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Detection of Tn*1545* conferring erythromycin resistance in clinical isolates of *Streptococcus pneumonoiae*

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Abstract

This study was conducted to investigate the genetic organization of erm-carrying Tn*1545* in clinical isolates of Streptococcus pneumonia. A total of fifteen isolates of *S. pneumoniae* isolates were examined and was found to contain Tn*1545* element. Susceptibility of these isolates to different antibiotics was also examined, results showed that these isolates are resistant to penicillin in percentage of 93%, then to streptomycin and trimethoprim (87%), clindamycin (73%), kanamycin (67%), erythromycin, tetracycline and azithromycin (60%), ciprofaxin and levofloxacin (53%).

Genomic DNA was extracted from *S. pneumoniae* isolates for detection Tn*1545* by using specific primers to amplify erm gene carried by this transposable element. Results showed that seven of *S. pneumoniae* isolates were found to contain Tn*1545* element giving them erythromycin resistance. erm gene encodes this antibiotic but does not mediate resistance to other antimicrobial agents. On the other hand, nucleotide sequence for erm gene was determined, and compared by alignment with the erm gene sequence located on the same transposable elements in standard strains of *S.pneumoniae* recorded in NCBI data base. Results of alignment showed 100% identity between these sequences..

Keywords: *S. pneumoniae* , Tn*1545*, Erythromycin resistance.

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Introduction

Streptococcus pneumoniae is a transient colonizer of the nasopharynx, with colonization peaking in the early years of life and declining into adulthood. It is associated with both invasive and non-invasive pneumococcal diseases. However, post-pneumococcal capsular vaccine (PCV) surveillance studies conducted in well-organized settings have shown increased pneumococcal resistance to erythromycin and other antibiotics, partly attributed to increased consumption of macrolides[1–3]. Pneumococcal resistance to macrolides is mediated by erythromycin ribosomal methylase B (*erm*(B)) encoding enzymes that methylate the 23S rRNA, thereby inhibiting macrolide binding[4]. The *erm*(B) confers resistance to macrolides, lincosamides, and Streptogramin B, producing MLSB phenotypes[5,6]. Macrolide efflux protein A and E, efflux pumps encoded by the *mef*(A) and *mef*(E) genes, and ribosomal mutations (23S rRNA), are other common causes of macrolide resistance in *Streptococcus pneumoniae*[4]. The *mef*(A/E) genes confer the M phenotype, exhibiting low level resistance to macrolides, but not resistance to lincosamides and streptogramin B. The macrolide resistance genes are commonly carried on mobile genetic elements, facilitating their easy intra- and interspecies dissemination[7,8]. The Tn*916* transposon family that contains the tetracycline resistance determinant *tet*(M), has frequently been reported to harbor macrolide resistance determinant genes [9]. In addition, there are pathogens, such as *Clostridium difficile*, which have risen to global prominence over the last few years and have the ability to acquire mobile genetic elements from enterococci, indicating the potential to acquire resistance to the last line of defense antibiotics, for example vancomycin and other glycopeptides[10,11]. These resistances are commonly acquired on mobile genetic elements such as conjugative plasmids and conjugative transposons, which are capable of broad host range transfer between pathogens[11] and between commensal and pathogenic bacteria. The Tn*916*/Tn*1545* family is responsible for a large proportion of the antibiotic resistance in these different pathogens. erm(B) resistance can be expressed by pneumococci either constitutively or inducibly[12]. Among clinical *S. pneumoniae* isolates with erm(B)-mediated erythromycin resistance, most are also resistant to tetracycline[13], this connection appears to reflect the widespread presence in pneumococcal populations of genetic elements (such as Tn*1545* or Tn*3872*) resulting from the insertion of erm(B)-containing DNA into conjugative transposons of the Tn*916* family, which typically carry tet(M)[(14]. According to the importance of Tn*1545* in spreading erythromycin resistance between bacterial isolates, this study was aimed to explore the prevalence of Tn*1545* in *S. pneumonia* isolates.

Materials and Methods

S. pneumonia Isolates

Clinical isolates of *S. pneumonia* were obtained from the department of molecular and medical biotechnology, those fifteen isolates were maintained on chocolate agar medium, Fresh culture of each bacterial isolate were obtained after inoculating Brain-Heart infusion broth medium and incubated at 37 °C with 5% CO_2 for 16 hrs.

