# CHAPTER V

# RESULTS

## 1. Clinical isolates

### 1.1 GGS isolates

A total of selected 60 GGS isolates from culture collections from patients attended King Chulalongkorn Memorial hospital from 1995 to 2000 were included in this study. They could be divided into 30 non-invasive and 30 invasive isolates. There are 17 males and 13 females in non-invasive isolates and 17 males and 13 females in invasive isolates. In the non-invasive group, there are 19 isolates (63.3%) from pus culture, 10 isolates (33.3%) from throat culture, and 1 isolate (3.3%) from eye culture. In the invasive group, there are 19 isolates (63.3%) from hemoculture, 7 isolates (23.3%) from joint fluid and 4 isolates (13.3%) from cerebrospinal fluid (CSF).

### 1.2 GCS isolates

A total of 52 GCS isolates from culture collections of patients attended King Chulalongkorn Memorial hospital from 1995 to 2000 were included in this study. They could be divided into 40 non-invasive and 12 invasive isolates. There are 17 males and 23 females in non-invasive isolates and 7 males and 5 females in invasive isolates. In the non-invasive group, there are 18 isolates (45%) from pus culture, 16 isolates (40%) from throat culture, 4 isolates (10%) from tissue wound, 1 isolate (2.5%) from eye and 1 isolate (2.5%) from urine culture. In the invasive group, there are 8 isolates (66.6%) from hemoculture, 2 isolates (16.6%) from joint fluid, 1 isolate (8.3%) from cerebrospinal fluid (CSF) and 1 isolate (8.3%) from lung tissue.

## 2. Streptococcal Characteristics

### 2.1 GGS isolates

Gram stain and culture on blood agar plate were done to confirm cellular and colony morphology of these organisms. All of 60 GGS isolates were grown on blood agar plate with β-hemolysis. The species of the *Streptococcus* were identified by biochemical and enzymatic characteristics with the API20 STREP (bioMerieux, Marcy-l' Etoile, France). C-carbohydrate group antigens were identified by a commercial available co-agglutination test (Phadebact, Boule Diagnostics AB, Huddinge, Sweden). *S. dysgalactiae subsp. equisimilis* were found the most both in non-invasive (29 isolates, 96.6%) and invasive (29 isolates, 96.6%) groups. Other species can also be found. For example, *S. constellatus* was found in 1 isolate (3.3%) from non-invasive isolates and *S. anginosus* was found in 1 isolate (3.3%) from invasive isolates (Table 6 and table 7).

Table 6: The general characteristics of the non-invasive GGS isolates included in this study

| No. | ID of clinical isolates | Year | Sex | Specimen | *Streptococcus* species identified by API20 STREP | % identity |
|---|---|---|---|---|---|---|
| 1 | G38-004 | 1995 | M | Throat swab | *S. dys. equisimilis* | 93.4 |
| 2 | G38-014 | 1995 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 3 | G38-019 | 1995 | M | Sputum | *S. dys. equisimilis* | 98.5 |
| 4 | G38-023 | 1995 | F | Discharge from breast | *S. dys. equisimilis* | 93.4 |
| 5 | G39-218 | 1996 | F | Pus | *S. dys. equisimilis* | 98.8 |
| 6 | G40-161 | 1997 | M | Pus | *S. dys. equisimilis* | 98.8 |
| 7 | G40-164 | 1997 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 8 | G40-167 | 1997 | F | Pus | *S. dys. equisimilis* | 98.5 |
| 9 | G40-179 | 1997 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 10 | G41-002 | 1998 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 11 | G41-006 | 1998 | F | Pus | *S. dys. equisimilis* | 98.5 |
| 12 | G41-010 | 1998 | M | Sputum | *S. dys. equisimilis* | 93.4 |
| 13 | G41-018 | 1998 | M | Pus | *S. dys. equisimilis* | 93.4 |
| 14 | G41-025 | 1998 | F | Pus | *S. dys. equisimilis* | 98.8 |
| 15 | G41-032 | 1998 | F | Pus | *S. dys. equisimilis* | 98.5 |
| 16 | G41-035 | 1998 | F | Pus from middle finger | *S. dys. equisimilis* | 98.5 |
| 17 | G41-042 | 1998 | F | Throat swab | *S. dys. equisimilis* | 91.9 |
| 18 | G41-050 | 1998 | M | Saline fluid | *S. dys. equisimilis* | 93.4 |
| 19 | G42-043 | 1999 | M | Sputum | *S. dys. equisimilis* | 93.4 |
| 20 | G42-060 | 1999 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 21 | G42-068 | 1999 | M | Sputum | *S. constellatus* | 97.0 |
| 22 | G42-072 | 1999 | F | Sputum | *S. dys. equisimilis* | 98.5 |
| 23 | G42-084 | 1999 | F | Throat swab | *S. dys. equisimilis* | 98.5 |
| 24 | G42-089 | 1999 | F | Pus | *S. dys. equisimilis* | 93.4 |
| 25 | G42-090 | 1999 | F | Pus | *S. dys. equisimilis* | 96.0 |
| 26 | G43-002 | 2000 | M | Eye swab | *S. dys. equisimilis* | 98.5 |
| 27 | G43-014 | 2000 | M | Pus | *S. dys. equisimilis* | 98.5 |
| 28 | G43-023 | 2000 | M | Sputum | *S. dys. equisimilis* | 98.0 |
| 29 | G43-040 | 2000 | M | Tonsil swab | *S. dys. equisimilis* | 98.5 |
| 30 | G43-049 | 2000 | F | Pus | *S. dys. equisimilis* | 98.5 |

Table 7: The general characteristics of the invasive GGS isolates included in this study

| No. | ID of clinical isolates | Year | Sex | Specimen | *Streptococcus* species identified by API20 STREP | % identity |
|---|---|---|---|---|---|---|
| 1 | G38-009 | 1995 | F | Synovial fluid | *S. dys. equisimilis* | 98.5 |
| 2 | G38-012 | 1995 | F | Blood | *S. dys. equisimilis* | 93.4 |
| 3 | G38-018 | 1995 | M | Synovial fluid | *S. dys. equisimilis* | 93.4 |
| 4 | G38-030 | 1995 | F | Ascitic fluid | *S. dys. equisimilis* | 98.5 |
| 5 | G38-032 | 1995 | M | Blood | *S. dys. equisimilis* | 98.5 |
| 6 | G38-034 | 1995 | M | Blood | *S. dys. equisimilis* | 93.4 |
| 7 | G38-035 | 1995 | M | Body fluid | *S. dys. equisimilis* | 97.7 |
| 8 | G39-064 | 1996 | F | Blood | *S. dys. equisimilis* | 97.7 |
| 9 | G39-222 | 1996 | F | Blood | *S. dys. equisimilis* | 97.7 |
| 10 | G40-006 | 1997 | M | Blood | *S. dys. equisimilis* | 93.4 |
| 11 | G40-010 | 1997 | F | Blood | *S. dys. equisimilis* | 80.0 |
| 12 | G40-159 | 1997 | M | Body fluid | *S. anginosus* | 96.0 |
| 13 | G40-178 | 1997 | F | Body fluid | *S. dys. equisimilis* | 98.5 |
| 14 | G40-182 | 1997 | M | Blood | *S. dys. equisimilis* | 93.4 |
| 15 | G40-186 | 1997 | F | Blood | *S. dys. equisimilis* | 93.4 |
| 16 | G41-008 | 1998 | F | Blood | *S. dys. equisimilis* | 93.4 |
| 17 | G41-012 | 1998 | M | Joint fluid | *S. dys. equisimilis* | 93.4 |
| 18 | G41-021 | 1998 | F | Body fluid | *S. dys. equisimilis* | 98.8 |
| 19 | G41-024 | 1998 | M | Joint fluid | *S. dys. equisimilis* | 93.4 |
| 20 | G41-046 | 1998 | M | Blood | *S. dys. equisimilis* | 97.7 |
| 21 | G41-059 | 1998 | M | Blood | *S. dys. equisimilis* | 93.4 |
| 22 | G42-041 | 1999 | M | Blood | *S. dys. equisimilis* | 96.0 |
| 23 | G42-054 | 1999 | M | Blood | *S. dys. equisimilis* | 93.2 |
| 24 | G42-056 | 1999 | M | Blood | *S. dys. equisimilis* | 86.5 |
| 25 | G42-062 | 1999 | M | Joint fluid | *S. dys. equisimilis* | 99.6 |
| 26 | G42-076 | 1999 | M | Joint fluid | *S. dys. equisimilis* | 93.4 |
| 27 | G43-005 | 2000 | F | Blood | *S. dys. equisimilis* | 90.3 |
| 28 | G43-036 | 2000 | M | Blood | *S. dys. equisimilis* | 90.3 |
| 29 | G43-041 | 2000 | F | Blood | *S. dys. equisimilis* | 90.3 |
| 30 | G43-064 | 2000 | F | Blood | *S. dys. equisimilis* | 90.3 |

### 2.2 GCS isolates

Gram stain and culture on blood agar plate were done to confirm cellular and colony morphology of these organisms. All of 40 non-invasive and 12 invasive GCS isolates were grown on blood agar plate with β-hemolysis. The species of the *Streptococcus* were identified by biochemical and enzymatic characteristics with the API20 STREP (bioMerieux, Marcy-l' Etoile, France). C-carbohydrate group antigens were identified by a commercial available co-agglutination test (Phadebact, Boule Diagnostics AB, Huddinge, Sweden). *S. dysgalactiae subsp. equisimilis* were found the most both in non-invasive (33 isolates, 82.5%) and invasive (11 isolates, 91.6%) groups. Other species can also be found. For example, *S. constellatus* were found in 5 isolates (12.5%) from non-invasive isolates and 1 isolate (8.3%) from invasive isolates. *S. intermedius* were found in 2 isolates (5%) from non-invasive isolates (Table 8 and table 9).

