore, resistance to non-beta-lactam drugs has become a major clinical problem. Macrolide resistance in *Streptococcus* spp. mainly occur by ribosomal modification, mediated by *erm* gene and drug efflux pumps, conferred by *mefA/mefE* genes [9]. In contrast, tetracycline resistance genes (*tet*) are diverse. The *tetM* and *tetO* genes encode ribosomal protection proteins, while *tetK* and *tetL* are involved in efflux pumps [10]. Furthermore, isolates of erythromycin-resistant streptococci frequently showed co-resistance to tetracycline or clindamycin. One reason might be associated with the presence of erythromycin- and tetracycline-resistance genes which were carried on transmissible transposons of Tn916-related families. These transposons were considered common transposons in streptococci [11].

The objectives of this study were to investigate carriage rate and antibiotic resistance patterns of *S. pneumoniae* and BHS isolated from healthy school-children as well as to evaluate the distribution of genetic determinants and related genetic elements associated with erythromycin- and tetracycline-resistance in these streptococci.

Materials and Methods

Sample Collection

This cross-sectional study was carried out from June to August 2014 for two boarding schools in a rural area of Thailand. Throat and nasal swabs were collected from 220 Thai school-children aged 3–15 years old. Children with fever, erythema, and swelling of the pharynx were

excluded. The study protocol was approved by the Institutional Review Board of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Thailand (MU-DT/PY-IRB 2014/DT025).

Bacteriological Methods

The swab used in sample collection was immediately streaked on streptococcal selective agar (Oxoid, USA) supplemented with 5 % horse blood (BD Biosciences, USA). The plates were then incubated at 37 °C in a 5 % CO₂ incubator for 24 h. Colonies showing beta- and alpha-hemolytic patterns were verified by gram staining for gram-positive cocci arranged in pairs or in chains. Beta-hemolytic colonies were tested with bacitracin susceptibility and a latex agglutination test (Oxoid, USA) for Lancefield classification. Alpha-hemolytic colonies were pursued with the optochin disc test (Oxoid, USA) and bile solubility test [12]. All strains were kept as 15 % glycerol stocks in a –80 °C freezer.

Antibiotic Susceptibility Test and Inducible Clindamycin Resistance Pattern

Isolates of *S. pneumoniae* and BHS were grown in Brain Heart Infusion (BHI) broth (Difco, USA) at 37 °C in a 5 % CO₂ incubator for overnight. The broth culture of each isolate was adjusted to a turbidity of 0.5 McFarland standards. After that, the prepared bacterial suspension were applied to the surface of Mueller–Hinton agar (Difco, USA) containing 5 % horse blood. The antibiotic discs to be tested (BD Diagnostics, USA) were placed on the plates. After incubation at 37 °C for 18 h, the diameter of the inhibition zone was measured and interpreted as susceptibility, intermediate, or resistance according to the Clinical Laboratory Standards Institute (CLSI) guidelines [13]. The antibiotic discs used in this study were penicillin (10 units), clindamycin (2 µg), erythromycin (15 µg), levofloxacin (5 µg), and tetracycline (30 µg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains for antibiotic susceptibility test. The antibiotic susceptibility test was done in duplicate.

Both *S. pneumoniae* and BHS isolates were also tested with the double disc diffusion method for an inducible clindamycin resistance phenotype, according to the CLSI guidelines [13]. A “D shape” of the clindamycin inhibition zone adjacent to the erythromycin disc was interpreted as an inducible clindamycin resistance phenotype (iMLS), whereas bacterial isolates with resistance to both antibiotics were interpreted as constitutive clindamycin resistance (cMLS). Finally, the bacterial isolates resistant to only erythromycin but not clindamycin were considered to have a M phenotype.

PCR Detection for *S. pneumoniae* and Erythromycin/Tetracycline Resistance Genes

Genomic DNA of bacterial isolates were extracted from overnight broth cultures using ArchivePure DNA Yeast and Gram-positive Bacteria Kit (5-prime, USA), according to manufacturer's instructions.

