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In-silico Analysis of the Genetic Diversity and Virulence Genes in *Streptococcus* species.

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ABSTRACT

The present study investigates the virulence property of *Streptococcus* species through *in silico* tools. *In silico* polymerase chain reaction analyzed fifteen virulence genes. Studies revealed that *lytA* is an obligatory gene as all the *Streptococcus pneumoniae* isolates analyzed in this study had the autolysin genes, *lytA*. Twenty isolates (17.09%) had the pneumolysin gene, *ply*, which is expressed on the surface of pneumococci. Nineteen isolates (16.24%) carried pneumococcal surface protein A (*pspA*) gene that has anticomplementary property. Among the three genes of *Streptococcus agalactiae*, the *bca* gene (1.71%) was encountered at a lower frequency compared to *cylE* (5.98%) and *sip* gene (7.69%). Isolate *Streptococcus uberis*0140J expressed both streptokinase, *skc* and plasminogen activator, *pauA* gene which differentiates *Streptococcus uberis* from other *Streptococcus* species. The glucosyltransferase, *gtfB* genes were present in three isolates (2.56%). Glutamate dehydrogenase genes (*gdh*), a diagnostic marker of *Streptococcus suis*, were present in 11.9% (n=14) of the isolates. Thirteen isolates (11.11%) had the muramidase released protein (*mrp*) and suilysin (*sly*) gene. Streptococcal superantigen (*ssa*), streptococcal pyrogenic toxin A (*speA*) and streptococcal invasion locus (*sil*) genes were present in 2.56%, 5.98% and 3.42% isolates, respectively. *In silico* pulsed field gel electrophoresis was able to group isolates into 15 genotypes at 80% cutoff value. Genotype 8 was more prevalent (24.9%) and also carried 7 virulent genes. Genotype 9 and 10 harboured mainly pneumolysin and autolysin genes but glucosyltransferase genes, *gtfB* were also present. Six genotypes harboured no virulence gene. This virulence gene profile generated here aids us to understand the virulence gene associated with disease in relation to the genotypes.

Keywords: *Streptococcus*, Polymerase chain reaction, Pulsed field gel electrophoresis, Virulence genes, Genotype.

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INTRODUCTION

Streptococci is a gram-positive organism and is important as a part of the normal microbiological flora. Two most important streptococci, *Streptococcus pyogenes* and *Streptococcus pneumoniae*, has been received attention for several decades. According to previous studies [1], virulence genes that are associated with the pathogenicity of *Streptococcus pneumoniae* are found on the surface of this bacterium. Capsule and other protein help pneumococci to escape the host immune defense [2]. Pneumolysin is an important virulence factor of pneumococci that are released by the action of autolysin [1]. Pneumolysin has several effects on the body as it stimulates the production of inflammatory cytokines [3]. Previous studies [4] found that bactericidal activity and neutrophils migration of the host is suppressed by pneumolysin. Pneumococcal pneumonia mostly occurs in children [1]. Streptococcal disease such as streptococcal toxic shock syndrome (STSS), food poisoning, Lyme disease and rheumatoid arthritis caused by *Streptococcus pyogenes* was encountered previously [5]. Superantigen interacts with T cells and accelerates cytokine response [6,7,8]. Structural homology of superantigen at the protein level was found between SpeA and SSA (49% similar) and SSA and SEB (60% similar) [9]. Superantigens also interact with TCR and class II MHC molecules by sharing conformational features. Previous studies [10] found that meningitis, pneumonia, septicemia and arthritis diseases in pigs are caused by *Streptococcus suis*. Virulence factors such as glutamate dehydrogenase (*gdh*), extracellular factor (*ef*), capsular polysaccharide (*cps*), murimidasereleased protein (*mrp*), suilysin (*sly*) play role in the pathogenicity of the isolates [11] and distinguish virulent from the avirulent isolates. *Streptococcus agalactiae* is associated with mastitis and other clinical infections as reported by [12]. It is also infected newborns by transmission through uterus when neonate aspirates contaminated amniotic fluids and results in low weight and full-term infants [13]. Virulence profile of 117 isolates analyzed in this study by *in silico* tools helps to understand the distribution of the genes that lead to the devastating effects of disease associated with streptococci.

MATERIALS AND METHODS

Strains used in the study: Strain used in the study are summarized in Table 1.

Table 1: Name of the isolates.

Serial	Isolate
1	NC_021485 *Streptococcus agalactiae* 09mas018883
2	NC_021195 *Streptococcus agalactiae* 2-22
3	NC_004116 *Streptococcus agalactiae* 2603V/R
4	NC_007432 *Streptococcus agalactiae* A909
5	NC_018646 *Streptococcus agalactiae* GD201008-001
6	NC_021486 *Streptococcus agalactiae* ILRI005
7	NC_021507 *Streptococcus agalactiae* ILRI112
8	NC_004368 *Streptococcus agalactiae* NEM316
9	NC_019048 *Streptococcus agalactiae* SA20-06
10	NC_022244 *Streptococcus anginosus* C1051
11	NC_022239 *Streptococcus anginosus* C238
12	NC_022238 *Streptococcus constellatus* subsp. *pharyngis* C1050
13	NC_022236 *Streptococcus constellatus* subsp. *pharyngis* C232
14	NC_022245*Streptococcus constellatus* subsp. *pharyngis* C818
15	NC_022532 *Streptococcus dysgalactiae* subsp. *equisimilis* 167
16	NC_019042 *Streptococcus dysgalactiae* subsp. *equisimilis* AC-2713
17	NC_017567 *Streptococcus dysgalactiae* subsp. *equisimilis* ATCC 12394
18	NC_012891 *Streptococcusdysgalactiae* subsp. *equisimilis* GGS_124 chromosome 1
19	NC_018712 *Streptococcus dysgalactiae* subsp. *equisimilis* RE378

