Clinical and microbiological analysis of beta hemolytic streptococci during 2006-2010 at Nagoya City University Hospital

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SUMMARY

Beta hemolytic streptococci (BHS) cause various infectious diseases from acute pharyngitis to streptococcal toxic shock syndrome. Though BHS infection has been increasing recently, the comprehensible investigation of the BHS isolated in tertiary hospital has not been performed. In this study, we investigated 918 BHS isolated in Nagoya City University Hospital during 2006-2010. The numbers of group A streptococcus (GAS), group B streptococcus (GBS), group C streptococcus (GCS), and group G streptococcus (GGS) isolates were 84, 715, 36, and 83, respectively. Only the number of GBS isolated from female was more than that from male. Sixty-one percent of GAS strains were isolated from under the 30-year-old patients. GBS were isolated most from age 30-39. Half of GGS were from over age 60. The number of the BHS isolates from outpatients was larger than that from inpatients. The clinical department from which BHS was isolated most was dermatology for GAS and GGS, ob-

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Abbreviations: BHS, beta hemolytic streptococci, GAS, group A streptococcus; GBS, group B streptococcus; GCS, group C streptococcus; GGS, group G streptococcus; PCG, benzylpenicillin; ABPC, ampicillin; SBT／ABPC, ampicillin-sulbactam; CVA／AMPC, amoxicillin-clavulanate; CTX, cefotaxime; CFPM, cefepime; MEPM, meropenem; VCM, vancomycin; EM, erythromycin; CLDM, clindamycin; TC, tetracycline; CP, chloramphenicol; LVFX, levofloxacin; ST, sulfamethoxazole-trimethoprim; FQ, fluoroquinolone; EUCAST, European committee on antimicrobial susceptibility testing; CLSI, clinical and laboratory standards institution; ICU, intensive care unit; ER, emergency room.

stetrics and gynecology for GBS, respiratory medicine for GCS, respectively. Ninety-three percent of GAS were identified as *Streptococcus pyogenes,* 59% of GCS were *Streptococcus anginosus,* and 78% of the GGS were *Streptococcus dysgalactiae* by the biochemical method. BHS in this study were largely susceptible to beta lactam antibiotics although 1-3% of GBS and GGS were resistant to each beta lactam antibiotics examined. In GAS and GGS, erythromycin and tetracycline resistant rates were approximately 30% and 50%, respectively. Non susceptible rate of levofloxacin ranged from 13-26% in BHS except GGS. We must pay attention to the antimicrobial susceptibility of BHS hereafter.

Key words: beta hemolytic streptococci, tertiary hospital, antimicrobial susceptibility

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INTRODUCTION

Streptococci are gram−positive spherical bacteria that characteristically form pairs or chains during growth（1）. Most species are facultative anaerobes, and some grow only in an atmosphere enhanced with carbon dioxide（2）. They are widely distributed in nature（1）. Some are members of the normal human flora; others are associated with important human diseases attributable in part to infection by streptococci（1）.

The differentiation of species within the genus is complicated, because several different schemes are used to classify the organisms（2）. Over many years, the classification of streptococci into major categories has been based on a series of observations; [1] colony morphology and hemolytic reactions on blood agar; [2] serologic specificity of the cell wall group-specific substances（Lancefield classification）and other cell wall or capsular antigens; [3] biochemical factors; and [4] ecologic features（1）. Additional biochemical tests and molecular genetics also have been used to study the relationships of streptococcal species to each other（1）. While standard biochemical and molecular criteria are used to classify streptococci into species, several additional classification schemes are also commonly utilized（3）.

The most common of these are based on the appearance of colonies grown on blood agar plates. Hemolysis is used as a guide for managing patients as well as an aid in classification of the bacterium to the species level（4）. Streptococci are classified into three groups by the types of enzymatic hemolysis on blood agar plates; alpha hemolytic group is characterized by incomplete lysis, with green pigment surrounding the colony. Beta hemolytic group is characterized by total lysis and release of hemoglobin and a clear area around the colony. Beta hemolytic streptococci（BHS）can be subdivided into large-colony and small-colony（＜0.5 mm in diameter）formers. *Streptococcus pyogenes*（Lancefield group A）, *Streptococcus agalactiae*（group B）, and *Streptococcus dysgalactiae subsp. equisimilis*（group C, G）belong to

large-colony formers（5）. *Streptococcus anginosus* belong to small-colony former（5）. Gamma hemolytic group is characterized by absence of lysis（2）.