Alpha-hemolytic streptococci resistant to the optochin test were further analyzed by PCR. The presence of two virulence genes of *S. pneumoniae* (pneumolysin; *ply*, and autolysin; *lytA*) was detected by specific primers [14]. Erythromycin resistance genes were detected with primers specific for *ermA*, *ermB* and *mefA/E* genes [15, 16]. Discrimination between *mefA* and *mefE* genes was performed with a *Bam*HI restriction enzyme reaction [15]. Tetracycline-resistant bacteria were tested for *tetM*, *tetO*, *tetL*, and *tetK* genes [17, 18]. *Streptococcus pyogenes* strains UR1092, BM137, and N1916 were used as positive control

for *ermA*, *ermB*, and *mefA/E* genes, respectively. Also, *Enterococcus faecalis* BM4110, *E. faecalis* P33, *S. pyogenes* BM137, and *Staphylococcus aureus* R16794 were used as positive controls for *tetO*, *tetL*, *tetM*, and *tetK*, respectively.

Detection and Discrimination Between Genetic Elements Carrying Erythromycin and/or Tetracycline Resistance Genes

To detect the transposons in Tn916-related family, primers for integrase (*int*-Tn), excisionase (*xis*-Tn) and *tetM* genes were used. The presence of *tnpA* and *tnpR* genes was associated with the Tn917 transposon. The *erm(B)*-carrying Tn916-like element (Tn6002, Tn6003/Tn1545, Tn3872), *mefE*-carrying Tn916-like element (Tn2009), and *ermB/mefE*-carrying Tn916-like element (Tn2010, Tn2017) were identified with various primer sets as shown in Fig. 1 and Table 1.

Fig. 1 Schematic representation of ORF map of Tn916, Tn916-like elements and composite transposon Tn3872. Short arrowheads indicate the positions of the primers used for detection of various types of genetic elements. J12 and J11 primers could amplify DNA region between *orf18* and *orf20* of Tn916. The lengths of DNA amplicons obtained can be divided into three groups. The first group represented Tn3872, Tn2017 and Tn2009 had a 0.8 kb amplicon. The second group had a 3.6 kb DNA product, which was found in Tn6002, Tn2010. The other group had a 7.9 kb amplicon, which represented

Tn6003/Tn1545. Tn916-like elements carrying mega element (Tn2009, Tn2010 and Tn2017) were identified with primers specific to the mega element, such as *orf5* and *orf7* primers, and primer designed to detect linkage of the mega element and Tn916, SG1/LTF primers. The Tn3872 was a composite element resulting from addition of *ermB*-carrying Tn917 into Tn916-transposon, so it was identified with INS917 primers binding DNA region at insertion site of Tn917 in *Orf9* of Tn916. This picture was modified from the study of Robert et al. [23] and Varaldo et al. [24]