Table 8: The general characteristics of the non-invasive GCS isolates included in this study

| No. | ID of clinical isolates | Year | Sex | Specimen | *Streptococcus* species identified by API20 STREP | % identity |
|---|---|---|---|---|---|---|
| 1 | C38-001 | 1995 | M | Submaxillary abscess | *S. constellatus* | 80.0 |
| 2 | C38-002 | 1995 | F | Cervix swab | *S. dys. equisimilis* | 97.7 |
| 3 | C38-003 | 1995 | F | Pus | *S. dys. equisimilis* | 98.5 |
| 4 | C38-004 | 1995 | M | Pus | *S. dys. equisimilis* | 93.4 |
| 5 | C38-005 | 1995 | M | Tissue wound | *S. dys. equisimilis* | 98.5 |
| 6 | C38-006 | 1995 | F | Urine culture | *S. dys. equisimilis* | 97.7 |
| 7 | C38-007 | 1995 | F | Throat swab | *S. dys. equisimilis* | 98.5 |
| 8 | C38-008 | 1995 | F | Throat swab | *S. intermedius* | 80.8 |
| 9 | C38-009 | 1995 | F | Sputum | *S. dys. equisimilis* | 99.3 |
| 10 | C38-010 | 1995 | F | Eye swab | *S. dys. equisimilis* | 98.5 |
| 11 | C39-022 | 1996 | F | Pus | *S. dys. equisimilis* | 98.8 |
| 12 | C41-001 | 1998 | M | Pus | *S. dys. equisimilis* | 97.7 |
| 13 | C41-004 | 1998 | F | Pus | *S. dys. equisimilis* | 98.5 |
| 14 | C41-005 | 1998 | F | Sputum | *S. dys. equisimilis* | 98.5 |
| 15 | C41-006 | 1998 | F | Nasal discharge | *S. dys. equisimilis* | 98.5 |
| 16 | C41-007 | 1998 | F | Discharge from breast | *S. dys. equisimilis* | 93.4 |
| 17 | C42-001 | 1999 | M | Throat swab | *S. dys. equisimilis* | 98.5 |
| 18 | C42-002 | 1999 | M | Throat swab | *S. constellatus* | 99.8 |
| 19 | C42-003 | 1999 | F | Pus | *S. dys. equisimilis* | 97.7 |
| 20 | C42-005 | 1999 | M | Maxillary sinus swab | *S. dys. equisimilis* | 93.4 |
| 21 | C42-006 | 1999 | M | Pus | *S. dys. equisimilis* | 80.0 |
| 22 | C42-007 | 1999 | M | Pus | *S. dys. equisimilis* | 93.4 |
| 23 | C42-008 | 1999 | M | Pus | *S. dys. equisimilis* | 80.0 |
| 24 | C42-010 | 1999 | F | Pus | *S. dys. equisimilis* | 93.4 |
| 25 | C43-001 | 2000 | F | Sputum | *S. dys. equisimilis* | 80.0 |
| 26 | C43-002 | 2000 | F | Tissue swab | *S. dys. equisimilis* | 93.4 |
| 27 | C43-004 | 2000 | M | Tissue at neck swab | *S. dys. equisimilis* | 93.4 |
| 28 | C43-005 | 2000 | M | Tonsil swab | *S. dys. equisimilis* | 93.4 |

| No. | ID of clinical isolates | Year | Sex | Specimen | *Streptococcus* species | % identity |
|---|---|---|---|---|---|---|
| 29 | C43-006 | 2000 | F | Pus | *S. dys. equisimilis* | 93.4 |
| 30 | C43-007 | 2000 | M | Sputum | *S. constellatus* | 97.0 |
| 31 | C43-008 | 2000 | M | Sputum | *S. dys. equisimilis* | 93.4 |
| 32 | C43-009 | 2000 | F | Tonsil swab | *S. dys. equisimilis* | 93.4 |
| 33 | C43-011 | 2000 | M | Sputum | *S. dys. equisimilis* | 96.0 |
| 34 | C43-012 | 2000 | M | Gastric swab | *S. dys. equisimilis* | 97.7 |
| 35 | C43-013 | 2000 | M | Sputum | *S. dys. equisimilis* | 93.4 |
| 36 | C43-014 | 2000 | F | Sputum | *S. constellatus* | 89.3 |
| 37 | C43-015 | 2000 | F | Pus | *S. constellatus* | 89.3 |
| 38 | C43-016 | 2000 | M | Lesion in lower lip | *S. dys. equisimilis* | 93.4 |
| 39 | C43-017 | 2000 | F | Throat swab | *S. dys. equisimilis* | 93.4 |
| 40 | C43-018 | 2000 | F | Tonsil swab | *S. intermedius* | 91.2 |

Table 9: The general characteristics of the invasive GCS isolates included in this study

| No. | ID of clinical isolates | Year | Sex | Specimen | *Streptococcus* species identified by API20 STREP | % identity |
|---|---|---|---|---|---|---|
| 1 | C38-011 | 1995 | M | Lung fluid | *S. dys. equisimilis* | 98.5 |
| 2 | C38-012 | 1995 | M | Blood | *S. dys. equisimilis* | 99.6 |
| 3 | C38-013 | 1995 | F | Body fluid | *S. dys. equisimilis* | 99.3 |
| 4 | C38-014 | 1995 | F | Blood | *S. dys. equisimilis* | 93.4 |
| 5 | C38-015 | 1995 | M | Blood | *S. dys. equisimilis* | 98.5 |
| 6 | C38-016 | 1995 | F | Blood | *S. dys. equisimilis* | 98.5 |
| 7 | C38-017 | 1995 | F | Blood | *S. dys. equisimilis* | 98.5 |
| 8 | C38-018 | 1995 | M | Blood | *S. dys. equisimilis* | 98.5 |
| 9 | C41-002 | 1998 | M | Plural fluid | *S. constellatus* | 97.6 |
| 10 | C41-003 | 1998 | M | Joint fluid | *S. dys. equisimilis* | 98.8 |
| 11 | C42-009 | 1999 | F | Blood | *S. dys. equisimilis* | 80.0 |
| 12 | C43-003 | 2000 | M | Blood | *S. dys. equisimilis* | 98.5 |

## 3. *Emm* gene amplification

### 3.1. Reference strains

In order to determine the specificity of primer1 and primer2 for *emm* gene. We use different groups of reference strains as positive and negative controls. *Emm* gene is present in *Streptococcus* group A, C, and G (Bisno, et al., 1996). R. C. Lancefield, J17A4 was a reference strain for GAS. M. Ciuca 'Chestle' and PHLS, 'Valente' were the reference strain for GCS and GGS, respectively. They were used as the positive control. M. Ciuca 'Lewis' and PHLS, 'O' Mahoney' were the reference strain for GBS and GFS, respectively. *Streptococcus pneumoniae* (ATCC49619) and *Staphylococcus aureus* (ATCC25923) were the reference strain. They were used as the negative control. The organisms were all obtained from *Streptococcus* Center in KANAKAWA Public Health Laboratory, Japan (Figure 8).

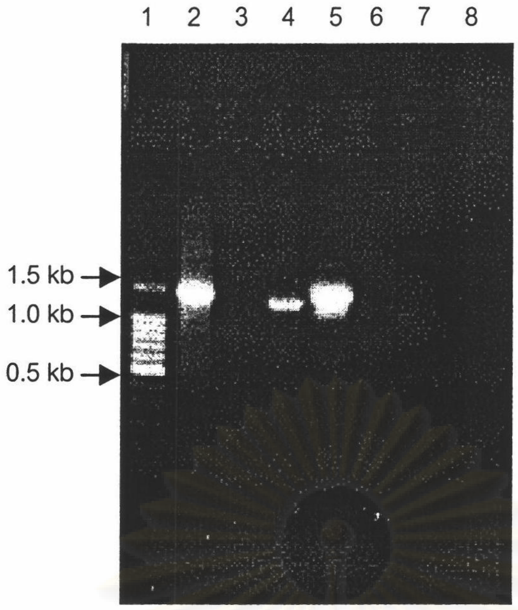

Figure 8: Determination of *emm* amplification from different groups of reference strains as positive and negative controls.

Lane 1: 100 bp DNA marker
Lane 2: GAS (R. C. Lancefield, J17A4)
Lane 3: GBS (M. Ciuca 'Lewis')
Lane 4: GCS (M. Ciuca 'Chestle')
Lane 5: GGS (PHLS, 'Valente')
Lane 6: GFS (PHLS, 'O' Mahoney')
Lane 7: *Streptococcus pneumoniae* ATCC49619
Lane 8: *Staphylococcus aureus* ATCC25923

PCR products of ~ 1 kb size were present in the positive control lanes as expected. The positive control is representing in lane 2 (GAS), 4 (GCS) and 5 (GGS) and negative control is representing in lane 3 (GBS), 6 (GFS), 7(*Streptococcus pneumoniae*) and 8 (*Staphylococcus aureus*).

## 3.2. Clinical isolates

### 3.2.1. Determination of the *emm* gene from GGS isolates

All of 30 non-invasive and 30 invasive GGS isolates can be amplified by PCR method modified from Beall and his colleges in 1996-1997. By running with electrophoresis in 1.5% agarose gel, the PCR product is approximately 1-1.5 bp as expected (Figure 9 and 10).

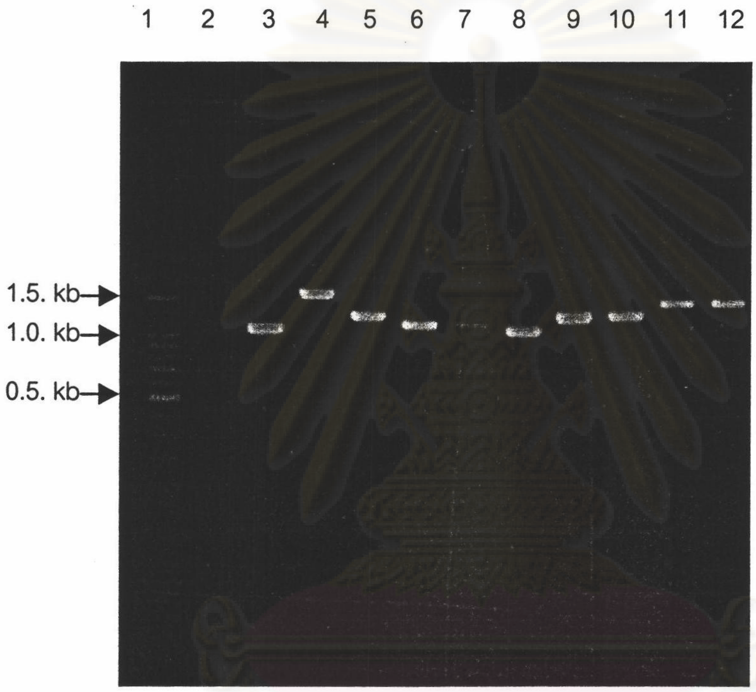

Figure 9: A representative picture of *emm* gene amplification of non-invasive GGS isolates

Lane 1 : 100 bp DNA marker
Lane 2 : negative control (double distill water)
Lane 3 : G38-014 isolate
Lane 4 : G39-218 isolate
Lane 5 : G40-161 isolate
Lane 6 : G41-025 isolate
Lane 7 : G41-032 isolate
Lane 8 : G42-043 isolate
Lane 9 : G42-068 isolate
Lane 10 : G42-072 isolate
Lane 11 : G43-014 isolate
Lane 12 : G43-049 isolate

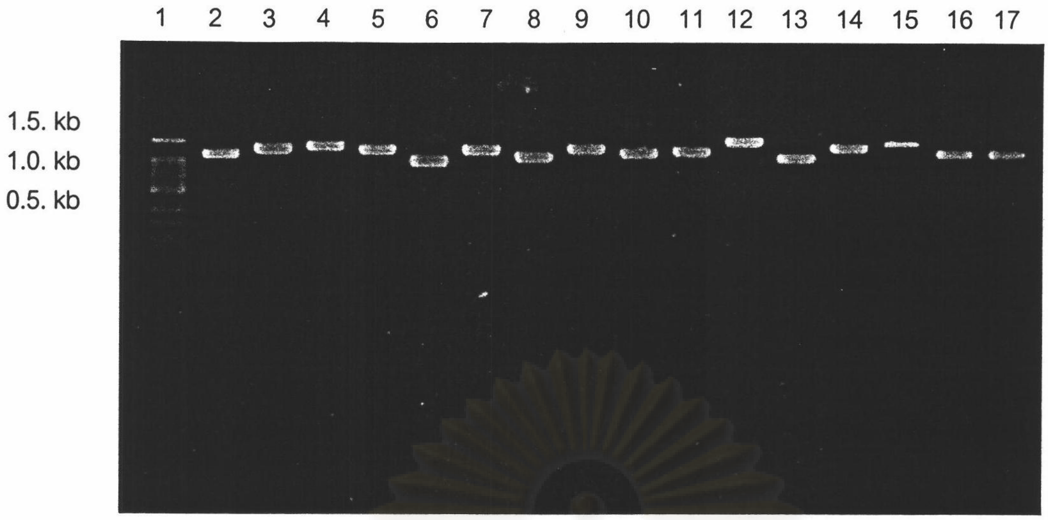

Figure 10: A representative picture of *emm* gene amplification of invasive GGS isolates.