20	NC_012471 *Streptococcus equi* subsp. *equi* 4047
21	NC_012470 *Streptococcus equi* subsp. *zooepidemicus*
22	NC_017582 *Streptococcus equi* subsp. *zooepidemicus* ATCC 35246
23	NC_011134 *Streptococcus equi* subsp. *zooepidemicus* str. MGCS10565
24	NC_013798 *Streptococcus gallolyticus* UCN34
25	NC_017576 *Streptococcus gallolyticus* subsp. *gallolyticus* ATCC 43143
26	NC_015215 *Streptococcus gallolyticus* subsp. *gallolyticus* ATCC BAA-2069
27	NC_009785 *Streptococcus gordonii* str. *Challis*substr. CH1
28	NC_016826 *Streptococcus infantarius* subsp. *infantarius* CJ18
29	NC_021314 *Streptococcus iniae* SF1
30	NC_022246 *Streptococcus intermedius* B196
31	NC_022237 *Streptococcus intermedius* C270
32	NC_018073 *Streptococcus intermedius* JTH08
33	NC_021900 *Streptococcus lutetiensis* 033
34	NC_016749 *Streptococcus macedonicus* ACA-DC 198
35	NC_013853 *Streptococcus mitis* B6
36	NC_018089 *Streptococcus mutans* GS-5
37	NC_017768*Streptococcus mutans* LJ23
38	NC_013928 *Streptococcus mutans* NN2025
39	NC_004350 *Streptococcus mutans* UA159
40	NC_021175 *Streptococcus oligofermentans* AS 1.3089
41	NC_015291 *Streptococcus oralis* Uo5
42	NC_015678*Streptococcus parasanguinis* ATCC 15912
43	NC_017905 *Streptococcus parasanguinis* FW213
44	NC_015558 *Streptococcus parauberis* KCTC 11537
45	NC_015600 *Streptococcus pasteurianus* ATCC 43144
46	NC_014498 *Streptococcus pneumoniae* 670-6B
47	NC_012468 *Streptococcus pneumoniae* 70585
48	NC_022655 *Streptococcus pneumoniae* A026
49	NC_014494 *Streptococcus pneumoniae* AP200
50	NC_011900*Streptococcus pneumoniae* ATCC 700669
51	NC_01058*Streptococcus pneumoniae* CGSP14
52	NC_008533 *Streptococcus pneumoniae* D39
53	NC_011072 *Streptococcus pneumoniae* G54
54	NC_010380 *Streptococcus pneumoniae*Hungary19A-6
55	NC_017591 *Streptococcus pneumoniae* INV104
56	NC_017593 *Streptococcus pneumoniae* INV200
57	NC_012466 *Streptococcus pneumoniae* JJA
58	NC_017592 *Streptococcus pneumoniae* OXC141
59	NC_012467 *Streptococcus pneumoniae* P1031
60	NC_003098 *Streptococcus pneumoniae* R6
61	NC_018594 *Streptococcus pneumoniae* SPNA45
62	NC_017769 *Streptococcus pneumoniae* ST556
63	NC_014251 *Streptococcus pneumoniae* TCH8431/19A

64	NC_003028 *Streptococcus pneumoniae* TIGR4
65	NC_012469 *Streptococcus pneumoniae* Taiwan19F-14
66	NC_018630 *Streptococcus pneumoniae* gamPNI0373
67	NC_015875 *Streptococcus pseudopneumoniae* IS7493
68	NC_018936 *Streptococcus pyogenes* A20
69	NC_01759 *Streptococcus pyogenes* Alab49
70	NC_021807 *Streptococcus pyogenes* HSC5
71	NC_020540 *Streptococcus pyogenes* M1 476 DNA
72	NC_002737 *Streptococcus pyogenes* M1 GAS
73	NC_008022 *Streptococcus pyogenes* MGAS10270
74	NC_006086 *Streptococcus pyogenes* MGAS10394
75	NC_008024 *Streptococcus pyogenes* MGAS10750
76	NC_017040 *Streptococcus pyogenes* MGAS15252
77	NC_017053 *Streptococcus pyogenes* MGAS1882
78	NC_008023 *Streptococcus pyogenes* MGAS2096
79	NC_004070 *Streptococcus pyogenes* MGAS315
80	NC_007297 *Streptococcus pyogenes* MGAS5005
81	NC_007296 *Streptococcus pyogenes* MGAS6180
82	NC_008021 *Streptococcus pyogenes* MGAS9429
83	NC_011375 *Streptococcus pyogenes* NZ131
84	NC_004606 *Streptococcus pyogenes* SSI-1
85	NC_009332 *Streptococcus pyogenes* str. Manfredo
86	NC_003485 *Streptococcus pyogenes* strain MGAS8232
87	NC_017594 *Streptococcus salivarius* 57.I
88	NC_015760 *Streptococcus salivarius* CCHSS3
89	NC_017595 *Streptococcus salivarius* JIM8777
90	NC_009009 *Streptococcus sanguinis* SK36
91	NC_022584 *Streptococcus* sp. I-G2
92	NC_022582 *Streptococcus* sp. I-P16
93	NC_009442 *Streptococcussuis* 05ZYH33
94	NC_009443 *Streptococcus suis* 98HAH33
95	NC_017622 *Streptococcus suis* A7
96	NC_012926 *Streptococcus suis* BM407
97	NC_017621 *Streptococcus suis* D12
98	NC_017620 *Streptococcus suis* D9
99	NC_017617 *Streptococcus suis* GZ1
100	NC_017618 *Streptococcus suis* JS14
101	NC_012925 *Streptococcus suis* P1/7
102	NC_018526 *Streptococcus suis* S735
103	NC_020526 *Streptococcus suis* SC070731
104	NC_012924 *Streptococcus suis* SC84
105	NC_017619 *Streptococcus suis* SS12
106	NC_017950 *Streptococcus suis* ST1
107	NC_015433 *Streptococcus suis* ST3

108	NC_022665 *Streptococcus suis* T15
109	NC_021213 *Streptococcus suis* TL13
110	NC_022516 *Streptococcus suis* YB51
111	NC_006449 *Streptococcus thermophilus* CNRZ1066
112	NC_017581 *Streptococcus thermophilus* JIM 8232
113	NC_008532 *Streptococcus thermophilus* LMD-9
114	NC_006448 *Streptococcus thermophilus* LMG 18311
115	NC_017927 *Streptococcus thermophilus* MN-ZLW-002
116	NC_017563 *Streptococcus thermophilus* ND03
117	NC_012004 *Streptococcus uberis* 0140J

Primer used in the study: Primer used in the study are summarized in Table 2.