Susceptibility testing

Susceptibility of *S. pneumonia* isolates against different antibiotics (penicillin, streptomycin, trimethoprim, tetracycline, trimethoprim, clindamycin, kanamycin, erythromycin, azithromycin, ciproflaxin and levofloxacin) was examined on Muller-Hinton agar medium according to the standard disc diffusion method[15]. These antibiotics were supplied by Bioanalyse/Turkey.

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Detection of transposable elements

Tn*1545* transposable element found in the fifteen isolates of *S. pneumonia* was detected by amplification of erythromycin resistance gene carried by this transposon using primers, TETM2: 5'-GAACTCGAACAAGAGGAAAGC-3' and TETM3: 5'- ATGGAAGCCCAGAAAGGAT-3', provided in lyophilized form, and were dissolved in sterilized distilled water to give a final concentration of 10 picomole/µl. PCR master mix supplied by promega\USA was prepared to be consisting of the following components:

Material	Concentration
PCR buffer (PH=8.5)	2X
$MgCl_2$	3 mM
dNTPs	400 mM
Taq DNA polymerase	5 units

Table (1): Optimum conditions for amplification of Tn*1545* .

Step	Temperature (°C)	Time	No. of cycles
Initial Denaturation	95	5 min.	1
Denaturation	95	30 sec.	30
Annealing	60	45 sec.	
Extension	72	45 sec.	
Final extension	72	7 min.	1

Results and Discussion

Antibiotics sensitivity of *S. pneumoniae*

Results of antibiotic susceptibility of *S. pneumoniae* illustrated in table (2) showed that multi-drug resistant was spread in the clinical isolates of *S. pneumoniae* as these isolates gave different resistant patterns to these antibiotics. Results indicated in table (2) showed that most of the bacterial isolates (93%) were resist to penicillin antibiotic, then to streptomycin (87%), then to tetracycline and trimethoprim (80%), then to clindamycin (73%), then to kanamycin (50%), then to erythromycin and azithromycin (40%), then to levofloxacin and ciproflaxin (20%). In recent years, therapy for *S. pneumoniae* has become tricky owing to the global rise in the spread of antibiotic resistance, particularly against first-line antibiotics such as erythromycin and penicillin as it was mentioned by Xu *et al.*[16]**.** Furthermore, results showed that the most

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resistant isolates are the isolates symbol D1, D3, D5, D6, D9 and D15 as they show resistance to all antibiotics used in this study, then D2 and D10 which was resist to nine antibiotics (90%), then D7 (80%), then D4, D8, D11 and D12 (40%), then D14 (30%), then D13 (10%). Resistance and multiresistance to a large group of antibiotics, including aminoglycosides, polypeptides and first-generation quinolones has been reported in several studies[17]. Findings of this study is consistent with those of previous studies showed an increase in antibiotic resistance rates, especially to erythromycin and penicillin[18,19].

Table (2): Antibiogram of *S. pneumoniae* clinical isolates; R: Resistance; S: Sensitive; AZI: Azithromycin; CIP: Ciproflaxin; CD: Clindamycin; E: Erythromycin; K: Kanamycin; LEV: Levofloxacin; P: Penicillin; S: Streptomycin; T: Tetracycline; TM: Trimethoprim.

Isolates No.	Azi	cip	CD	E	K	LEV	P	S	T	TM
D1	R	R	R	R	R	R	R	R	R	R
D2	R	S	R	R	R	R	R	R	R	R
D3	R	R	R	R	R	R	R	R	R	R
D4	S	R	R	S	S	S	R	S	S	R
D5	R	R	R	R	R	R	R	R	R	R
D6	R	R	R	R	R	R	R	R	R	R
D7	R	S	R	R	R	S	R	R	R	R
D8	S	S	S	S	R	S	R	R	R	S
D9	R	R	R	R	R	R	R	R	R	R
D10	R	S	R	R	R	R	R	R	R	R
D11	S	S	S	S	S	S	R	R	R	R
D12	S	S	S	S	S	S	R	R	R	R
D13	S	S	R	S	S	S	S	S	S	S
D14	S	R	S	S	S	S	R	R	S	S

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D15	R	R	R	R	R	R	R	R	R	R

Tn*1545* is a conjugative shuttle transposon detected in multi-resistant clinical isolates of *S. pneumoniae*, carrying erythromycin gene encoding coresistance to macrolide, lincosaide and streptogramin B antibiotic[20].