Hemolytic streptococci can be divided into serologic groups, and certain groups can be subdivided into types（1）. Several antigenic substances are found and group-specific cell wall antigen is one of them（1）. This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping (Lancefield groups)（1）. The serologic classification scheme was developed by Lancefield in 1933 for differentiating beta hemolytic strains（2）. Most beta hemolytic strains and some alpha hemolytic and non hemolytic strains possess group-specific antigens, most of which are cell wall carbohydrates（2）. The Lancefield classification scheme provides a useful framework for considering the pathogenic range of the streptococci（3）. Group A streptococci（GAS）are one of the most frequent human pathogens capable of producing a wide variety of diseases. Such infections range from suppurative sequelae like pharyngitis, impetigo, necrotizing fasciitis, to more severe and life-threatening disease like streptococcal toxic shock syndrome（STSS）. Group B streptococci（GBS）are the most frequent cause of sepsis and meningitis in newborn infants（3）. Group A and B streptococci have been regarded as important streptococci which show medically obvious pathogenicity to humans, and the isolation of these streptococci has been placed great importance in laboratory medicine（6）. Group C and G streptococci resemble the group A streptococci but cause a number of different diseases in animals like horses, cows, cats, dogs, and pigs（3）. Though the group C and G streptococci had been regarded as weak pathogenic bacteria, it was reported that these streptococci as well as group A streptococci caused STSS in recent years（6）.

BHS is clinically important bacteria and it was reported that BHS infection has been increasing recently (http://strep.umin.jp/index.html). Although invasive BHS have been well-analyzed until now worldwide, the analysis of comprehensive BHS in tertiary hospital has not been performed yet. The objective of this study was to elucidate the characteristic of BHS isolates in Japanese tertiary hospital and to clarify the trend of BHS there.

MATERIALS AND METHODS

Strains and clinical data collection

A total of 918 streptococci isolates were obtained from various clinical specimens at Nagoya City University Hospital between April 2006 and March 2010. Nagoya City University Hospital, Japan, is an 807-bed tertiary hospital in mid-Japan. We used medical records appended to clinical species for the analysis of clinical feature at Nagoya City University Hospital. We considered several isolates from the same region of the same patient as one isolate per one patient for the analysis in this study. All streptococcal isolates were identified by

standard conventional biochemical methods or the VITEK™ system (bioMe'rieux, Durham NC, USA). We determined the identification of these streptococcus species by Streptogram (Wako Pure Chemicals, Osaka, Japan) according to the manufacturer's instructions. We also determined the Lancefield serologic grouping of these streptococci by test kits (*Oxoid DR 585*; Oxoid, Cambridge, UK). Our experimental design was approved by the ethics committee at Nagoya City University.

Antimicrobial susceptibility analysis

BHS isolates were examined for antibiotic susceptibilities of 14 antibiotics as follows; PCG, benzylpenicillin; ABPC, ampicillin; SBT/ABPC, ampicillin-sulbactam; CVA/AMPC, amoxicillin-clavulanate; CTX, cefotaxime; CFPM, cefepime; MEPM, meropenem; VCM, vancomycin; EM, erythromycin; CLDM, clindamycin; TC, tetracycline; CP, chloramphenicol; LVFX, levofloxacin; ST, sulfamethoxazole-trimethoprim. Antimicrobial susceptibility was tested using MicroScan (Siemens Japan, Tokyo, Japan), and these results were interpreted according to the EUCAST criteria (http://www.eucast.org/).

Statistical analysis

We conducted the statistical analysis with the chi-squared test or Fisher's exact test when appropriate. Differences were considered significant when p was < 0.05.