Table 2: Primers used for virulence gene detection.

Virulence factor	Gene	Primer sequence (5' to 3')	Amplicon size (bp)	References
Autolysin	*lytA*	CAACCGTACAGAATGAAGCGG TTATTCGTGCAATACTCGTGCG	319	[14]
Pneumolysin	*ply*	ATTTCTGTAACAGCTACCAACGA GAATTCCCTGTCTTTTCAAAGTC	348	[14]
Pneumococcal surafece protein A	*pspA*	CTTTCTGCAATCATTCTTG GCCTTCTTTACCTTGTTCTGC	834	[15]
Alpha C protein	*bca*	TAACAGTTATGATACTTCACAGAC ACGACTTTCTTCCGTCCACTTAGG	535	[16]
Beta hemolysin	*cylE*	TGACATTTACAAGTGACGAAG TTGCCAGGAGGAGAATAGGA		[17]
Surface immunogenic protein	*sip*	ACTATTGACATCGACAATGGCAGC GTTACTGTCAGTGTTGTCTCA	267	[18]
Streptokinase	*skc*	TCCGGATTTTGGGTCCTTAGCCA AGTCGACTTTGCGCCTGATGCAC	475	[18]
Plasminogen activator	*pauA*	TGCTACTCAACCATCAAAGGTTGC TAGCAGTCTCAGTAGGATGAGTGA	440	[18]
Muramidase-released protein	*mrp*	CAGATGTGGACCGTAGACC GGATAATCACCAGCAGGAA	316	[19]
Suilysin	*sly*	GTGAAAACATGAAAGGATAAA CCAGATTACTCTATCACCTCA	1524	[19]
Glutamate dehydrogenase	*gdh*	AAGTTCCTCGGTTTTGAGCA GCAGCGTATTCTGTCAAACG	566	[20]
Glucosyltransferase	*gtfB*	ACTACACTTTCGGGTGGCTTGG CAGTATAAGCGCCAGTTTCATC	517	[21]
Streptococcal superantigen	*ssa*	TGAGGTAATTGGGGAGATGA CTAATTCTTGAACAGTGACTTG	621	[22]
Streptococcal pyrogenic exotoxin A	*speA*	CCAAGCCAACTTCACAGATC CCCTTCATGATTTGTTACCCC	309	[22]
Streptococcal invasion locus	*sil*	GGAGTTGGTTTATCAAATGTCAG ATCTGCCACAAAGACTGATCAAG	638	[23]

PCR amplification: *In silico* PCR amplification was done in the website http://insilico.ehu.eus/PCR/ [24, 25].

PFGE digestion: Pulsed field gel electrophoresis(PFGE) digestion and construction of the dendrogram was done in the website http://insilico.ehu.es/digest/. The enzyme used for the digestion was *ApaI* [24, 25].

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RESULTS AND DISCUSSION

Pulsed field gel electrophoresis (PFGE) analysis with *ApaI* digestion was performed in the website http://insilico.ehu.es/digest/. Recognition sequence was G_GGCC'C. Lambda ladder was used to compare the band size. Different band fragments were separated in 1.2% agarose gel. This *in silico* PFGE analysis was able to group 117 isolates into 15 genotypes (Fig 1). 80% genetic similarity was chosen as a cutoff value. According to previous studies [26], *S. uberis* isolates were divided into 10 major groups at 80% cutoff values. Genotype 8 was more prevalent with 24.79% (n=29) of the isolates (Fig 2). Genotype 6 contained 13.68% (n=16) of the isolates. Genotype 13 and 14 were found to contain 11.97% and 10.26% of the isolates.