Lane 1 : 100 bp DNA marker
Lane 2 : G38-009 isolate
Lane 3 : G38-030 isolate
Lane 4 : G39-064 isolate
Lane 5 : G39-222 isolate
Lane 6 : G40-006 isolate
Lane 7 : G40-159 isolate
Lane 8 : G41-008 isolate
Lane 9 : G41-012 isolate
Lane 10 : G41-024 isolate
Lane 11 : G41-046 isolate
Lane 12 : G42-041 isolate
Lane 13 : G42-054 isolate
Lane 14 : G42-062 isolate
Lane 15 : G43-005 isolate
Lane 16 : G43-041 isolate
Lane 17 : G43-064 isolate

### 3.2.2. Determination of the *emm* gene from GCS isolates

In non-invasive GCS groups, only 21 out of 40 isolates can be amplified for *emm* gene (Figure 11). In invasive GCS groups, only 3 out of 12 isolates can be amplified (Figure 12). By running with electrophoresis in 1.5% agarose gel, the PCR product is approximately 1-1.5 bp as expected.

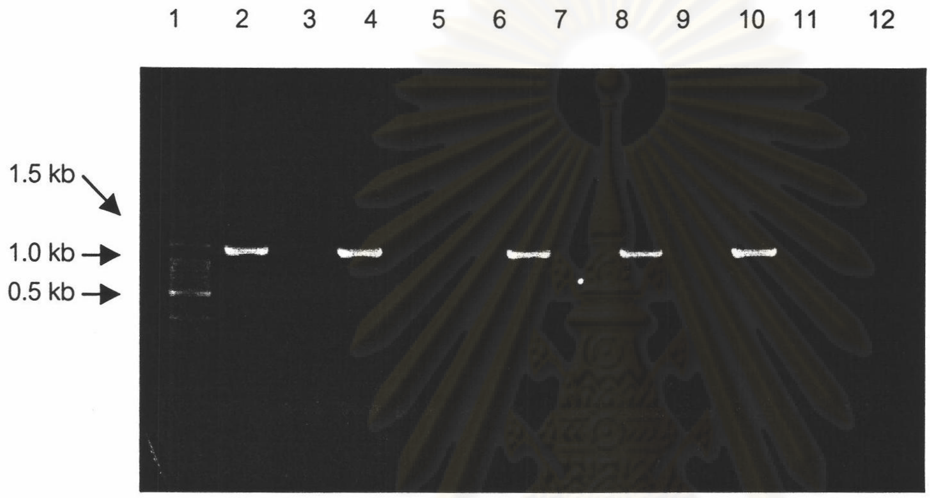

Figure 11: A representative picture of *emm* gene amplification of non-invasive GCS isolates

Lane 1 : 100 bp DNA marker
Lane 2 : C38-004 isolate
Lane 3 : C38-006 isolate
Lane 4 : C38-007 isolate
Lane 5 : C38-009 isolate
Lane 6 : C41-004 isolate
Lane 7 : C42-001 isolate
Lane 8 : C42-005 isolate
Lane 9 : C42-007 isolate
Lane 10 : C43-001 isolate
Lane 11 : C43-004 isolate
Lane 12 : C43-007 isolate

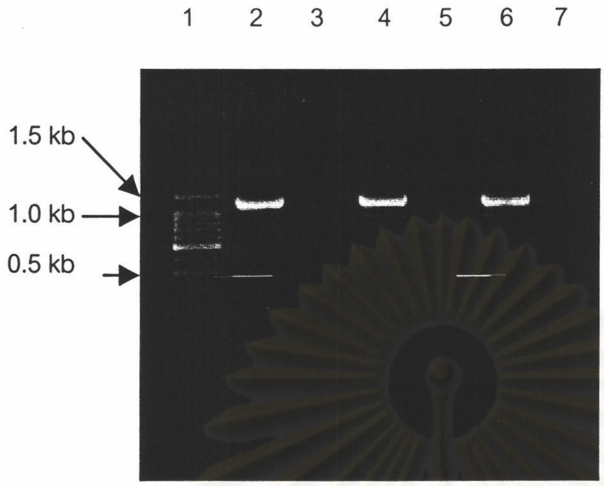

Figure 12: A representative picture of *emm* gene amplification of invasive GCS isolates

Lane 1 : 100 bp DNA marker

Lane 2 : C38-014 isolate

Lane 3 : C38-013 isolate

Lane 4 : C38-015 isolate

Lane 5 : C41-002 isolate

Lane 6 : C41-003 isolate

Lane 7 : C43-003 isolate

### 3.3. Non amplified *emm* gene products from GCS isolates

From 40 non-invasive GCS isolates, *emm* gene from 21 isolates can be amplified. From 12 invasive GCS isolates, there are only 3 isolates that can be amplified by primer 1 and primer 2. In summaries, primer 1 and primer 2 can not amplify 19 non-invasive and 9 invasive isolates. The species of those that cannot be amplified were shown in table 10 and table 11. In non-invasive groups, 12 isolates *were S. dysgalactiae subsp. equisimilis*, 5 isolates were *S. constellatus* and 2 isolates *were S. intermedius.* In invasive groups, 8 isolates were *S. dysgalactiae subsp. equisimilis* and 1 isolate was *S. constellatus.* In conclusion, there was no obvious correlation between species and *emm* amplification. Another possibility is that *emm* gene of those isolate may have polymorphism at primer site. Using different set of primers proved this problem. MF2 (GGATCCATAA GGAGCATAAA AATGGCTA) and MR1 (TGATAGCTTA GTTTTCTTCT TTGCGTTTT) from publications by A. Podbielski and colleagues were used in this study. However, MF2 and MR1 can not amplify any of the 19 non-invasive and 9 invasive GCS isolates (data not shown). Last hypothesis, which is the most likely one, is that those isolates may not have *emm* gene, which have to be proved by hybridization using conserved *emm* sequence. However, we did not performed hybridization in this study.

Table 10: The *Streptococcus* species of 19 non-invasive GCS isolates, which are non-amplified for *emm* gene

| ID of clinical isolate | *Streptococcus* species |
|---|---|
| C38-001 | *S. constellatus* |
| C38-002 | *S. dys. equisimilis* |
| C38-003 | *S. dys. equisimilis* |
| C38-006 | *S. dys. equisimilis* |
| C38-008 | *S. intermedius* |
| C38-009 | *S. dys. equisimilis* |
| C38-010 | *S. dys. equisimilis* |
| C41-004 | *S. dys. equisimilis* |
| C41-006 | *S. dys. equisimilis* |
| C42-002 | *S. constellatus* |
| C42-005 | *S. dys. equisimilis* |
| C42-006 | *S. dys. equisimilis* |
| C42-008 | *S. dys. equisimilis* |
| C43-001 | *S. dys. equisimilis* |
| C43-007 | *S. constellatus* |
| C43-008 | *S. dys. equisimilis* |
| C43-014 | *S. constellatus* |
| C43-015 | *S. constellatus* |
| C43-018 | *S. intermedius* |

Table 11: The *Streptococcus* species of 9 invasive GCS isolates, which are non-amplified for *emm* gene

| ID of clinical isolate | *Streptococcus* species |
|---|---|
| C38-011 | *S. dys. equisimilis* |
| C38-012 | *S. dys. equisimilis* |
| C38-013 | *S. dys. equisimilis* |
| C38-016 | *S. dys. equisimilis* |
| C38-017 | *S. dys. equisimilis* |
| C38-018 | *S. dys. equisimilis* |
| C41-002 | *S. constellatus* |
| C42-009 | *S. dys. equisimilis* |
| C43-003 | *S. dys. equisimilis* |

## 4. *Emm* sequence types

Amplified *emm* gene products were sequenced by automated sequencer (ABI 310) using Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Then sequencing results were compared to the collection of *emm* gene previously described in the GenBank and CDC database.

### 4.1. *Emm* sequence type from GGS isolates

#### 4.1.1. Non-invasive GGS isolates

From 30 isolates, there are 15 different *emm* types. Nine types from 18 isolates have been previously described; namely, *STC5345, H46A, STG6, STG485, STC839, STC6979, STG653, STC74A*, and *STG840*. One type from 3 isolates (G41-050, G41-010, and G42-089) is identified in this study as *emm* type *STC5345.1 variant* (accession number = AF485843), which is 96% homology to *STC5345*. Five types from 9 isolates are identified as novel *emm* types. The first type named *NSG62647* found in 3 isolates (G38-014, G39-218, and G42-089) has 85% homology to *STG62647* (accession number = AF485839). The second type named *NSG10* found in 2 isolates (G42-043 and G42-090) has 70% homology to *STG10* (accession number = AF485837). The third type named *NSG480.1* found in 2 isolates (G41-035 and G42-090) has 32% homology to *STG480.1* (accession number = AF485845). The forth type named *NSGemm3.1* found in 1 isolate (G41-025) has 87% homology to *emm3.1* (accession number = AF485846). The last type named *NSG1969.1* found in 1 isolate (G42-068) has 89% homology to *ST1969* (accession number = AF485859) (Table 12).

In this group, the common *emm* types that were identified in more than 1 isolates include *STC5345* (5 isolates, 16.6%), *H46A* (3 isolates, 10%), *STC5345.1 variant* (3 isolates, 10%), *NSG62647* (3 isolates, 10%), *STG6* (2 isolates, 6.6%), *STG485* (2 isolates, 6.6%), *STC839* (2 isolates, 6.6%), *NSG10* (2 isolates, 6.6%), and *NSG480.1* (2 isolates, 6.6%).