RESULT

At first, we evaluated the relationship between clinical patients' features and Lancefield serologic groups of BHS. Figure 1 reveals that the number of female patients was larger than that of male patients with respect to GBS ($p < 0.05$). Although the number of male patients tended to be larger than that of female patients in other BHS group, there was no significant difference. The relationship between patient age group and BHS is shown in Figure 2. The age range was grouped every 10 years old. In GAS, the number of patients less than 30s was larger than that of 30s and over. Twenty-five percent of GBS were isolated from patients of 30s. Twenty-four percent of GBS were isolated from patients of 70s and over. In GGS, the number of patients of 60s and over was more than that under 60s. Figure 3 represents that the number of inpatients was significant less than that of outpatients in GAS and GBS ($p < 0.05$). Figure 4 shows the relationship between clinical department and BHS. Dermatology was the most frequent clinical department from which GAS and GGS were isolated (Fig. 4(a), and 4(d)). Thirty-five percent of GBS were isolated from obstetrics and gynecology department (Fig. 4(b)). The clinical department, from which GCS was isolated most
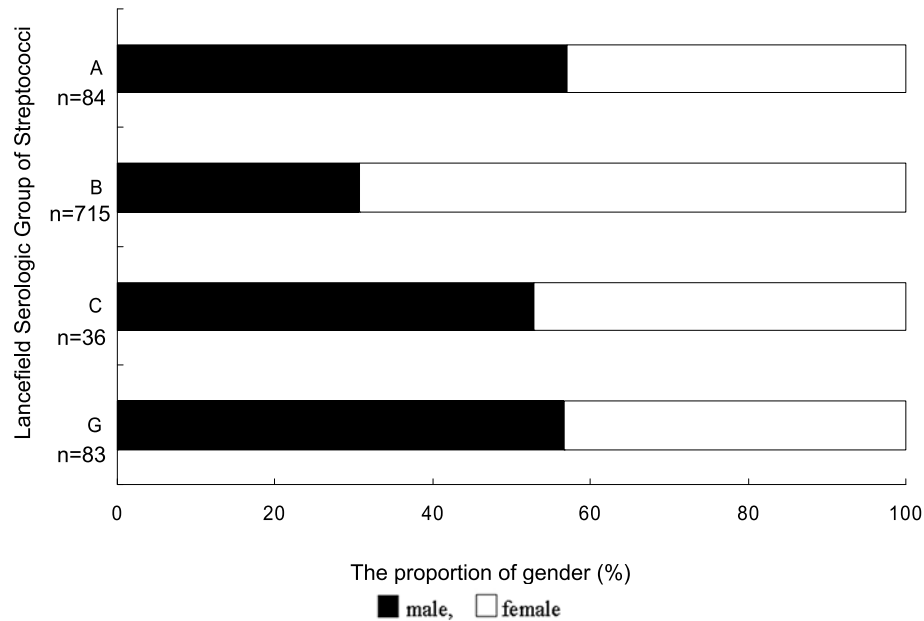
FIG. 1 Prevalence of gender proportion in beta hemolytic streptococci
（a）group A streptococci,（b）group B streptococci,（c）group C streptococci,（d）group G streptococci
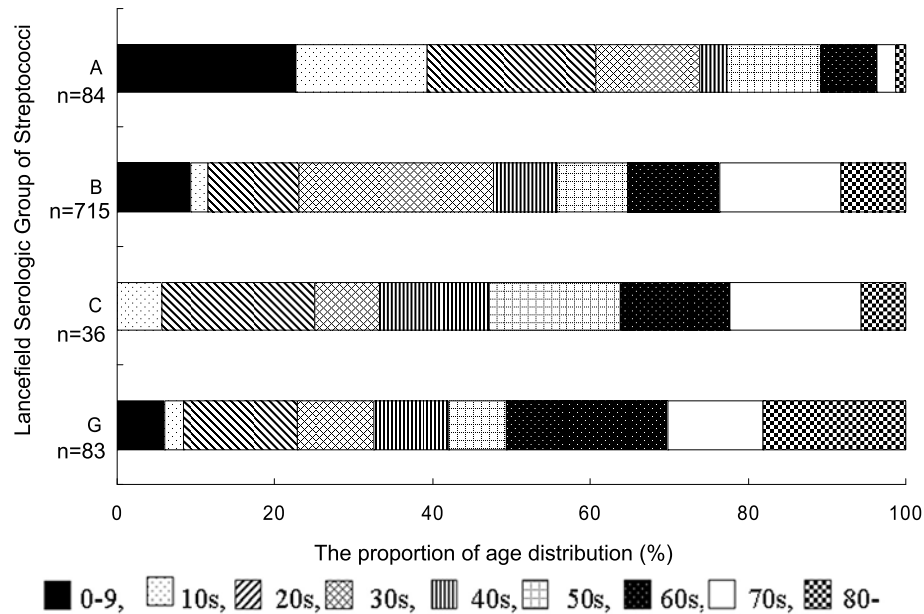
FIG. 2 Prevalence of age distribution in beta hemolytic streptococci
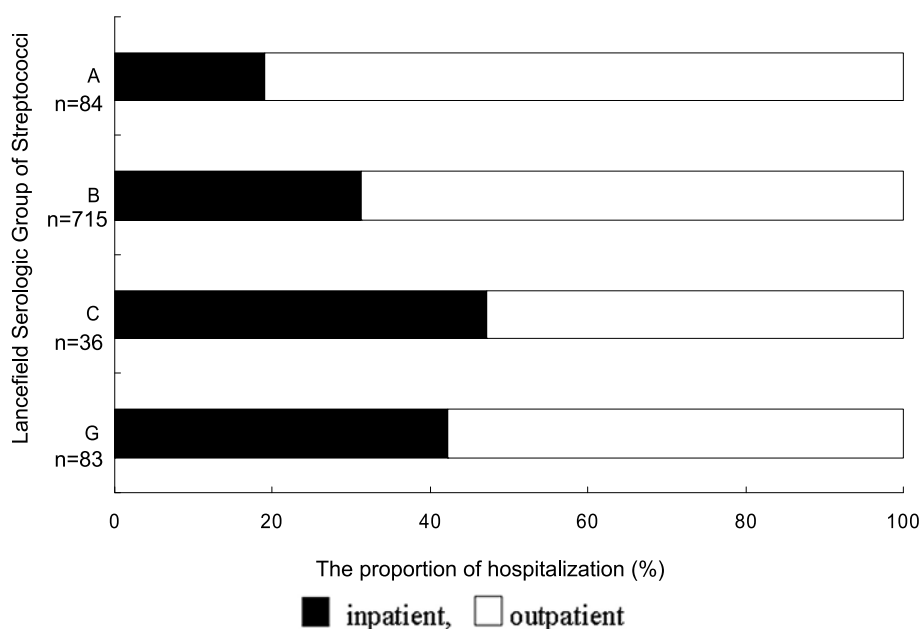（a）group A streptococci,（b）group B streptococci,（c）group C streptococci,（d）group G streptococci