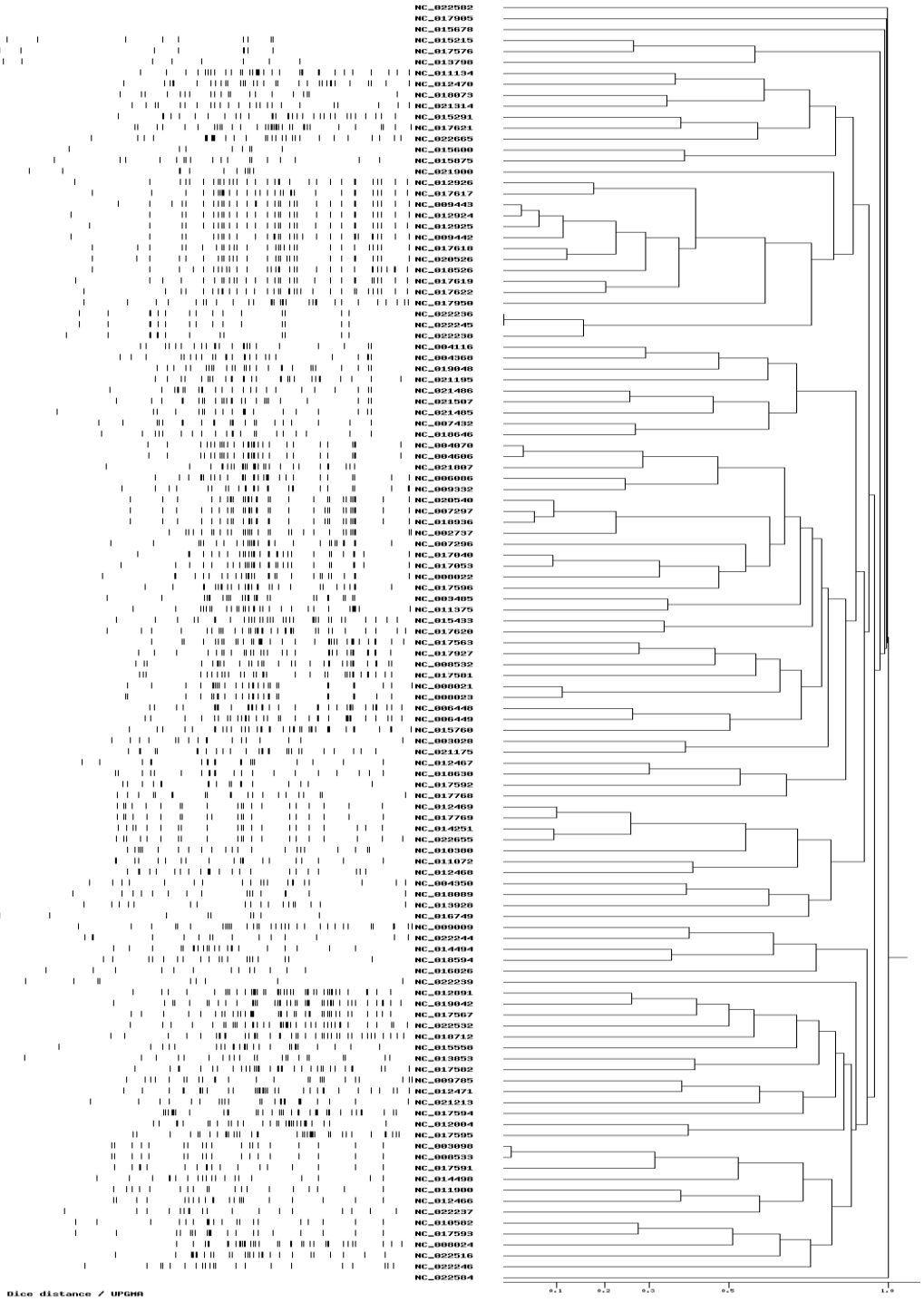

Fig 1: Phylogenetic diversity of *Streptococcus* spp. identified by PFGE.

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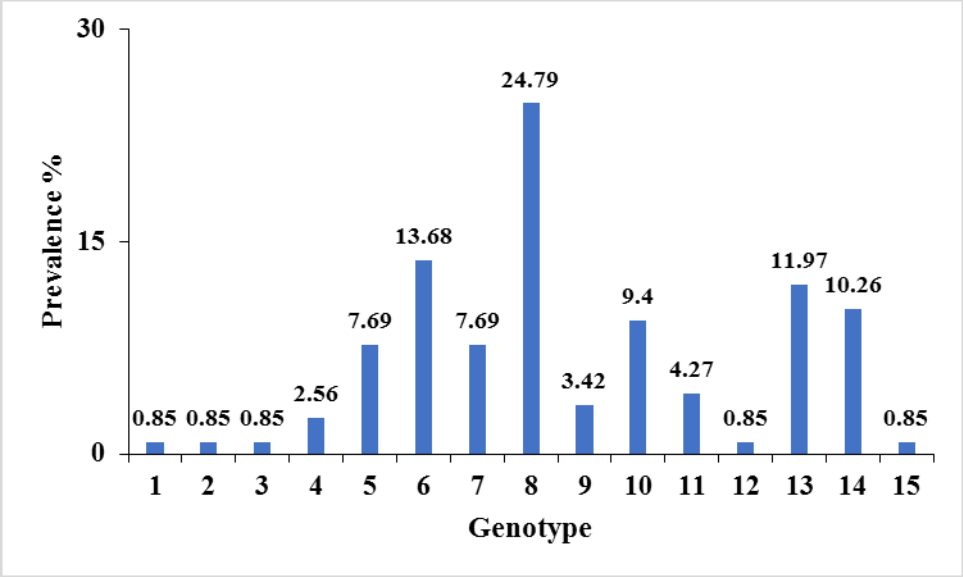

Fig 2: Prevalence of Genotypes.

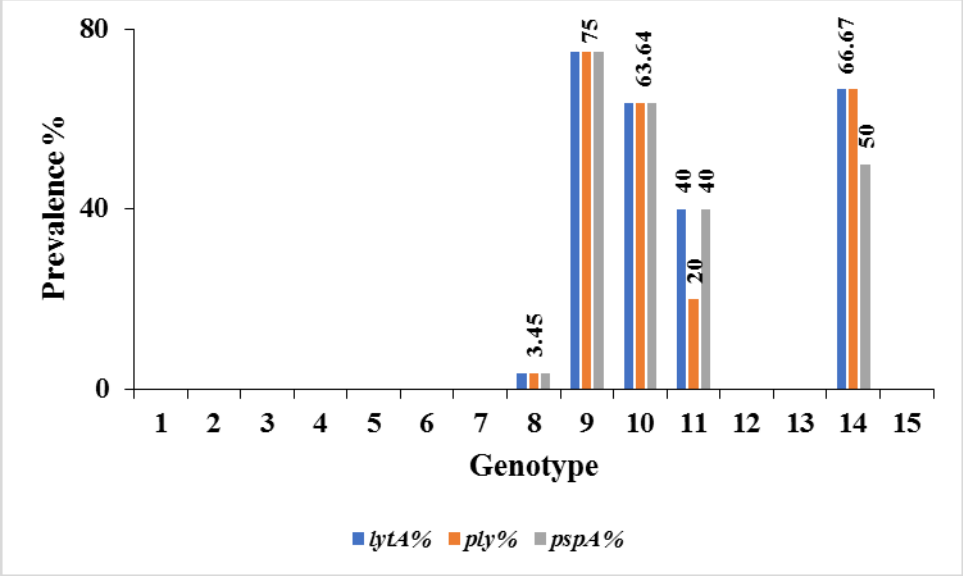

Fig 3: Genotypic distribution of autolysin, pneumolysin and pneumococcal surface protein A.