Table 12: *Emm* types from non-invasive GGS isolates

| Previously identified | | *Emm* variant type | | Novel *emm* type | |
|---|---|---|---|---|---|
| *Emm* type | No. of isolates | *Emm* type | No. of isolates | *Emm* type | No. of isolates |
| *STC5345* | 5 | *STC5345.1 variant* | 3 | *NSG62647* | 3 |
| *H46A* | 3 | | | *NSG10* | 2 |
| *STG6* | 2 | | | *NSG480.1* | 2 |
| *STG485* | 2 | | | *NSGemm3.1* | 1 |
| *STC839* | 2 | | | *NSG1969.1* | 1 |
| *STC6979* | 1 | | | | |
| *STC653* | 1 | | | | |
| *STC74A* | 1 | | | | |
| *STG840* | 1 | | | | |

### 4.1.2. Invasive GGS isolates

From 30 isolates, there are 22 different *emm* types. Ten types from 15 isolates have been previously described, 6 types from 8 isolates are *emm variant* types and 6 types from 7 isolates are novel *emm* types. Ten types that are similar to previously described *emm* sequence types are *STC5345, H46A, STG6, STG485, STC839, STC6979, STG653, STC1376, STG4831, and NSRT17*. Six types from 8 isolates are *emm variant* types. The first type named *STC5345.1 variant* found in 2 isolates (G41-024 and G40-159) has 96% homology to *STC5345* (accession number = AF485853). The second type named *STG166B.1 variant* found in 2 isolate (G38-009 and G43-041) has 99% homology to *STG166B* (accession number = AF485851). The third type named *STG166B.2 variant* found in 1 isolate (G38-032) has 97% homology to *STG166B* (accession number = AF485852). The forth type named *STG643 variant* found in 1 isolate (G42-041) has 99% homology to *STG643* (accession number = AF485853). The fifth type named *emm23 variant* found in 1 isolate (G38-030) has 95% homology to *emm23* (accession number = AF485848). The last type named *STC5345.2* *variant* found in 1 isolate (G42-062) has 95% homology to *STC5345* (accession number = AF485857).

Six types from 7 isolates are novel *emm* types. The first type named *NSG62647* found in 1 isolate (G39-064) has 85% homology to *STG62647* (accession number = AF485842). The second type named *NSG1969.2* found in 1 isolate (G40-182) has 90% homology to *ST1969* (accession number = AF485847). The third type named *NSGemm100.1* found in 1 isolate (G41-008) has 89% homology to *emm100.1* (accession number = AF485849). The forth type named *NSG5345* found in 1 isolate (G40-186) has 94% homology to *STC5345* (accession number = AF485856). The fifth type named *NSG1741* found in 1 isolate (G41-021) has 64% homology to *STC1741* (accession number = AF485850). The last type named *NSG93464* found in 2 isolate (G43-005 and G43-036) has 49% homology to *STG93464* (accession number = AF516905) (Table 13).

In this group, the common *emm* types that were identified include *STC6979* (6 isolates, 20%), *STC5345.1 variant* (2 isolates, 6.6%), *STG166B.1 variant* (2 isolates, 6.6%), and *NSG93464* (2 isolates, 6.6%).

Table 13: *Emm* type from invasive GGS isolates

| Previously identified | | *Emm* variant type | | Novel *emm* type | |
|---|---|---|---|---|---|
| *Emm* type | No. of isolates | *Emm* type | No. of isolates | *Emm* type | No. of isolates |
| *STC6979* | 6 | *STC5345.1 variant* | 2 | *NSG93464* | 2 |
| *STG6* | 1 | *STG166B.1 variant* | 2 | *NSG62647* | 1 |
| *STC5345* | 1 | *STG166B.2 variant* | 1 | *NSG1969.2* | 1 |
| *STG485* | 1 | *STG643 variant* | 1 | *NSGemm100.1* | 1 |
| *STC839* | 1 | *Emm23 variant* | 1 | *NSG5345* | 1 |
| *H46A* | 1 | *STC5345.2 variant* | 1 | *NSG1741* | 1 |
| *STG653* | 1 | | | | |
| *STC1376* | 1 | | | | |
| *STG4831* | 1 | | | | |
| *NSRT17* | 1 | | | | |

### 4.1.3. Summary of *emm variant* types of GGS isolates

The summaries of *emm variant* types identified from GGS in this study were shown in table 14. There are 6 types from 11 isolates. *STC5345.1 variant* types were mostly found with 3 isolates from non-invasive and 2 isolates from invasive groups. *STG166B.1 variant* was mostly found with 2 isolates from invasive groups. *STG166B.2, STG643, STC5345.2, and emm23 variant* types were each only found in 1 isolates from invasive groups. The sequence alignments of *emm variant* types were shown in figure 13 A-F.

Table 14: *Emm variant* types of GGS isolates

| *Emm* type | ID of clinical isolates | Most homology To *emm* type | % homology | Number of isolates | | Accession number |
|---|---|---|---|---|---|---|
| | | | | Non-invasive | Invasive | |
| *STC5345.1 variant* | G40-159,<br>G41-010,<br>G41-024,<br>G41-050,<br>G42-089 | *STC5345* | 96 | 3 | 2 | AF485843 |
| *STG166B.1 variant* | G38-009,<br>G43-041 | *STG166B* | 99 | - | 2 | AF485851 |
| *STG166B.2 variant* | G38-032 | *STG166B* | 96 | - | 1 | AF485852 |
| *STG643 variant* | G42-041 | *STG643* | 99 | - | 1 | AF485853 |
| *STC5345.2 variant* | G42-062 | *STC5345* | 95 | - | 1 | AF485857 |
| *Emm23 variant* | G38-030 | *Emm23* | 95 | - | 1 | AF485848 |

A: *Emm* sequence alignment of *STG643 variant* compare to *STG643*

(99% homology, Accession number = AF485853)

Base differences are underlined.

| | |
|---|---|
| *STG643* | TTAGGGGCAGGATTAGCGGTTAACCAAACAGAAGTTAAGGCGGAGAG |
| *STG643 variant* | TTAGGGGCAGGATTAGCGGTTAACCAAACAGAAGTTAAGGCGGAGAG |
| *STG643* | TGTTGATATTGAGAAAATCAGAGAAGAAGCCTTAAAAGAAGTTATTGGC |
| *STG643 variant* | TGTTGATATTGAGAAAATCAGAGAAGAAGCCTTAAAAGAAGTTATTGAC |
| *STG643* | AGAATGGACTATGGTCAACTAAGTAATACTTTAGCTGGATCATTCCGTG |
| *STG643 variant* | AGAATGGACTATGGTCAACTAAGTAATACTTTAGCTGGATCATTCCGTG |
| *STG643* | AGAATAGTGCGTTAAAAGAAACCAT |
| *STG643 variant* | AGAATAGTGCGTTAAAAGAAACCAT |

B: *Emm* sequence alignment of *STG166B.1 variant* compare to *STG166B*

(99% homology, Accession number = AF485851)

Base differences are underlined.

| | |
|---|---|
| *STG166B* | GTAGGAGCAGGGTTTGCGAATCAAACAGAGGTTAAGGCTGATAGATATAC |
| *STG166B.1 variant* | TTAGGGGCAGGGTTTGCGAATCAAACAGAGGTTAAGGCTGATAGATATAC |
| *STG166B* | CGAAGCTAAAAATGAAGTACTAGATAATAACTATGTACCGCTTAGAAGGTG |
| *STG166B.1 variant* | CGAAGCTAAAAATGAAGTACTAGATAATAACTATGTACCGCTTAGAAGGTG |
| *STG166B* | GTATTCGACTTATATTGAAAAAGAACGTCTTCGTAAGGAAAATGAACAACTT |
| *STG166B.1 variant* | GTATTCGACTTATATTGAAAAAGAACGTCTTCGTAAGGAAAATGAACAACTT |
| *STG166B* | GACGAACTAGTTCGTAA |
| *STG166B.1 variant* | GACGAACTAGTTCGTAA |

C: *Emm* sequence alignment of *STG166B.2 variant* compare to *STG166B*

(96% homology, Accession number = AF485852)

Base differences are underlined.

| | |
|---|---|
| *STG166B* | GTAGGAGCAGGGTTTGCGAATCAAACAGAGGTTAAGGCTGATAGATATAC |
| *STG166B.2 variant* | TTAGGGGCAGGGTTTGCGAATCAAACAGAGGTTAAGGCTGATAGATATAC |
| *STG166B* | CGAAGCTAAAAATGAAGTACTAGATAATAACTATGTACCGCTTAGAAGGTG |
| *STG166B.2 variant* | CGAAGCTAAAAATGAAGTACTAGATAATAACTATGTACCGCTTAGAAGGTG |
| *STG166B* | GTATTCGACTTATATTGAAAAAGAACGTCTTCGTAAGGAAAATGAACAACTT |
| *STG166B.2 variant* | GTATTCGACTTATATTGAAAAAGAACGTCTTCGTAAGGAAAATGAACAACTT |
| *STG166B* | GACGAACTAGTTCGTAA |
| *STG166B.2 variant* | GACGAACTAGTCAGAAT |

D: *Emm* sequence alignment of *STC5345.1 variant* compare to *STC5345*

(96% homology, Accession number = AF485843)

Base differences are underlined.

| | |
|---|---|
| *STC5345* | TTAGGGGCAGGAT TAGCAAGCGGGCAGATAGTAAAAGCAGATAGTAA |
| *STC5345.1 variant* | TTAGGGGCAGGGCTAGTAGCAGGGCAGACAGTAAAAGCAGATAGTAA |
| *STC5345* | CGATGCCGCTATTGTAGTACAGCCCCAATTTATTGAGAAAGAGATAGC |
| *STC5345.1 variant* | CGATGCCGCTATTGTAGTACAGCCCCAATTTATTGAGAAAGAGATAGC |
| *STC5345* | AGGTCTCAACAATAAAATTCAAAAATTAGAAAAAGAAAACGACTTACTTA |
| *STC5345.1 variant* | AGGTCTCAACAATAAAATTCAAAAATTAGAAAAAGAAAACGACTTACTTA |
| *STC5345* | ATGATTCACTTCTTAAAACTGAACA |
| *STC5345.1 variant* | ATGATTCACTTCTTAAAACTGAACA |

E: *Emm* sequence alignment of *emm23 variant* compare to *emm23*

(95% homology, Accession number = AF485848)

Base differences are underlined.

| | |
|---|---|
| *Emm23* | TTAGGGACAGGAT TAGCAAGCCAAACAGAAGTTAAGGCGGATGGGGA |
| *Emm23 variant* | TTAGGGGCAGGACTAGCAAGCCAAACAGAAGTTAAGGCGGATGGGGA |
| *Emm23* | AGCTAGGGACGTTGTTCCTGAACTTGTTGCAAACAATCT TGGCTTACTG |
| *Emm23 variant* | AT CTAGGGACGTTGTTTCTGAACTTGTTGCAAACAATCG TGACTTACTG |
| *Emm23* | AGGAAAAGAGTAGCGAGACTTCAAG CAGAGTTAAAAACTAAAGAAGAA |
| *Emm23 variant* | AAGAAAAGAGTAGCGAGACTTCAAA CAGAGTTAAAAACTAAAGAAGAA |
| *Emm23* | AAGTTACGTAAACTGGACTTAGCTCT |
| *Emm23 variant* | AAGTTACGTGAACTGGACTTAGCTCT |