Tn*1545* was detected in clinical isolates of *S. pneumoniae* by amplification of erythromycin gene using specific primers. Results illustrated in figure (1) showed an amplified product of 740 bp appeared after electrophoresis on agarose gel (1%) represents erm gene carried by Tn*1545*, this transposable element was detected in seven isolates of *S. pneumoniae* (D1, D2, D3, D6, D7, D9, and D15) out of the total isolates (15 isolate). All these isolates are resistance to erythromycin and this result explain the source of erythromycin resistant phenotype in these seven isolates conferred by Tn*1545*. Mean white, results indicated in table (2) showed that there are other two erythromycin resistant isolates (D5 and D10) possess a chromosomal or plasmid copy of erythromycin resistance gene. Furthermore, there are six isolates of *S.pneumoniae* (D4, D8, D11, D12, D13, and D14) were sensitive to erythromycin among the total resistant isolates, and were unable to grow on enrichment medium containing this antibiotic.

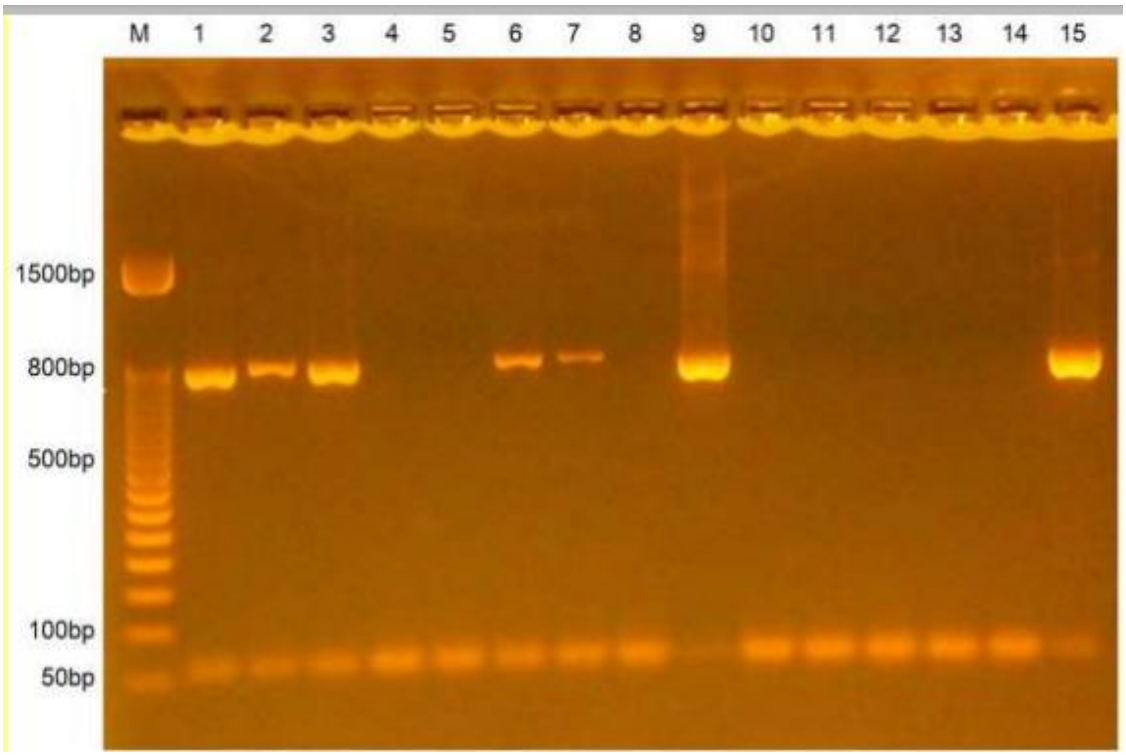

Figure (1): Erythromycin gene of Tn*1545* detected after amplification of genomic DNA for *S.pneumoniae* isolates and electrophoresis on agarose gel (1%) for one hour. Lane (M): Ladder marker; Lane (1-15): Bacterial isolates of *S. pneumonia.*

Nucleotide sequence of erythromycin gene in Tn*1545* was illustrated in figure (2). Alignment of these sequences specified for *S.pneumoniae* isolates with transposon sequences of *S.pneumoniae* standard

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strains recorded in NCBI are illustrated in figure (3). Results of alignment showed that erythromycin gene sequence of Tn*1545* in these seven bacterial isolates was identical (100% identity) with chromosomal erythromycin gene sequences in different standard strains of *S.pneumoniae* and with genomic erythromycin resistance gene in other standard strains of this bacterium which supports the results concluded in this study that the seven isolates of *S. pneumoniae* harboring chromosomal or plasmid copy of Tn*1545* conferring erythromycin resistance which may cause high-level resistance to aminoglycoside through the acquisition of the mobile genetic elements[21,22].