Virulence factors may contribute to the pathogenicity of the isolates. Autolysin, *lytA* is one of major virulence factor of *Streptococcus pneumoniae*. Autolysins, encoded by N-acetylmuramoyl-L-alanine amidase, disrupt the peptidoglycan layer of the cell wall [27]. Autolysin contributes to *Streptococcus pneumoniae* pathogenesis by invading pneumococci and releasing lethal toxin. An autolysin deficient *S. pneumoniae*has been identified that have the attenuated virulence property and contributes to the pathogenesis of the pneumococcal disease [28]. Out of the 117 isolates analyzed for the autolysin gene, 21 isolates (17.95%) were found to express *lytA* gene with 319 bp gene product. All the *Streptococcus pneumoniae* isolates analyzed in present study harboured the autolysin, *lytA* gene. The results of the current study were agreement with another study was done by [14]. So, it is an obligatory gene of *Streptococcus pneumoniae* isolates. Another virulence factor pnuemolysin, (*ply*) is released by the action of autolysin and play role in the early pathogenesis of pneumococcal infection [29]. Alveolar and capillary boundary is disrupted by the action of pneumolysin, *ply* and provided nutrient for bacterial growth [30]. Isolates were also tested for pneumolysin gene and found that 20 isolates had the *ply* gene with an approximate length of 348 bp. Hence the prevalence of percentage was 16.24. Out of the 21 *Streptococcus pneumoniae* isolates, *ply* gene was absent only in isolate *Streptococcus pneumoniae* SPNA45. Autolysin and pnuemolysin genes are the major virulence factor and present in almost all *Streptococcus pneumoniae*. That's why they can be used as a target for vaccine development. The *pspA*

gene is present in the cell wall of pneumococci [31]. Anticomplementary property of *pspA* has been observed by previous findings [32] and also found that *pspA* reduced the complement mediated clearance and phagocytosis of *S. pneumoniae*. The identification of *pspA* gene was characterized by the presence of 834 bp gene product. Nineteen isolates had the *pspA* gene and the prevalence was 16.24%. Out of the 21 *Streptococcus pneumoniae* isolates, *pspA* gene was absent in isolate *Streptococcus pneumoniae* INV200 and *Streptococcus pneumoniae* JJA. Genotypic distribution of these three genes was almost similar and found in genotype 8, 9, 10, 11, and 14 (Fig 3). Seventy-five percent isolates present in genotype 9 harboured *lytA*, *ply* and *pspA* genes. Genotypic distribution of these three genes was different in genotype 11 and 14. Twenty percent isolates present in genotype 11 carried the *ply* gene.

The *bca* gene is alpha C protein present in *Streptococcus agatactiae*. The *bca* gene is required for the entry of streptococci into the host cell [33]. Only two isolates (*Streptococcus agalactiae* A909, *Streptococcus agalactiae* GD201008-001) had the *bca* gene. Hence the prevalence of percentage was 1.71%. The *cylE* gene contributes to the development of meningitis by the systemic spread of the streptococci was documented by [34]. Seven isolates (5.98%) were found to possess the *cylE* gene with 248 bp gene product. So, these isolates were responsible for the development of meningitis. The *sip* gene, encoded by surface immunogenic protein, is responsible for raising an antibody response and found on the surface of *S. agalactiae* [18]. All of the nine (.69%) *S. agalactiae* isolates analyzed in the present study harboured the *sip* gene. The *bca*, *cylE* and *sip* genes were present in only genotype 7. The *sip* gene was more prevalent than *bca* and *cylE* gene (Fig 4). Lower level of *bca* gene was encountered in genotype 7 (22.22%).

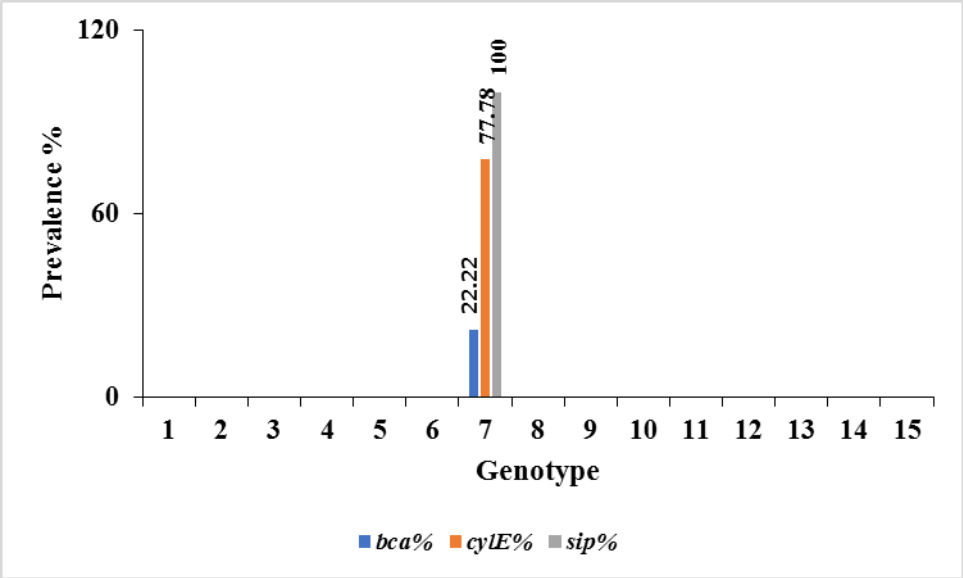

Fig 4: Genotypic distribution of alpha C protein, beta hemolysin and surface immunogenic protein.

According to previous study [18], streptokinase, *skc* gene is needed for colonization and degradation of extracellular matrix. Streptokinase possesses an intrinsic species specificity [35]. Streptokinase is used for the identification of *S. uberis*. Only one isolate harboured the *skc* gene. Hence the prevalence was 0.85%. Streptokinase is an important virulence factor of *S. uberis* that allows them to grow on the bovine mammary gland [36]. Previous studies [36, 37] indicated that plasminogen activator, *pauA* of *S. uberis* converts plasminogen to plasmin in blood plasma and tissue and also differentiate closely related *Streptococcus uberis* species. Only one isolate (*Streptococcus uberis* 0140J) harboured the *pauA* gene with 440 bp gene product. So, isolate *Streptococcus uberis* 0140J harboured both *skc* and *pauA* gene. Only three isolates carried 51 bp gene product for *gtfB* gene. Hence the prevalence was 2.56%. The *skc* and *pauA* genes were present in the same number in only genotype 13 (7.14%) (Fig 5). The *gtfB* genes of *Streptococcus mutans* were present in genotype 9 and 10. Twenty five percent isolates present in genotype 9 carried the *gtfB* gene.