F: *Emm* sequence alignment of *STC5345.2 variant* compare to *STC5345*

(95% homology, Accession number = AF485857)

Base differences are underlined.

| | |
|---|---|
| *STC5345* | TTAGGGGCAGGAT TAGCAAGCGGGCAGAT ----- AGTAAAAGCAGA |
| *STC5345.2 variant* | TTAGGGGCAGGGCTAGTAGCA GGGCAGGGCAGACAGTAAAAGCAGA |
| *STC5345* | TAGTAACGATGCCGCTATTGTAGTACAGCCCCAATTTATTGAGAAAGAG |
| *STC5345.2 variant* | TAGTAACGATGCCGCTATTGTAGTACAGCCCCAATTTATTGAGAAAGAG |
| *STC5345* | ATAGCAGGTCTCAACAATAAAATTCAAAAATTAGAAAAAGAAAACGACTT |
| *STC5345.2 variant* | ATAGCAGGTCTCAACAATAAAATTCAAAAATTAGAAAAAGAAAACGACTT |
| *STC5345* | ACTTAATGATTCACTTCTTAAAACT |
| *STC5345.2 variant* | ACTTAATGATTCACTTCTTAAAACT |

Figure 13 A-F: The nucleotide alignment of *emm* variant types of GGS isolates in this study. (A) *STG643 variant* found in G42-041 isolate compare to *STG643*. There is 1 base pair point mutation (99% homology). (B) *STG166B.1 variant* found in G38-009 isolate compare to *STG166B*. There are 2 base pair point mutation (99% homology). (C) *STG166B.2 variant* found in G39-032 isolate compare to *STG166B*. There are 6 base pair point mutation (96% homology). (D) *STC5345.1 variants* found in G40-159, G41-010, G41-024, G41-050, and G42-089 isolates compares to *STC5345*. There are 7 base pair point mutation (96% homology). (E) *Emm23 variant* found in G38-030 isolate compare to *emm23*. There are 9 base pair point mutation (95% homology). (F) *STC5345.2 variant* found in G42-062 isolate compare to *STC5345*. There are 8 base pair point mutation and 1 base pair insertion (95% homology). Base differences are underlined.

### 4.1.4. Summary of novel *emm* types of GGS isolates

The summaries of novel *emm* types identified from GGS isolates in this study were shown in table 15. There are 9 types from 14 isolates. *NSG62647* novel types were mostly found with 3 isolates from non-invasive and 1 isolate from invasive groups. *NSG10* were found with 2 isolates from non-invasive groups. *NSG480.1* were found with 2 isolates from non-invasive groups. *NSG93464* were found with 2 isolates from invasive groups. *NSGemm3.1*, *NSG1969.1* novel types were each only found in 1 isolate from non-invasive groups. *NSG1969.2*, *NSGemm100.1*, *NSG5345*, and *NSG1741* novel types were each only found in 1 isolate from invasive groups. The sequence alignments of novel *emm* types were shown in figure 14 A-I.

Table 15: Novel *emm* types of GGS isolates

| *Emm* type | ID of clinical isolates | Most homology to *emm* type | % homology | Number of isolates | | Accession number |
|---|---|---|---|---|---|---|
| | | | | Non-invasive | Invasive | |
| *NSG62647* | G38-014,<br>G39-064,<br>G39-218,<br>G40-161 | *STG62647* | 85 | 3 | 1 | AF485839 |
| *NSG10* | G42-043,<br>G42-090 | *STG10* | 70 | 2 | - | AF485837 |
| *NSG480.1* | G41-002,<br>G41-035 | *STG480.1* | 32 | 2 | - | AF485845 |
| *NSGemm3.1* | G41-025 | *Emm3.1* | 87 | 1 | - | AF485846 |
| *NSG1969.1* | G42-068 | *ST1969* | 89 | 1 | - | AF485859 |
| *NSG93464* | G43-005,<br>G43-036 | *STG93464* | 49 | - | 2 | AF516905 |
| *NSG1969.2* | G40-182 | *ST1969* | 90 | - | 1 | AF485847 |
| *NSGemm100.1* | G41-008 | *Emm100.1* | 89 | - | 1 | AF485849 |
| *NSG5345* | G40-186 | *STC5345* | 94 | - | 1 | AF485856 |
| *NSG1741* | G41-021 | *STC1741* | 64 | - | 1 | AF485850 |

A: *Emm* sequence alignment of *NSG5345* compare to *STC5345*

(94% homology, Accession number = AF485856)

Base differences are underlined.

*STC5345* TTAGGGGCAGGAT TAGCAAGC GGGCAGATAGTAAAAGCAGATAGTAACGATGC
*NSG5345* TTAGGGGCAGGGCTAGTAGCA GGGCAGACAGTAAAAGCAGATAGTAACGATGC

*STC5345* CGCTATT- GTAGTACAGCCCCAATTTATT - - GAGAAAGAGATAGCAGGTCTCAAC
*NSG5345* CGCTATTAGTAGTACAGCCCCAATTTACTTAGAGAAAGAGATAGCAGGTCTCAAC

*STC5345* AATAAAATTCAAAAATTAGAAAAAGAAAACGACTTACTTAATGATTCACTTCTTAAA
*NSG5345* AATAAAATTCAAAAATTAGAAAAAGAAAACGACTTACTTAATGATTCACTTCTTAAA

*STC5345* ACTGA
*NSG5345* ACTGA

B: *Emm* sequence alignment of *NSG1969.2* compare to *ST1969*

(90% homology, Accession number = AF485847)

Base differences are underlined.

*ST1969* TTAGGGGCAGGTTTTACGAATCAAACAGAGGTTAAAGCTAACGAGAATGGTAGT
*NSG1969.2* TTAGGGGCAGGATTTACGAATCAAACAGAGGTTAAAGCTAACGAGAATGGTAGT

*ST1969* CCTAGGGAAGTTATAGAAGATCTTGCAGCAAACAATCCCAAAGTACAAAATATAC
*NSG1969.2* CCTAGGGAAGTTATAGAAGAACTTGCAGCAAAAAATCCCGTAA TACAAAATATAC

*ST1969* GTTTACGTCACGAAAACGAGAACTTAAAAGCGAGATTAGAGAATGCAATGAAT G
*NSG1969.2* GTTTACGTTCCGAAAACCAGAAATTAAAAGAGAGTCTAGAGAATGCAATGGACG

*ST1969* TTGCGGA
*NSG1969.2* TTGCAGG

C: *Emm* sequence alignment of *NSGemm100.1* compare to *emm100.1*

(89% homology, Accession number = AF485849)

Base differences are underlined.

*Emm100.1* GTAGCAGGGCAGACAGTTAATGCTGACTCGCTAGCTAATGTAGGAGAAGATTTAT
*NSGemm100.1* GTAGCAGGGCAGACAGTTAATGCTGACTCGCTAGCTAATGTAGGAGAAGATTTAT

*Emm100.1* TAGCAGAAGTTAAGGAAATTACTGACGCAAATAAGATTCTGGAAGACGCTCTAAA
*NSGemm100.1* TAGCAGAAGTTAAGGAAATTACTGACGCAAATAAGATTCTGGAAGACGCTCTAAA

*Emm100.1* TAGGACTATAGAAGAGAAAATCAAGTCAGATGCAAATAAGACTCTGAAACAAGCT
*NSGemm100.1* TAGGACTATAGAAGAGAAAATCAAGTCAGATGCTGACTACAAAAAACAAATT GGT

*Emm100.1* CT AGA
*NSGemm100.1* GAAGT

D: *Emm* sequence alignment of *NSG1969.1* compare to *ST1969*

(89% homology, Accession number = AF485859)

Base differences are underlined.

*ST1969* TTAGGGGCAGGT TTTACGAATCAAACAGAGGTTAAAGCTAACGAGAATGGTAGT
*NSG1969.1* TTAGGGGCAGGA TTTACGAATCAAACAGAGGTTAAAGCTAACGAGAATGGTAGT

*ST1969* CCTAGGGAAGTTATAGAAGATCTTGCAGCAAACAATCCCAAAGTACAAAATATAC
*NSG1969.1* CCTAGGGAAGTTATAGAAGAACTTGCAGCAAAAATGCCCGTAATACAAAATATAC

*ST1969* - GTTTACGTCACGAAAACGAGAACTTAAAAGCGAGATTAGAGAATGCAATG AAT
*NSG1969.1* AGTTTACGTTCCGAAAACCAGAAATTAAAAGA GAGTCTAGAGAATGCAATGGAC

*ST1969* GTTGCGG
*NSG1969.1* GTTGCAG

E: *Emm* sequence alignment of *NSGemm3.1* compare to *emm3.1*

(87% homology, Accession number = AF485846)

Base differences are underlined.

*Emm3.1* TTAGGGACAGGA CTGGTAGCAGGGCAGACAGTAAAGGCAGATGCTAGGAGTGT
*NSGemm3.1* TTAGGGGCAGGGCTAGTAGCA GGGCAGACAGTAAAGGCAGATGTTGGTA ATGT

*Emm3.1* TAATGGAGAGTTTCC TAGACATGTTAAATTAAAAAATGAAATTGAG AACTTGTTAG
*NSGemm3.1* TAA TGGAGAGTATCATAGACATACTAAATTAAAAAGTGAAATTGAGGACTTGTTAG

*Emm3.1* ATCAGGTTACACAATTATATACTAAACATAAT AGTAATTACCAACA ATATAATGCA
*NSGemm3.1* ATCAGGTTACAGAATTATATAGTACACATAATCA TAATTACCAACGATATGATT CA

*Emm3.1* CAAGC
*NSGemm3.1* GAAGC

F: *Emm* sequence alignment of *NSG62647* compare to *STG62647*

(85% homology, Accession number = AF485839)

Base differences are underlined.

*STG62647* TTAGGGGCAGGACTAGCAAGCCAAACTGAAGTTAAGGCGGAAGAGAATGAGAG
*NSG62647* TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGCGGAAGAGAATGAGAG

*STG62647* ATTAAGACAAGCCAAAGAACAAGCTTTACAAGAGGTTATTGACCAAAT GCCCTA
*NSG62647* ATTAAGACAAGCCAAAGAACAAGCTTTACAAGAGGTTCTTAGAAACACGCCCTA

*STG62647* TGATCATCTAAAAAAT ACTTTAGCTGGGGCATTCCGTAAGAATCAGGAAAATGAA
*NSG62647* TGATGACCTAAAAAATGCTTACGCTGGAGCATTCCGTAAGAATGATGAATTA GAA

*STG62647* CGAGATTT
*NSG62647* AAAACCTA

G: *Emm* sequence alignment of *NSG10* compare to *STG10*

(70% homology, Accession number = AF485837)

Base differences are underlined.