Table 1 Primers used for discrimination of genetic elements

Primer name	Sequence (5′–3′)	Product size (bp)	References
Detection for <i>tetM</i> and silence form of <i>tetM</i> gene			
<i>tetM</i> sense ^a	ATG CTT TGT ATG CCT ATG GTT ATG	1990	This study
<i>tetM</i> antisense	ATC CGA CTA TTT GGA CGA CGG		
TETM2 ^b	GAA CTC GAA CAA GAG GAA ATC	1293	[18]
Detection for Tn916 transposon			
<i>int</i> -Tn sense	GCG TGA TTG TAT CTC ACT	1012	[19]
<i>int</i> -Tn antisense	GAC GCT CCT GTT GCT TCT		
<i>xis</i> -Tn sense	AAG CAG ACT GAG ATT CCT A	194	[19]
<i>xis</i> -Tn antisense	GCG TCC AAT GTA TCT ATA A		
Detection for Tn917 or Tn3872 transposon			
<i>TnpA</i> sense	ATG GCT ATG AAA AGA AT	848	[20]
<i>TnpA</i> antisense	AAA AAA GCA ATA AGT AAA		
<i>TnpR</i> sense	TTT TGG CTA TGC TCG AGT G	303	[20]
<i>TnpR</i> antisense	ATT CTT TTC ATA GCC AT		
INS917 sense ^c	ACT TGA CGG TGA CAT CTC TC	600	This study
INS917 antisense ^c	GGT TGA GTA CCT TTT CAT TCG TTA A		
Detection for mega element			
<i>Orf5</i> sense	TAA TAC AAG TAC ATG ACT CAC C	585	[21]
<i>Orf5</i> antisense	TCC TAA ATA TTG TGC GAA CAT C		
<i>Orf7</i> sense	TCA ATT TAT CAA TAA ATG AAA TGA G	160	[21]
<i>Orf7</i> antisense	ATA CTT CCA ATT CGA TGC CAG		
SG1	CTC ACT GCA CCA GAG GTG TA	1007	[21]
LTF	GCA GAG TAT ACC ATT CAC ATC GAA GTT CCA C		
Discrimination between Tn916-related families			
J11	CTA CCG CAC TTC GTT TGG TGT AC	800/3600/7900	[22]
J12	ATT CCC ATT GAA GAC GCA GAA GT		

^a Included leader sequence and coding region of *tetM* gene

^b The forward primer amplified silent *tetM*, which lacked leader sequence of *tetM* gene

^c Detected insertion site of Tn917 in *orf9* of Tn916-element

Results

Carriage Rates of Pneumococci and BHS

Seventy-seven isolates of pneumococci (35 %) were isolated from nasal and throat swabs of 220 Thai school-children enrolled in this study. Six isolates of these 77 pneumococci (8 %) were identified as optochin-resistant (zone diameter <13 mm) and showed positive bile solubility. These optochin-resistant isolates were further analyzed to be *S. pneumoniae* using PCR detection of the *lytA* and *ply* genes. A total of six isolates were positive for both *lytA* and *ply* genes.

Fifty-four BHS isolates (25 %) detected from throat swab samples of 220 children were classified according to the Lancefield groupings. Group A (8 isolates, 15 %), group C (27 isolates, 50 %), group F (3 isolates, 6 %), and group G (16 isolates, 30 %) were identified. Our results

indicated groups C and G as the predominant BHS group recovered from school-children. Whereas, group B BHS was not found in this study population.

Antibiotic Susceptibility Rates and Patterns

Forty-five from the total 77 pneumococci isolated (58 %) were identified as antibiotic nonsusceptible isolates. Forty-nine percent of these nonsusceptible pneumococci showed multidrug-resistance phenotypes, as shown in Table 2. Thirty-two pneumococcal isolates (42 %) exhibited erythromycin nonsusceptible phenotypes. Moreover, 34 isolates of the total 77 isolates (44 %) showed tetracycline resistance. We found only two isolates exhibiting reduced susceptibility to penicillin. No isolates were resistant to levofloxacin.

Thirty-two of the 54 BHS isolates (59 %) were antibiotic-nonsusceptible isolates. Approximately 44 % of the

Table 2 Antibiotic susceptibility patterns of *S. pneumoniae* and beta-hemolytic streptococci included in this study

Organisms (No. of isolates)	Mono-drug ^a nonsusceptible				Multi-drug nonsusceptible ^a					
	P	L	E	T	E + P	E + T	E + CC	T + CC	E + T + CC	E + T + P
<i>S. pneumoniae</i> (77)	2 (3 %)	0 (0 %)	10 (13 %)	13 (17 %)	1 (1 %)	14 (18 %)	0 (0 %)	0 (0 %)	6 (8 %)	1 (1 %)
Beta-hemolytic streptococci (54)	0 [0 %]	0 [0 %]	2 [4 %]	16 [30 %]	0 [0 %]	7 [13 %]	2 [4 %]	1 [3 %]	4 [7 %]	0 [0 %]
Group A (8)	0	0	0	3	0	0	0	0	0	0
Group C (27)	0	0	2	7	0	7	1	0	4	0
Group F (3)	0	0	0	0	0	0	0	0	0	0
Group G (16)	0	0	0	6	0	0	1	1	0	0