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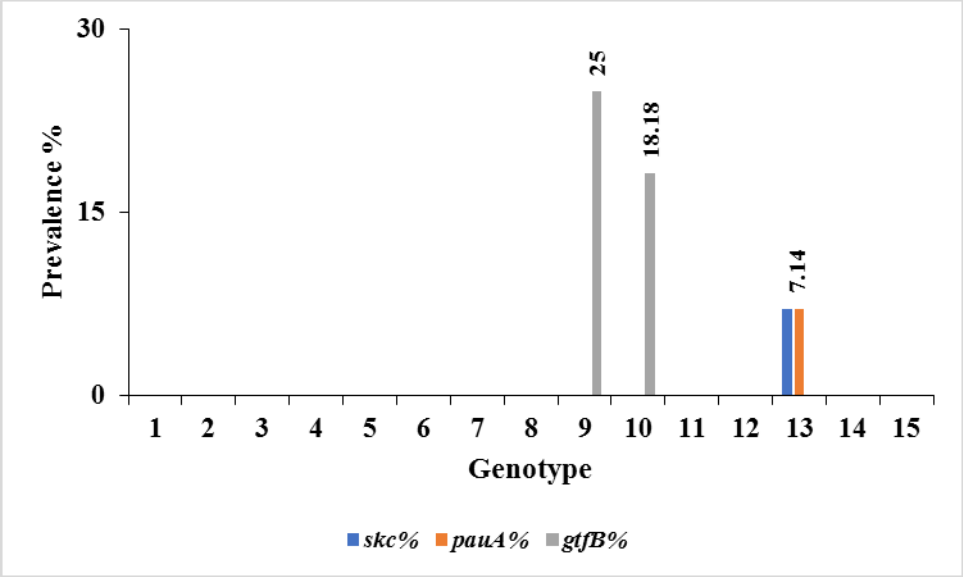

Fig 5: Genotypic distribution of streptokinase, plasminogen activator and *gtfB* gene.

The *mrp* gene is the major virulence factor of *S. suis* serotype 2 [39, 40]. Out of the 117 *Streptococcus* isolates analyzed, 13 isolates harboured the *mrp* gene with 316 bp gene product. The prevalence of *mrp* gene was 11.11%. Suilysin is putative virulence gene of *S. suis* and has a cytolytic function. Previous hypothesis [41] found that invasion of eukaryotic cell and inhibition of complement mediated opsonization is mediated by suilysin gene, *sly*. Thirteen isolates (11.11%) were found to harbour the *sly* gene and produce 1524 bp gene product. This *sly* gene is also a protective antigen as it had toxicity on Hep-2 cells, endothelial cells and vero cells [42]. Fourteen (11.97%) isolates carried the *gdh* gene with 566 bp gene product. Glutamate dehydrogenase, *gdh* gene is a diagnostic marker for the confirmation of *S. suis* [43]. The *mrp*, *sly* and *gdh* genes were present in the same number in genotype 5 (11.15%) (Fig 6). Seventy five percent isolates present in genotype 6 carried *sly* and *gdh* gene and 62.5% isolates present in genotype 6 harboured *mrp* gene. The *mrp* and *gdh* gene were also present in genotype 8.

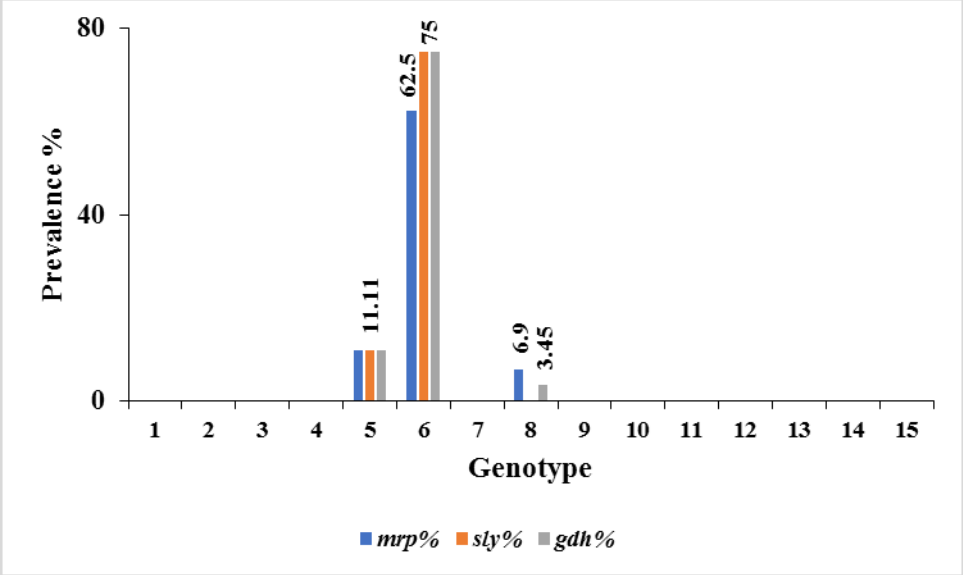

Fig 6: Genotypic distribution of muramidase released protein, suilysin and glutamate dehydrogenase gene.

Previous studies [44] found that polyclonal activation of streptococcal pyrogenic toxins (SPE) secretes large amounts of cytokine that is responsible for toxic shock. Three isolates harboured the streptococcal pyrogenic exotoxin A (*speA*) with 309 bp gene product. Hence the prevalence was 2.56%. The *sil* gene controls the spread of *Streptococcus pyogenes* into deeper tissue and play role in DNA transformation [45, 46]. Only

four isolates (3.42%) were found to express *sil* gene with 638 bp gene product. So, these four isolates may be involved in DNA transformation. Only three isolates were found to express *ssa* gene with 621 bp gene product. Previous studies [9] demonstrated that SSA proteins shares 60.2% homology with staphylococcal enterotoxin B. Genotype 8 carried all these three genes but their prevalence was different. The *speA* gene was most prevalent and 24.13% isolates present in genotype 8 carried *speA* gene (Fig 7). Prevalence of *sil* and *ssa* genes within the genotype 8 was 10.34 and 6.9%, respectively. Genotype 14 harboured only *sil* gene and 7.14% isolates present in genotype 14 carried the *sil* gene.