*STG10* TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGC TGAGGGGAAGTTCAA
*NSG10* TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGC GGAGGGGAGTA–CAA

*STG10* CTCAACTCTACGATAACTGGAACTGGAATAACAATTG AA -TAGAACTT CTGAGC
*NSG10* CTCAACACTACGATAACTATCTAAA GGAAGCAGAAGAGGCTAGAACTGCTAA AC

*STG10* TAGGTAAT AAA TTACGAAAT CTAGAAACAAGC GTTTCTAACCTTGA AGATT TGA
*NSG10* TAAT TGATGGGTTGGCAAAGCTAGATGCAGAA GTTTCTAACCTTGA GCAGCTGA

*STG10* TGAACAGTCT
*NSG10* TGGACCAACT

H: *Emm* sequence alignment of *NSG1741* compare to *STC1741*

(64% homology, Accession number = AF485850)

Base differences are underlined.

*STC1741* TTAGGGGCAGGATTAGCAAGCCAAACTGAAGTTAAGGCGGAGATGATTACTGA
*NSG1741* TTAGGGGCAGGATTAGCAAGCCAAACTGAAGTTAAGGCGGAGAT - - - TAGTGC

*STC1741* AGCTCAATACAAACGAAAGTTG GCAGAAGAGGAGGATAGAGCTCTTGAACTAG
*NSG1741* AGCTCAATACAAACAAAA GGAAGCAGAAGATAGA GCTTCTAAA CTAGAACGTA

*STC1741* AACGTAAATT GTACAAAAT ATCATCAGATGTTGATCACCTTGAAATGTTG ATGA
*NSG1741* AATTGCACCAGCTATCATCAGAATTTAC TCATCTTGAAA TGTTGATGGAAGATA

*STC1741* AAAATATT CA
*NSG1741* TTCAGAAAGG

I: *Emm* sequence alignment of *NSG93464* compare to *STG93464*

(49% homology, Accession number = AF516905)

Base differences are underlined.

*STG93464* GTAGCGGTTGCTCTAACAGTTGTAGGAGCAGGGTTAGTAGCAGGGCAGACAG
*NSG93464* GTAGCGGTTGGTCTAACAGTTGTAGGAGCAGGGTTAGTCGCAGGGCAGACAG

*STG93464* CTAAGGCAGACAGCATGGAAGTTAGTGGGCAAAGCATAGAGGTAACACATAT A
*NSG93464* TTAA TGCTGAC TCGCTAGCTAA TGTACGGAGAAGAGTTATTAGC AGAAGCTAA

*STG93464* AA ACCAGAAGAAGGTCTAACTGTGGTAACTGACGAT TCTGATATCT T AAAAGAA
*NSG93464* GGAAATT ACTGACGCA TACAAGATTCTGGAAGACGCTCTAAATAGGGACTTTCG

*STG93464* AAATTA A GCAAG
*NSG93464* CAAGAGA CATCC

J: *Emm* sequence alignment of *NSG480.1* compare to *STG480.1*

(32% homology, Accession number = AF485845)

Base differences are underlined.

*STG480.1* TTGACAGTCGT AGGAGCAGGATTAGTAGCAGGGCAGACAGTAAAGGCCAATAA
*NSG480.1* TTAGGAGCAGGATT AGTA ACAAATACTACT GT GAACGCAAATTC AT CTAATAA

*STG480.1* TAGTGAAGAT ATT ACTTCGA TGATGCCTAT TTTGAGTGGTGTTGGTTCAAGTAAT
*NSG480.1* CGAAAACTT TCGAAAAGAAGCAGGTAGATATCAAGATAT TCTTAGTAAACTTAGG

*STG480.1* GCAGTTGATTCTAAC T TTGCTGCGGATCAACTTGC TAAGAGAATGAATGAT CCG
*NSG480.1* GGCT TTAGTTCCCGTTCC CAAGACCTAAT GAGACGTTAT AGTTCT ATTGAGAAT

*STG480.1* AAAGCGAT
*NSG480.1* AGACCCGA

Figure 14 A-J: The nucleotide alignment of novel *emm* types of GGS isolates in this study. (A) *NSG5345* found in G40-186 isolate compare to *STC5345.* There are 8 base pair point mutation and 2 base pair insertion (85% homology). (B) *NSG1969.2* found in G40-182 isolate compare to *ST1969.* There are 17 base pair point mutation (90% homology). (C) *NSGemm100.1* found in G41-008 isolate compare to *emm100.1.* There are 18 base pair point mutation (89% homology). (D) *NSG1969.1* found in G42-068 isolate compare to *ST1969.* There are 18 base pair point mutation and 1 base pair insertion (89% homology). (E) *NSGemm3.1* found in G41-025 isolate compare to *emm3.1.* There are 22 base pair point mutation (87% homology). (F) *NSG62647* found in G38-014, G39-064, G39-218, and G40-161 isolates compares to *STG62647.* There are 26 base pair point mutation (85% homology). (G) *NSG10* found in G42-043 and G42-090 isolates compares to *STG10.* There are 49 base pair point mutation, 1 base pair deletion and 1 base pair insertion (70% homology). (H) *NSG1741* found in G41-021 isolate compare to *STC1741.* There are 62 base pair point mutation and 1 base pair deletion (64% homology). (I) *NSG93464* found in G43-005 and G43-036 isolates compare to *STG93464.* There are 86 base pair point mutation (49% homology). (J) *NSG480.1* found in G41-035 and G41-002 isolates compares to *STG480.1.* There are 116 base pair point mutation (32% homology). Base differences are underlined.

### 4.1.5. Comparison of *emm* types between non-invasive and invasive groups from GGS isolates

The comparisons of *emm* type from invasive and non-invasive GGS isolates were shown in table 16. The frequencies of *emm* types that were found in more than 1 isolates compared between invasive and non-invasive GGS isolates in this study were shown in figure 15. *STC6979* (6 isolates) was found more frequently in invasive groups. *STC5345* (5 isolates) was found more frequently in non-invasive GGS. However, the difference is not statistically significance by chi-square analysis. Twenty-two different *emm* types were found in invasive GGS isolates and 15 different *emm* types were found in non-invasive GGS isolates. In invasive GGS isolates can be found the different *emm* type more than in non-invasive GGS isolates. Some *emm* types can found only invasive GGS isolates. For example, *STC1376, STG4831, NSRT17, STG643 variant, STG166B.1 variant, STG166B.2 variant, emm23 variant, STC5345.2 variant, NSG1969.2, NSG5345, NSGemm100.1, NSG1741,* and *NSG93464.* Some *emm* types can be found only non-invasive GGS isolates. For example, *NSG10, NSG480.1, STC74A, STG840, NSGemm3.1,* and *NSG1969.1.*

Table 16: The frequencies of *emm* type from invasive and non-invasive, which found in this study

| No. | *Emm* type | Number of isolates | |
|---|---|---|---|
| | | Invasive (%) | Non-invasive (%) |
| 1 | *STC6979* | 6(20) | 1(3.3) |
| 2 | *STC5345* | 1(3.3) | 5(16.6) |
| 3 | *STC5345.1 variant* | 2(6.6) | 3(10) |
| 4 | *H46A* | 1(3.3) | 3(10) |
| 5 | *NSG62647* | 1(3.3) | 3(10) |
| 6 | *STG6* | 1(3.3) | 2(6.6) |
| 7 | *STG485* | 1(3.3) | 2(6.6) |
| 8 | *STC839* | 1(3.3) | 2(6.6) |
| 9 | *STG166B.1 variant* | 2(6.6) | - (-) |
| 10 | *NSG93464* | 2(6.6) | - (-) |
| 11 | *NSG10* | - (-) | 2(6.6) |
| 12 | *NSG480.1* | - (-) | 2(6.6) |
| 13 | *STG653* | 1(3.3) | 1(3.3) |
| 14 | *STC1376* | 1(3.3) | - (-) |
| 15 | *STG4831* | 1(3.3) | - (-) |
| 16 | *NSRT17* | 1(3.3) | - (-) |
| 17 | *STG643 variant* | 1(3.3) | - (-) |
| 18 | *STG166B.2 variant* | 1(3.3) | - (-) |
| 19 | *NSG1969.2* | 1(3.3) | - (-) |
| 20 | *emm23 variant* | 1(3.3) | - (-) |
| 21 | *NSG5345* | 1(3.3) | - (-) |
| 22 | *STC5345.2 variant* | 1(3.3) | - (-) |
| 23 | *NSGemm100.1* | 1(3.3) | - (-) |
| 24 | *NSG1741* | 1(3.3) | - (-) |
| 25 | *STC74A* | - (-) | 1(3.3) |
| 26 | *STG840* | - (-) | 1(3.3) |
| 27 | *NSGemm3.1* | - (-) | 1(3.3) |
| 28 | *NSG1969.1* | - (-) | 1(3.3) |

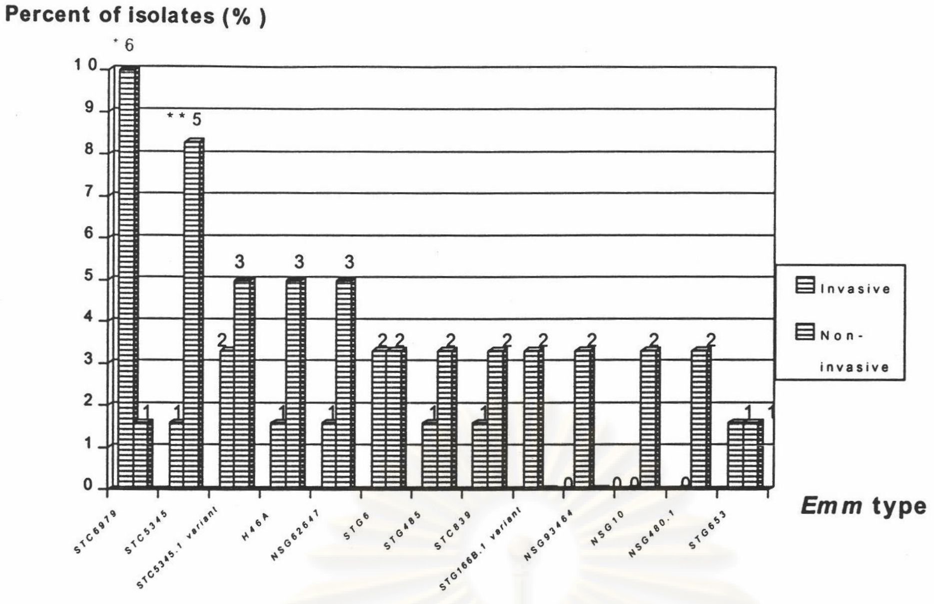

Figure 15: Frequencies of *emm* types that were found in more than 1 isolate compared between invasive and non-invasive GGS isolates

## 4.2. *Emm* sequence type from GCS isolates

### 4.2.1. Non-invasive GCS isolates

From 40 isolates, 21 isolates can be amplified. From 21 isolates, there are 9 different *emm* types. Four types from 14 isolates have been previously described and 5 types from 7 isolates are novel *emm* types (Table 17). Four types from 14 isolates have been previously described; namely, *ST245, STC74A, H46A*, and *STG4545*. Five types from 7 isolates are identified as novel *emm* types. The first type named *NSC401* found in 3 isolates (C38-005, C38-007, and C42-001) has 78% homology to *STCK401* (accession number = AF485831). The second type named *NSC11.1* found in 1 isolate (C41-007) has 48% homology to *STG11* (accession number = AF485828). The third type named *NSC11.2* found in 1 isolate (C42-007) has 46% homology to *STG11* (accession number = AF485829). The forth type named *NSC11.3* found in 1 isolate (C43-011) has 47% homology to *STG11* (accession number = AF485830). The last type named *NSC11.4* found in 1 isolate (C38-004) has 45% homology to *STG11* (accession number = AF485835) (Table 17).