E erythromycin, *T* tetracycline, *P* penicillin, *CC* clindamycin, *L* levofloxacin

^a Numbers in parentheses indicate percentages of *S. pneumoniae* isolates with each kind of drug non-susceptible, while numbers in brackets show percentages of BHS isolates with each kind of drug non-susceptible

nonsusceptible BHS isolated exhibited multidrug-resistant phenotypes (Table 2). Fifteen isolates of BHS (28 %) exhibited the erythromycin nonsusceptible phenotype. Furthermore, 28 of the total 54 BHS isolates (52 %) showed tetracycline resistance. All identified BHS isolates were 100 % sensitive to penicillin and levofloxacin. Group C streptococci made up the great majority of the BHS with the antibiotic-nonsusceptible phenotype.

Genetic Determinants for Erythromycin- and Tetracycline-Resistant Streptococci

Overall of the 32 erythromycin-resistant pneumococci, no isolates carried *ermA*, 10 isolates (31 %) possessed *ermB*, and 26 isolates (81 %) were positive for the *mefA/E* gene. Among the *mefA/E*-positive isolates, all of them carried *mefE* gene. There were 6 isolates (19 %) with both *ermB* and *mefE* genes. For the 34 tetracycline-resistant isolates, PCR revealed *tetM* as the most predominant tetracycline resistance gene, which was detected in almost all of the tetracycline-resistant pneumococci isolates (32/34 isolates, 94 %). The detections of other tetracycline resistance genes were as follows: three isolates of *tetK*, two isolates of *tetO*, and three isolates of *tetL*. Six isolates of pneumococci contained more than one type of *tet* gene (two for *tetM/tetK*, two for *tetM/tetL*, and two for *tetM/tetO* patterns).

Among the 15 BHS isolates with the erythromycin resistance phenotype, the resistance genes identified were *ermB* (60 %), and *mefE* (73 %). No isolates had the *ermA* or *mefA* genes. Erythromycin resistance genotype patterns were identified as four for *ermB*, six for *mefE*, and five for *ermB/mefE* genes. In the 28 tetracycline-resistant isolates, the *tetM* gene, which was also the most common *tet* gene in BHS, was found in 26 isolates (93 %) of tetracycline-resistant isolates. The second most common *tet* gene was *tetO* (21 % of the isolates), followed by *tetL* and *tetK* (18 and 4 % of the isolates, respectively). Ten isolates of BHS

contained more than one type of *tet* gene (three for *tetM/tetL*, four for *tetM/tetO*, two for *tetM/tetK/tetL*, and one for *tetM/tetO/tetL* patterns).

Inducible Clindamycin Resistance Phenotype

The macrolide-resistant streptococci showed a different pattern of clindamycin resistance phenotypes. Therefore, a total of 32 erythromycin-resistant pneumococcal isolates were tested with the double disc diffusion test or D zone test. Six isolates (18.75 %) of erythromycin-resistant pneumococci showed the constitutive clindamycin resistance phenotype (cMLS), while four isolates (12.5 %) showed inducible clindamycin resistance (iMLS) or positive D zone test results. Twenty-two isolates (68.75 %) showed the M phenotype. As for BHS, among 15 erythromycin-resistant BHS isolates, six isolates (40 %) exhibited a constitutive clindamycin resistance phenotype and three isolates (20 %) showed positive D zone test results. Six BHS isolates (40 %) were M phenotypes. The genotype patterns of various clindamycin resistance phenotypes, cMLS and iMLS were *ermB* or *ermB/mefE* genotypes. As the M phenotype contained only the *mefE* gene.