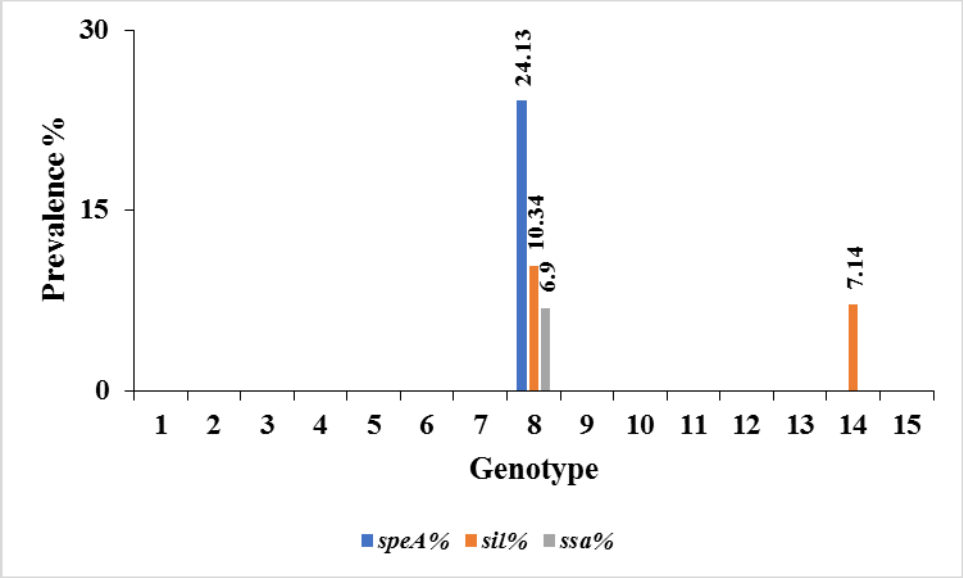

Fig 7: Genotypic distribution of *speA*, *sil* and *ssa* genes.

CONCLUSION

This study helps to predict virulence profile based on genotyping. The epidemiological data generated here aid to understand the disease associated genes and also helps to a develop vaccines.

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REFERENCES

[1] AlonsoDeVelasco E, Verheul, AF, Verhoef J, Snippe H. (1995). *Streptococcus pneumoniae*: virulence factors, pathogenesis, and vaccines. Microbiol Rev 1995; 59(4): 591-603.

[2] Neeleman C, Geelen S, Aerts P, Van Tilburg M, Watson D, Verhoef J, Fleer A, Van Dijk H. Virulence of Streptococcus pneumoniae is associated with a regulator factor H, abstr. 548. In Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC, 1993; P 219

[3] Houldsworth S, Andrew PW, Mitchell TJ. Pneumolysin stimulates production of tumor necrosis factor alpha and interleukin-1b by human mononuclear phagocytes. Infect Immun 1994; 62: 1501–1503.

[4] Paton JC, Ferrante A. Inhibition of human polymorphonuclear leukocyte respiratory burst, bactericidal activity, and migration by pneumolysin. Infect. Immun 1983; 41: 1212–1216.

[5] Kotb M.Bacterial pyrogenic exotoxins as superantigens. ClinMicrobiol Rev 1995; 8: 411–426.

[6] Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 1990; 17: 251–272

[7] Kotb M. Role of superantigens in the pathogenesis of infectious diseases and their sequelae. CurrOpin Infect Dis 1992; 5: 364–374.

[8] Kotzin BL, Leung DY, Kappler J, Marrack P. Superantigens and their potential role in human disease AdvImmunol 1993; 54: 99–166

[9] Reda KB, Kapur V, Mollick JA, Lamphear JG, Musser JM, Rich RR. Molecular characterization and phylogenetic distribution of the streptococcal superantigen gene (*ssa*) from *Streptococcuspyogenes*. Infect Immun 1994; 62: 1867–1874.

[10] Staats JJ, Feder I, Okwumabua, Chengappa MM. *Streptococcus suis*: past and present. Vet Res Commun 1997; 21(6): 381-407

[11] Lu LL, Li R, Zheng YZ, Jiang YQ. Research for virulence factors of *Streptococcus suis* type 2. Chinese J Health Lab Tech 2008; 18(3): 570-572.

[12] Hillerton JE, Leigh JA, Ward PN, Coffey TJ. *Streptococcus agalactiae* infection in dairy cows. Vet Rec 2004; 154: 671-672.

[13] Mullaney DM. Group B Streptococcal infections in newborns. J Obstet Gynecol Neonatal Nurs. 2001; 30:649–658.

[14] Sourav S, Patricia A, Sharma S, Kanungo R, Jayachandran S, Prashanth K. Detection of pneumolysin and autolysin genes among antibiotic resistant *Streptococcus pneumoniae* in invasive infections. Indian J Med Microbiol 2010; 10: 34-39

[15] Anthony J, Scott G, Marston EL, Hall AJ, Marsh K. Diagnosis of pneumococcal pneumonia by *psaA* PCR analysis of lung aspirates from adult patients in Kenya. J ClinMicrobiol 2003; 41(6): 2554–2559.

[16] Manning SD, Ki M, Marrs CF, Kugeler KJ, Borchardt SM, Baker CJ, Foxman B. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. BMC Infect Dis 2006; 6(1): 116.

[17] Bergseng H, Bevanger L, Rygg M, Bergh K. Real-time PCR targeting the sip gene for detection of group B streptococcus colonization in pregnant women at delivery. J Med Microbiol 2007; 56: 223-228.

[18] Nithinprabhu K, Isloor SK, Hegde R, Suryanarayana WS, *et al.* Standardization of PCR and phylogenetic analysis of predominant streptococcal species isolated from subclinical mastitis. International Symposium on – Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation and XVIIth Annual Convention of Indian Society of Veterinary Immunology and Biotechnology (ISVIB), Bikaner, Rajasthan, 2010; 50: 47.