In this group, the common *emm* types that were identified in more than 1 isolates include *ST245* (9 isolates, 42.9%), *NSC401* (3 isolates, 14.3%), *STC74A* (2 isolates, 9.5%), and *H46A* (2 isolates, 9.2%) (Figure 15).

Table 17: *Emm* type from non-invasive GCS isolates

| Previously identified | | Novel *emm* types | |
|---|---|---|---|
| *Emm* type | No. of isolates | *Emm* type | No. of isolates |
| *ST245* | 9 | *NSC401* | 3 |
| *STC74A* | 2 | *NSC11.1* | 1 |
| *H46A* | 2 | *NSC11.2* | 1 |
| *STG4545* | 1 | *NSC11.3* | 1 |
| | | *NSC11.4* | 1 |

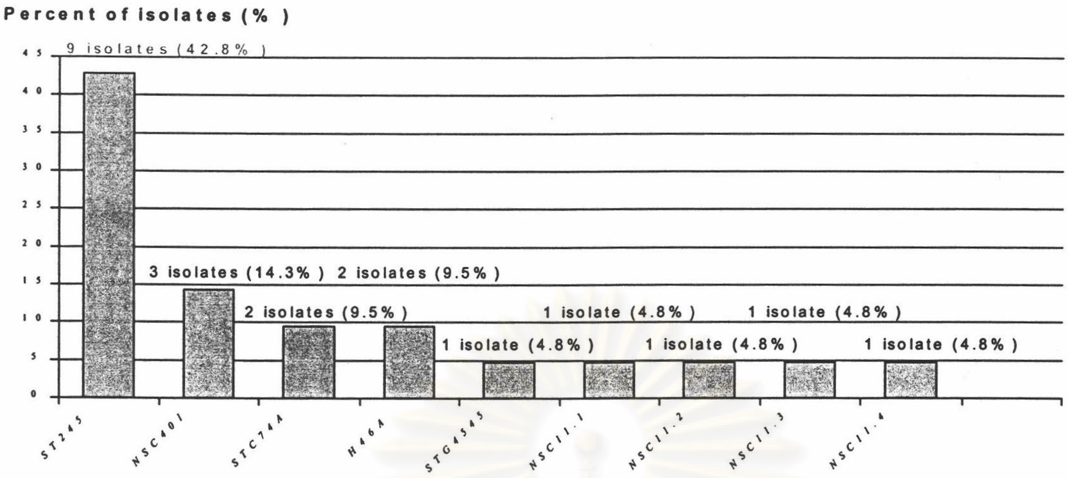

Figure 16: Frequencies of *emm* type in GCS non-invasive isolates

### 4.2.2. Invasive GCS isolates

From 12 isolates, only 3 isolates can be amplified. From 3 isolates, 3 different *emm* types were identified. *STC74A* found in 1 isolate (C38-015) from hemoculture. Two types from 2 isolates are novel *emm* types. The first type named *NSC103* found in 1 isolate (C38-014) from hemoculture has 41% homology to *STIL103* (accession number = AF485836). The second type named *NSC2764* found in 1 isolate (C41-003) from joint fluid has 40% homology to *STL2764* (accession number = AF485834) (Table 18).

Table 18: *Emm* types from invasive GCS isolates

| Previously identified | | Novel *emm* types | |
|---|---|---|---|
| *Emm* type | No. of isolates | *Emm* type | No. of isolates |
| *STC74A* | 1 | *NSC103* | 1 |
| | | *NSC2764* | 1 |

### 4.2.3. Summary of novel *emm* types of GCS isolates

The summaries of novel *emm* types identified from GCS isolates in this study were shown in table 19. There are 7 types form 9 isolates. *NSC401* novel types were mostly found with 3 isolates from non-invasive groups. *NSC11.1*, *NSC11.2*, *NSC11.3*, and *NSC11.4* novel types were each only found in 1 isolate from non-invasive groups. *NSC103 and NSC2764* novel types were each only found in 1 isolate from invasive groups. The sequence alignments of novel *emm* type were show in figure 17 A-G.

Table 19: Novel *emm* types of GCS isolates

| *Emm* type | ID of clinical isolates | Most homology to *emm* type | % homology | Number of isolates | | Accession number |
|---|---|---|---|---|---|---|
| | | | | Non-invasive | Invasive | |
| *NSC401* | C38-005, C38-007, C42-001 | *STCK401* | 78 | 3 | - | AF485831 |
| *NSC11.1* | C41-007 | *STG11* | 48 | 1 | - | AF485828 |
| *NSC11.2* | C42-007 | *STG11* | 46 | 1 | - | AF485829 |
| *NSC11.3* | C43-011 | *STG11* | 47 | 1 | - | AF485830 |
| *NSC11.4* | C38-004 | *STG11* | 45 | 1 | - | AF485835 |
| *NSC103* | C38-014 | *STIL103* | 44 | - | 1 | AF485836 |
| *NSC2764* | C41-003 | *STL2764* | 40 | - | 1 | AF485834 |

A: *Emm* sequence alignment of *NSC401* compare to *STCK401*

(78 % homology, Accession number = AF485831)

Base differences are underlined.

*STCK401* TTAGGGGCAGGTTTTGCGAATCAAACAGAGGTTAAGGCTCAAGAAAATACAGAT
*NSC401* TTAGGGGCAGGTTTTGCGAATCAAACAGAGGTTAAGGCTCAAGAAAATACTGAT

*STCK401* AAATATGACAGTTGGGTTAAAGATAGAGAAGAGGAACGTGCAGGTACTTTAAAT
*NSC401* CAATATGACAAATGGT TTAAAACGAGAGAAGAGGAACGTTT AAGTACCTTACTT

*STCK401* AATGATCTGAGACAGCTTGAAGGCAAAGTCAGAAATCTTAGAAGTATGATGCAT
*NSC401* TTAAGCATGAACCGGCTTGATAGCGAAGTCCAAAATCTTATTGA TATGATGGAT

*STCK401* GAACTTTG
*NSC401* AAACTTCA

B: *Emm* sequence alignment of *NSC11.1* compare to *STG11*

(48% homology, Accession number = AF485828)

Base differences are underlined.

*STG11* TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGCGGCGGAGAATACATA
*NSC11.1* TTAGGGGCAGGATTAGCGAATCAAGCAGAAGTTAAAGC CAAT GAGTATAACAA

*STG11* CGATAGATGGAAAGCACAAACAGAA GAGGCTAGAACTGATAA ACTAATTGCAG
*NSC11.1* CTGGTTA AAAACAAGCGAAGAAAAA AGA CACATAAT TT ACAACACAATTTAT C

*STG11* GGTTTGCAAACCTAGATGCAGACGTTACT AACCTTGGAAAGATGATGGACGAA
*NSC11.1* AAATCTTAAAGTGAGTGTTC GGCGGCTAGAAGATTAT ATGA ATGGACTT CTTA

*STG11* CTTC AGAAGC
*NSC11.1* GCCAGAAGAA

C: *Emm* sequence alignment of *NSC11.3* compare to *STG11*

(47% homology, Accession number = AF485830)

Base differences are underlined.

| | |
|---|---|
| *STG11* | TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGCGGCGGAGAATACATA |
| *NSC11.3* | TTAGGGGCAGGATTAGCGAATCAAGCAGAAGTTAAAGC CAAT GAGTATAACAA |
| | |
| *STG11* | CGATAGATGGAAAGCACAAACAGAAGAGGGCTAGAACTGATAA ACTAATTGCA |
| *NSC11.3* | CTGGTT AAAAACAAGTGAAGAACAAAG AA CACATAA TTT ACAACACAATTTAT |
| | |
| *STG11* | GGGTTTGCAAACCT AGATGCAGACGT TACT AACCTTGGAAAGATGATGGACGA |
| *NSC11.3* | CAA ATCTTAAA GTGAGTG TTC GGCGGCTAGAAGATTAT ATGA ATGGACTTCTT |
| | |
| *STG11* | AC TTC A GAAGC |
| *NSC11.3* | AGCCAG AAGAA |

D: *Emm* sequence alignment of *NSC11.2* compare to *STG11*

(46% homology, Accession number = AF485829)

Base differences are underlined.

| | |
|---|---|
| *STG11* | TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAG GCGGCGGAGA ATACAT |
| *NSC11.2* | TTAGGGGCAGGATTAGCGAATCAAGCAGAAGTTAAA GCCA AT GAGT ATAACA |
| | |
| *STG11* | ACGATAGATGGAAAGCACA AACAGAAGAGGGCTAGAACTGATAAACT AATTGC |
| *NSC11.2* | ACTGGTT AAAAACAAGCGGAAAA ACTT GCA CACATAAT TT ACAACACAATTTA |
| | |
| *STG11* | AGGGTTTGCAAACCTAGATGCAGACGT TACT AACCTTGGAAAGATGATGGACG |
| *NSC11.2* | TC AAATCTTAAAGTGAGTGTTC GGCGGCTAGAAGATTAT ATGA ATGGACTT CT |
| | |
| *STG11* | AAC TTC A GAAGC |
| *NSC11.2* | T AGCCAG AAGAA |

E: *Emm* sequence alignment of *NSC11.4* compare to *STG11*

(45% homology, Accession number = AF485835)

Base differences are underlined.

| | |
|---|---|
| *STG11* | TTAGGGGCAGGATTAGCAAGCCAAACAGAAGTTAAGGCGGCGGAGAATACATA |
| *NSC11.4* | TTAGGGGCAGGATTAGCGAATCAAGCAGAAGTTAAA GCCAAT GAGTATAACAA |

| | |
|---|---|
| *STG11* | CGATAGATGGAAAGCACA AAC AGAAGAGGGCTAGAACT GAT AAACT AATTGC |
| *NSC11.4* | CTGGTTA AAAACAAGCGGAGGACCT TGC ACACATAA TT T ACAACAC AATTTA |

| | |
|---|---|
| *STG11* | AGGGTTTGCAAACCT AGATGCAGACGT TACT AACCTTGGAAAGAT GATGGAC |
| *NSC11.4* | TCAA  ATCTT AAAGTGAGTGTTCGGCGGCTAGAAGA TTATATG AAT GGACTT C |

| | |
|---|---|
| *STG11* | GAAC TTCAGAAGC |
| *NSC11.4* | TT AGCCAGAAGAA |

F: *Emm* sequence alignment of *NSC103* compare to *STIL103*

(44% homology, Accession number = AF485836)