Genetic Elements Recovered from *S. pneumoniae* and BHS

In the mono-drug resistant streptococci, a mega element carrying the *mefE* gene was responsible for almost all erythromycin-resistant isolates of streptococci (11/12 isolates, 92 %), while the Tn916 transposon was involved in tetracycline-resistant streptococci (28/29 isolates, 97 %). The poly-drug resistant streptococci were found to have the following diverse types of Tn916-like transposons as shown in Table 3. Among the 21 erythromycin- and tetracycline-resistant pneumococci, Tn2009 was the most

Table 3 Association between resistance genes, transposase genes and related genetic elements responsible for erythromycin- and/or tetracycline-resistant phenotypes

Phenotypes ^a	Genotypes	No. of isolates	Sizes (kb) of DNA region between <i>orf20/orf18</i>	Presence of		Linkage of mega element and Tn916	ORFs of Tn917		Insertion site of Tn917 in <i>orf9</i> of Tn916	Presumed genetic elements
				ORFs of mega element	ORFs of Tn916		<i>int</i> -Tn	<i>xis</i> -Tn		
<i>S. pneumoniae</i>										
Mono-drug resistance										
Erythromycin, E (10)	<i>meE</i>	9	0.8	+		+	-	-	-	MEGA element
Tetracycline, T (13)	<i>tetM</i>	13	0.8	-		-	+	+	-	Tn916
Poly-drug resistance										
E + T co-resist (11)	<i>meE/tetK</i>	1	0.8	+		+	-	-	-	MEGA element
	<i>meE/tetM</i>	10	0.8	+		+	+	-	-	Tn2009
E + CC ^b + T (10)	<i>ermB/tetM</i>	3	0.8	-		-	+	+	+	Tn3872
		1	3.6	-		-	+	+	-	Tn6002
	<i>ermB/meE/tetM</i>	5	0.8	+		+	+	+	+	Tn2017
	<i>ermB/meE/tetL</i>	1	0.8	+		+	-	-	+	Tn917 + MEGA
<i>Beta-hemolytic streptococci</i>										
Mono-drug resistance										
Erythromycin, E (2)	<i>meE</i>	2	0.8	+		+	-	-	-	MEGA element
Tetracycline, T (16)	<i>tetM</i>	15	0.8	-		-	+	+	-	Tn916
Poly-drug resistance										
E + T co-resist (4)	<i>meE/tetM</i>	4	0.8	+		+	+	+	-	Tn2009
E + CC ^b (2)	<i>ermB/tetM</i> ^c	2	0.8	-		-	+	+	+	Tn3872-like
E + CC ^b + T (7)	<i>ermB/tetM</i>	2	3.6	-		-	+	+	-	Tn6002
	<i>ermB/meE/tetM</i>	5	0.8	+		+	+	+	+	Tn2017

^a Numbers in parentheses indicate the number of bacteria isolates with each kind of drug non-susceptible phenotype^b Is constitutive or inducible clindamycin resistance^c Is silent *tetM* gene

common transposon identified in 48 % of the resistant isolates, followed by Tn2017, with a proportion of 24 %. While, Tn2017 was the most prevalent transposon present in 38 % of the resistant BHS isolates, followed by Tn2009 (31 %).

Discussion

In the study population, the carriage rates of pneumococci and BHS among healthy school-children were 35 and 25 %, respectively. The previous studies reported carrier rates of pneumococci in children as 2–10 % in Italy, 21–23 % in the USA, 20–70 % in Asia [25] and for the carrier rates of BHS: 8 % in Croatia and 16 % in India [26, 27]. One meta-analysis study reported that the pool prevalence of group A streptococcus (GAS) carriage in children of all ages was 12 % (95 % CI 9–14 %) [28]. Our data showed that the GAS carriage rate was 4 %, while the carriage rate of group C and group G streptococci (GCS/GGS) was about 20 % of the study population. The GCS/GGS were considered opportunistic pathogens that cause both pharyngo-tonsillitis and invasive streptococcal diseases, for example rheumatic fever and bacteremia.