1	CATCAACACATCGAGGTCAGTCTGAACTTTGCGGAAAAG	40
41	TTTTCAAAATTGAGTATTCGGAAAAAAGACAGCGTC TTG	80
81	CATATATA CGTCTTTATAGTGGCGTACTGCATTTGC GAG	120
121	ATTCGGTTAGAATATCGGAAAAGGAAAAAATAAAAATTA	160
161	CAGAAATGTATACTTCAATAAATGGTGAATTATGTAA AA	200
201	TCGATAAGGCTTATTCCGGGGAAATTGTTATTTTGCA G A	240
241	ATGAGTTTTTGAAGTTAAATAGTGT TCTTGGAGATAC AA	280
281	AGCTATTGCCACAGAGAGAGAGAATTGAAAATCCC C T C	320
321	CTCTGCTGCAAACGACTGTTGAACCGAGCAAACCTCAAC	360
361	AAAGGGAAATGTTACTTGATGCACTTTTAGAAATCTC CG	400
401	ACAGTGACCCGCTTCTGCGATATTATGTGGATTCTGC G A	440
441	CAC ATGAAATCATACTTTCTTTCTTAGGGAAA GTACAAA	480
481	TGGAAGTGACTTGTGCTCTGCTGCAAGAAAAGT ATC ATG	520
521	TGGAGATAGAAATAAAAGAGCCTACAGTCATTTAT ATG G	560
561	GGAGTAAAAGACATTTTACTAGAGCTATTCAATCGC A TT	600
601	ATTGGTGCTTAAATAAAACCGTTCTTTTGTGGA ATATA A	640
641	GTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCA A A	680
681	CGAA ATAAAAGAGCCTACAGTCATTTATATGGAAAGACC	720
721	GTTAAAAAAAGCAGAGTATACCATTCACATCGAAGTTC C	760
761	ACCGAATCCT	771

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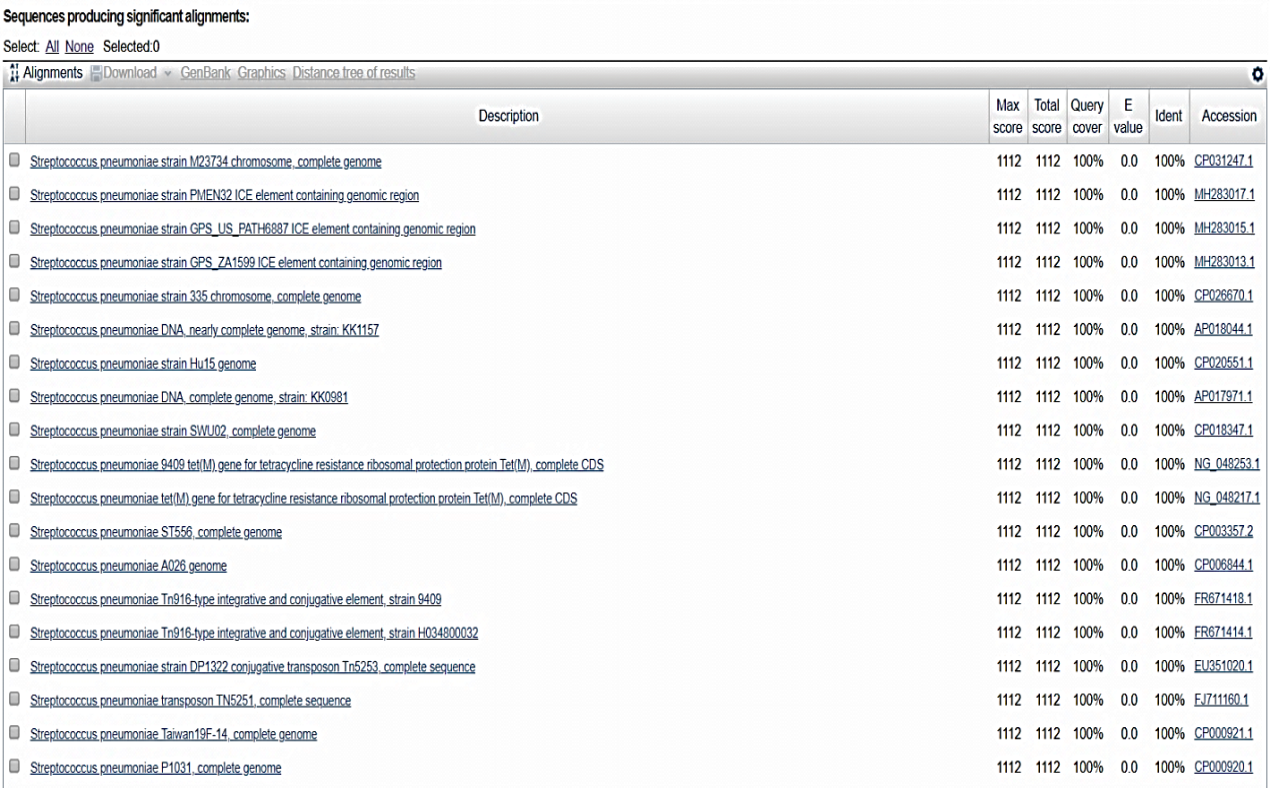
Figure (2): Nucleotide sequence of erythromycin gene of Tn*1545* carried by *S.pneumoniae* isolates