Base differences are underlined.

| | |
|---|---|
| *STIL103* | TTAGGGGCAGGTTTT GCGAATCAAACAGAGGTTAAGGCTAACGGTGATGGTAAT |
| *NSC103* | TTAGGGGCAGGATTAGCGAATCAAGCAGAAGTTAAAGCCAA TGAGTATAACAAC |

| | |
|---|---|
| *STIL103* | CCTATACGTCACGAAAA CAAGGACTTA AAAGCGAGATTAGA GAATGCAATGGA |
| *NSC103* | TGGTTAAAAACAA GCTGTGGAAC TTGCACACAT A AT TTACAAC ACAATT TATC |

| | |
|---|---|
| *STIL103* | AGTTGCAGGAAGAGATT TTAA  GAGAGCTGAAGAACTTGAAAAAGCAAAACAAG |
| *NSC103* | AAATCTTAA  AGTGAG TGTTCGGCGGCTA GAAGATTATATGAAT GGACTTCTT A |

| | |
|---|---|
| *STIL103* | CCTTA  GAAGA |
| *NSC103* | GCCAGA AGAA |

G: *Emm* sequence alignment of *NSC2764* compare to *STL2764*

(40% homology, Accession number = AF485834)

Base differences are underlined.

*STL2764* CTAGGGGCAGGATTAGTTGCTAAT ACTAATG AAGTTGGGGCACTCACGATT ACT
*NSC2764* CTAGGGGCAGGATTAGTCTAGCCAAACCGAAAGAATCTA GAGAAGTAACTGATC

*STL2764* AGGTCTATGGCAAAAGATCCGGAAAAACTAAAAGCACTTGCTGAAACTTATGAAG
*NSC2764* AGTTGGTTG _CAAA_GATGTGGAAAAATAAACTT GATGCTGCAAAACTTGAAAAT

*STL2764* TAGAAAACCATAAGTT AACAAATGAGAATGGTAAGTTAA CAAGTGAGAATGACA
*NSC2764* GAAAAGTTAAAAAGTGATATT GAGAGTT TAAAAGAAGCAATTAAA AGTTATGTT

*STL2764* AGTTAACAA
*NSC2764* GAAAGTATT

Figure 17 A-G: The nucleotide alignment of novel *emm* types of GCS isolates in this study. (A) *NSC401* found in C38-005, C38-007, and C42-001 isolates compares to *STCK401*. There are 37 base pair point mutation (78% homology). (B) *NSC11.1* found in C41-007 isolate compare to *STG11*. There are 89 base pair point mutation (48% homology). (C) *NSC11.3* found in C43-011 isolate compare to *STG11*. There are 90 base pair point mutation (47% homology). (D) *NSC11.2* found in C42-007 isolate compare to *STG11*. There are 92 base pair point mutation (46% homology). (E) *NSC11.4* found in C38-004 isolate compare to *STG11*. There are 93 base pair point mutation (45% homology). (F) *NSC103* found in C38-014 isolate compare to *STIL103*. There are 96 base pair point mutation (44% homology). (G) *NSC2764* found in C41-003 isolate compare to *STL2764*. There are 100 base pair point mutation and 2 base pair deletion (40% homology). Base differences are underlined.

## 5. *Emm* type and site of infections

### 5.1. GGS isolates

The *emm* types identified from each sites of infections from non-invasive and invasive GGS isolates were shown in table20 and table 21, respectively. In pus culture, 11 types from 19 isolates (63.3%) were found. *STC5345* (15.8%) and *NSG62647* (15.8%) were the most common found. In throat culture, 10 types from 10 isolates (33.3%) were found and eye culture found 1 type from 1 isolate (3.3%) was found. *STC5345* can be found in all 3 sites of non-invasive infections. In blood culture, 15 types from 19 isolates (63.3%) were found and *STC6979* (21.1%) was the most common found. In body fluid, 7 types from 7 isolates (23.3%) were found and joint fluid, 4 types from 4 isolates (13.3%) were found. *STC6979* can be found in all 3 site of invasive infections.

Table 20: Sites of infections and *emm* types of GGS non-invasive isolates

| Site of infection | No of patients (%) | *Emm* type (no) |
|---|---|---|
| Pus | 19 (63.3) | *11 types:*<br>*STC5345 (3), NSG62647 (3), STG6 (2), H46A (2), STC5345.1 variant (2), NSG480.1 (2), STC74A (1), STC839 (1), STG485 (1), NSG10 (1), NSGemm3.1 (1)* |
| Throat | 10 (33.3) | *10 types:*<br>*STC6979 (1), H46A(1), STC839 (1), STC5345 (1), STG485 (1), STG840 (1), STG653 (1), STC5345.1 variant (1), NSG10 (1), N SG1969.1 (1)* |
| Eye | 1 (3.3) | *1 type:*<br>*STC5345 (1)* |

Table 21: Sites of infections and *emm* types of GGS invasive isolates

| Site of infection | No of patients (%) | *Emm* type (no) |
|---|---|---|
| Blood | 19 (63.3) | *15 types:*<br>*STC6979 (4), STG485 (1), STC1376 (1), H46A (1), STG653 (1), STG4831 (1), NSRT17 (1), STG166B.1 variant (1), STG166B.2 variant (1), STG643 variant (1), NSG62647 (1), NSG1969.2 (1), NSG5345 (1), NSGemm100.1 (1), NSG93464 (2)* |
| Body fluid | 7 (23.3) | *7 types:*<br>*STC6979 (1), STC839 (1), STG6 (1), STG166B.1 variant (1), STC5345.1 variant (1), emm23 variant (1), NSG1741 (1)* |
| Joint fluid | 4 (13.3) | 4 types:<br>*STC6979* (1), *STC5345* (1), *STC5345.1 variant* (1), *STC5345.2 variant* (1) |

### 5.2. GCS isolates

The *emm* type identified from each site of infections from invasive and non-invasive GCS isolates were shown in table 22. For example, in pus culture, 7 types from 7 isolates were found and *ST245* (30%) was the most common found. *ST245* can be found in 3 sites of infections include pus, throat, and tissue culture.

Table 22: Site of infection and *emm* type of GCS non-invasive and invasive isolates

| Site of infection | No of patients (%) | *Emm* type (no) |
|---|---|---|
| Pus | 10 (41.6) | *ST245 (3), H46A (2), STC74A (1), STG4545 (1), NSC11.4 (1), NSC11.1 (1), NSC11.2 (1)* |
| Throat | 8 (33.3) | *ST245 (4), NSC401 (2), STC74A (1), NSC11.3 (1)* |
| Tissue | 3 (12.5) | *ST245 (2), NSC401 (1)* |
| Blood | 2 (8.3) | *STC74A (1), NSC103 (1)* |
| Joint fluid | 1 (4.2) | *NSC2764 (1)* |

In conclusion, there are no particular *emm* types of GGS and GCS isolates tend to be frequently associated with anatomic site of infections. In addition, the same *emm* types can associate with various sites of infections.

## 6. *Emm* type and the number of isolates in each year

### 6.1. GGS isolates

The *emm* types that were identified non-invasive and invasive GGS isolates divided by year were shown in table 23. In 1995, 8 types from 11 isolates (72.7%) were found and *STC6979* were the most common type (4 isolates, 36.3%). In 1996, 2 types from 3 isolates (66.6%) were found. In 1997, 8 types from 10 isolates (80%) were found. In 1998, 10 types from 15 isolates (66.6%) were found and *STC5345.1 variant* were the most common type (3 isolates, 20%). In 1999, 9 types from 12 isolates (75%) were found and *STC5345* were the most common type (3 isolates, 25%). In 2000, 9 types from 9 isolates (100%) were found.

Table 23: The number of isolates and *emm* type of GGS isolates divided by year

| Year | No. of patients (%) | *Emm* type (no) |
|---|---|---|
| 1995 | 11 (18.3) | 8 types:<br>*STC6979 (4), H46A (1), STC74A (1), STC839 (1), STG166B.1 variant (1), STG166B.2 variant (1), emm23 variant (1), NSG62647 (1)* |
| 1996 | 3 (5) | 2 types:<br>*NSG62647 (2), STG485 (1)* |
| 1997 | 10 (16.6) | 8 types:<br>*H46A (2), STG6 (2), STC1376 (1), STC5345 (1), STC5345.1 variant (1), NSG62647 (1), NSG1969.2 (1), NSG5345 (1)* |
| 1998 | 15 (25) | 10 types:<br>*STC5345.1 variant (3), STC839 (2), STC6979 (2), NSG480.1 (2), STC5345 (1), H46A (1), STG653 (1), NSGemm3.1 (1), NSGemm100.1 (1), NSG1741 (1)* |
| 1999 | 12 (20) | 9 types:<br>*STC5345 (3), NSG10 (2), STG485 (1), STC6979 (1), STG4831 (1), STC5345.1 variant (1), STC5345.2 variant (1), STG643 variant (1), NSG1969.1 (1)* |
| 2000 | 9 (15) | 9 types:<br>*STC5345 (1), STG6 (1), STG485 (1), STG653 (1), STG840 (1), NSRT17 (1), STG166B.1 variant (1), NSG93464 (2)* |

### 6.2. GCS isolates

The *emm* types that were identified from non-invasive and invasive GCS isolates divided by year were shown in table 24. In 1995, 4 types from 5 isolates (80%) were found. In 1996, 1 type from 1 isolate (100%) was found. In 1998, 4 types from 4 isolates (100%) were found. In 1999, 4 types from 4 isolates (100%) were found. In 2000, 3 types from 10 isolates (30%) were found and *ST245* were the most common found (8 isolates, 80%).

Table 24: The number of isolates and *emm* type of GCS isolates divided by year

| Year | No. of patients (%) | *Emm* type (no) |
|---|---|---|
| 1995 | 5 (20.8) | 4 types:<br>*NSC401 (2), STC74A (1), NSC11.4 (1), NSC103 (1)* |
| 1996 | 1 (4.2) | 1 types:<br>*STC74A (1)* |
| 1997 | - | - |
| 1998 | 4 (16.6) | 4 types:<br>*STC74A (1), H46A (1), NSC11.1 (1), NSC2764 (1)* |
| 1999 | 4 (16.6) | 4 types:<br>*ST245 (1), H46A (1), NSC401 (1), NSC11.2 (1)* |
| 2000 | 10 (41.6) | 3 types:<br>*ST245 (8), STG4545 (1), NSC11.3 (1)* |

In conclusion, there was no correlation between the particular *emm* type with year of isolates ands the same *emm* type can be associated with various years of isolates.