In this study, we found that 8 % (6/77 isolates) of the pneumococci isolates exhibited optochin resistance and positive bile solubility tests. They were identified as *S. pneumoniae* with PCR reactions for *lytA* and *ply* genes. In the last few decades, many studies have reported the presence of optochin-resistant pneumococci in many regions [29, 30]. It was hypothesized that the horizontal transfer of genes between *S. pneumoniae* and other oral streptococci were involved. Furthermore, the study of Pikis et al. [30] revealed point mutations in the α - or γ -subunit of membrane protein H^+ -ATPase, which lead to a lack of interaction between optochin and the H^+ -ATPase protein. Therefore, the optochin susceptibility test could not be used as the single test for differentiation between *S. pneumoniae* and mitis group streptococci.

High rates of nonsusceptibility to macrolide and tetracycline in *S. pneumoniae* and BHS could be explained by the adaptation of bacteria under antibiotic selective pressure. Some examples of events causing selective pressure were increasing use of macrolide in medical treatment, which was significantly correlated with an increase in macrolide-resistant *S. pneumoniae* [31] as well as heavy usage of tetracycline in medicine, agriculture, and livestock management.

The pattern of erythromycin- and tetracycline-resistance genes identified in pneumococcal isolates was similar to BHS isolates. In most of the identified erythromycin-resistant population, *ermB* and *mefE* were the predominant erythromycin resistance genes. This finding was consistent

with previous reports, which found high prevalences of *ermB* and *mefE* genes in streptococci [32, 33]. The *mefE* is more common in *S. pneumoniae* and viridans streptococci than *mefA* [34]. Furthermore, the presence of the *ermA* gene was not detected in some populations of pneumococci [15]. Therefore, *mefE*-carrying mega elements might be more widespread in streptococci than *mefA*-carrying elements including Tn1207. Among the identified tetracycline resistance genes in this population, *tetM* was still the most prevalent *tet* gene. This observation might be due to the fact that *tetM* was carried within transposons of the Tn916-related family, which had a broad host range [23].

Two tetracycline-susceptible BHS isolates were found to have silent *tetM*. This silent form was resulted from the absence of the leader sequence of the *tetM* gene, which could affect *tetM* gene expression. The silent form of *tetM* gene was carried by Tn3872. This finding was consistent with the study of Brenciani et al. [35], which reported that some isolates of *Streptococcus pyogenes* contained silent *tetM* carrying Tn3872-like transposon.

The present study demonstrated horizontal gene transfer might have a major role in the spread of antibiotic resistance genes, Tn916-like elements served as significant vehicles for transmission of resistance genes in both pneumococcal and BHS isolates. Tn916-related family were large family of integrative and conjugative transposons, which had ability to transfer between various gram-positive bacteria by mean of conjugation [36]. The element could excise out of and integrate into bacterial chromosome. The Tn916-related elements carried conserved genes involving transposition process, *int*-Tn and *xis*-Tn genes as well as conjugative process. Whereas antibiotic resistance genes are quite varied. Nevertheless, almost all members of Tn916-related family contained *tetM* gene. The Tn916-like elements also incorporated other genetic elements such as *ermB*-carrying cassette, macrolide efflux genetic assembly (*mega*) element and *ermB*-carrying Tn917 [23, 36]. Furthermore the Tn916-like elements also contained other antibiotic resistance genes, such as the *aphA-3* gene for kanamycin resistance and the *cat* gene for chloramphenicol resistance as seen in Tn6003/Tn1545 and Tn5253, respectively [23]. Under selective pressure of antibiotic use might lead to the existence of transposons in bacterial cells. Therefore, antibiotic-resistant isolates of *S. pneumoniae* and BHS in children could be important reservoirs for the resistance genes carried by transposon. Furthermore, this study indicated the dissemination of antibiotic-resistance genes in bacteria not only occurred in hospital but also in community setting. This event had impact on treatment for streptococcal infection.

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