[19] Rui P, Zhang ZZ, Ma ZJ, Fang H, Yang WJ, Zhang XZ, Jia QH. Detection of virulence-associated factors of *Streptococcus suis* serotype 2 by PCR assay in Hebei, Province of China. African J Microbiol Res 2012; 6(5): 1061-1064.

[20] Okwumabua O, O'Connor M, Shull E. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. *FEMS Microbiol. Lett 2003;* 218: 79–84.

[21] Oho T, Yamashita Y, Shimazaki Y, Kushiyama M, Koga T. Simple and rapid detection of *Streptococcus mutans* and *Streptococcus sobrinus* in human saliva by polymerase chain reaction. Oral MicrobiolImmunol 2000; 15: 258–262.

[22] Commons R, Rogers S, Gooding T, Danchin M, Carapetis J, Robins-Browne R, Curtis N. Superantigen genes in group A streptococcal isolates and their relationship with *emm* types. J Med Microbiol 2008; 57(10): 1238-1246.

[23] Jing HB, Ning BA, Hao HJ, Zheng YL, Chang D, Jiang W, Jiang YQ. Epidemiological analysis of group A streptococci recovered from patients in China. J Med Microbiol 2006; 55(8): 1101-1107.

[24] San Millán RM, Martínez-Ballesteros I, Rementeria A, Garaizar J, Bikandi J. Online exercise for the design and simulation of PCR and PCR-RFLP experiments. BMC Res Notes 2013; 6(1), 513.

[25] Bikandi J, San Millán R, Rementeria A, Garaizar J. *In silico* analysis of complete bacterial genomes: PCR, AFLP–PCR and endonuclease restriction. Bioinformatics 2004; 20(5): 798-799.

[26] Reinoso EB, Lasagno MC, Odierno LM. Genetic patterns of *Streptococcus uberis* isolated from bovine mastitis. Rev Argent Microbiol 2015; 47(2): 108-111.

[27] Rogers HJ, Perkins HR, Ward JB. Formation of cell wall polymers. In: Nombela C, editor. Microbial cell wall and membranes. London, United Kingdom: Chapman & Hall, Ltd, 1980; pp. 437–460.

[28] Ng EW, Costa JR, Samiy N, Ruoff KL, Connolly E, Cousins FV, *et al*. Contribution of pneumolysin and autolysin to the pathogenesis of experimental pneumococcal endophthalmitis. Retina 2002; 22: 622-32.

[29] Jedrzejas MJ. Pneumococcal virulence factors: structure and function. MicrobiolMolBiol Rev 2001; 65(2): 187-207.

[30] Rubins JB, Janoff EN. Pneumolysin: a multifunctional pneumococcal virulence factor. J Lab Clin Med 1998; 131: 21–27.

[31] McDaniel LS, Scott G, Kearney JF, Briles DE. Monoclonal antibodies against protease-sensitive pneumococcal antigens can protect mice from fatal infection with *Streptococcus pneumoniae*. J Exp Med 1984; 160: 386–397

[32] Briles DE, Hollingshead SK, Swiatlo E, Brooks-Walter A, Szalai A, Virolainen A, McDaniel L S, Benton KA, White P, Prellner K, Hermansson A, Aerts PC, Van Dijk H, Crain MJ. PspA and PspC: their potential for use as pneumococcal vaccines. Microb Drug Resist 1997; 3: 401–408.

[33] Bolduc, GR, Baron MJ, Gravekamp C, Lachenauer CS, Madoff LC. The alpha C protein mediates internalization of group B streptococcus within human cervical epithelial cells. Cell Microbiol 2002; 4: 751-758.

[34] Doran, KS, LIU GY, NIZET V. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J Clin Invest 2003; 112: 736-744.

[35] Mccoy HE, Broder CC, Lottenberg R. Streptokinases produced pathogenic by group C streptococci demonstrate species-specific plasminogen activation. J Infect Dis 1991; 164: 515-521

[36] Leigh JA, Field TR. *Streptococcus uberis* resists phagocytosis despite the presence of bound immunoglobulin. XIIth Lancefield Syposium on streptococci and streptococcal diseases, St Petersberg, Russia, 1993.

[37] Leigh JA. *Streptococcus uberis*: A permanent barrier to the control of bovine mastitis. Vet J 1999; 157: 225-238

[38] Leigh JA. Vaccines against bovine mastitis due to *Streotococcusuberis* current status and future prospectects, In: Biology of the Mammary Gland, 2000, pp. 307-311

[39] Wisselink HJ, Smith HE, Stockhofe-Zurwieden N, Peperkamp K, Vecht U.Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. Vet Microbiol 2000; 74:237-248.

[40] Vecht U, Wisselink HJ, Jellema ML, Smith HE. Identification of 2 proteins associated with virulence of *Streptococcus suis* type 2. Infect Immun 1991; 59:3156-3162.

[41] Chabot-Roy G, Willson P, Segura M, Lacouture S, Gottschalk M.Phagocytosis and killing of *Streptococcus suis* by porcine neutrophils.MicrobPathog 2006; 41:21-32

[42] Staats JJ, Plattner BL, Stewart GC, Changappa MM. Present of the *Streptococcus suis*suilysin gene and expression of MRP and EF correlates with high virulence in *Streptococcus suis* type 2 isolates. Vet Microbiol 1999; 70(3-4): 201-211

[43] Okwumabua, O, O'Connor, M, Shull, E. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. FEMS Microbiol Lett 2003; 218(1): 79-84.

[44] Olsen RJ, Shellburne SA, Musser JM. Molecular mehanisms underlying group A streptococcal pathogenesis. Cell Microbiol 2009; 11(1): 1-12.

[45] Hidalgo-Grass C, Ravins M, Dan-Goor M, Jaffe J, Moses AE. A locus of group A streptococcus involved in invasive disease and DNA transfer. MolMicrobiol 2002; 46: 87–99.

[46] Hidalgo-Grass C, Dan-Goor M, Maly A. Effect of a bacterial pheromone peptide on host chemokine degradation in group A streptococcal necrotizing soft-tissue infections. Lancet 2004; 363: 696–703.