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptococcus pneumoniae strain M23734 chromosome, complete genome	1112	1112	100%	0.0	100%	CP031247.1
Streptococcus pneumoniae strain PMEN32 ICE element containing genomic region	1112	1112	100%	0.0	100%	MH283017.1
Streptococcus pneumoniae strain GPS_US_PATH6887 ICE element containing genomic region	1112	1112	100%	0.0	100%	MH283015.1
Streptococcus pneumoniae strain GPS_ZA1599 ICE element containing genomic region	1112	1112	100%	0.0	100%	MH283013.1
Streptococcus pneumoniae strain 335 chromosome, complete genome	1112	1112	100%	0.0	100%	CP026670.1
Streptococcus pneumoniae DNA, nearly complete genome, strain: KK1157	1112	1112	100%	0.0	100%	AP018044.1
Streptococcus pneumoniae strain Hu15 genome	1112	1112	100%	0.0	100%	CP020551.1
Streptococcus pneumoniae DNA, complete genome, strain: KK0981	1112	1112	100%	0.0	100%	AP017971.1
Streptococcus pneumoniae strain SWU02, complete genome	1112	1112	100%	0.0	100%	CP018347.1
Streptococcus pneumoniae 9409 tet(M) gene for tetracycline resistance ribosomal protection protein Tet(M), complete CDS	1112	1112	100%	0.0	100%	NG_048253.1
Streptococcus pneumoniae tet(M) gene for tetracycline resistance ribosomal protection protein Tet(M), complete CDS	1112	1112	100%	0.0	100%	NG_048217.1
Streptococcus pneumoniae ST556, complete genome	1112	1112	100%	0.0	100%	CP003357.2
Streptococcus pneumoniae A026 genome	1112	1112	100%	0.0	100%	CP006844.1
Streptococcus pneumoniae Tn916-type integrative and conjugative element, strain 9409	1112	1112	100%	0.0	100%	FR671418.1
Streptococcus pneumoniae Tn916-type integrative and conjugative element, strain H034800032	1112	1112	100%	0.0	100%	FR671414.1
Streptococcus pneumoniae strain DP1322 conjugative transposon Tn5253, complete sequence	1112	1112	100%	0.0	100%	EU351020.1
Streptococcus pneumoniae transposon TN5251, complete sequence	1112	1112	100%	0.0	100%	FJ711160.1
Streptococcus pneumoniae Taiwan19F-14, complete genome	1112	1112	100%	0.0	100%	CP000921.1
Streptococcus pneumoniae P1031, complete genome	1112	1112	100%	0.0	100%	CP000920.1

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Figure (3): Alignment of erythromycin gene of Tn*1545* carried by *S. pneumoniae* isolates with erythromycin gene carried by standard strains of the same bacteria recorded in NCBI.

Conclusion

Tn*1545* carrying erythromycin resistance genes was detected in multidrug resistance isolates of *S. pneumoniae* isolated from pharyngitis and tonsillitis infections

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