FIG. 3   Prevalence of hospitalized proportion in beta hemolytic streptococci
（a）group A streptococci,（b）group B streptococci,（c）group C streptococci,（d）group G streptococci

frequently, was respiratory medicine（Fig. 4（c））. We represent that the relationship between isolation sites and BHS in Figure 5. Twenty-one percent of GAS were isolated from pharyngeal mucus（Fig. 5（a））. The site that GBS isolated most was urine（Fig 5（b））. One-third of GCS was isolated from sputum（Fig. 5（c））. We found GGS were isolated most frequently from pus（Fig. 5（d））. Two percent of GBS and 5% of GGS were only isolated from blood（Fig. 5（b）, and 5（d））. Next we clarified the relationship between Lancefield serological group of BHS and the streptococcal nomenclature by streptogram kit（Figure 6）. Although the majority of GAS was *Streptococcus pyogenes*, the small numbers of GAS were *Streptococcus dysgalactiae subsp. dysgalactiae*（Fig. 6（a））. Twenty-eight percent of GCS were *Streptococcus anginosus*（Fig 6（b））. Seventy-eight percent of GGS were *Streptococcus dysgalactiae* and *Streptococcus anginosus*. We found *Streptococcus equi subsp. zooepidemicus,* which caused zoonosis, in the small numbers of GGS（Fig. 6（c））.

We also analyzed the antimicrobial susceptibility of BHS in this study. The susceptibility of GAS against all beta lactam was effective. However, the MIC 90 of EM, CLDM, TC, and LVFX in GAS were＞2, ＞1, ＞4, and 2, respectively（Table 1）. The susceptibility of GBS against CLDM was effective. The susceptibility of GBS against penicillin was intermediate pattern. The MIC 90 of EM, TC, and LVFX in GBS were 2, ＞4, and ＞8, respectively（Table 2）. The susceptibility of GCS against EM and CLDM was effective. We also found lower sus-
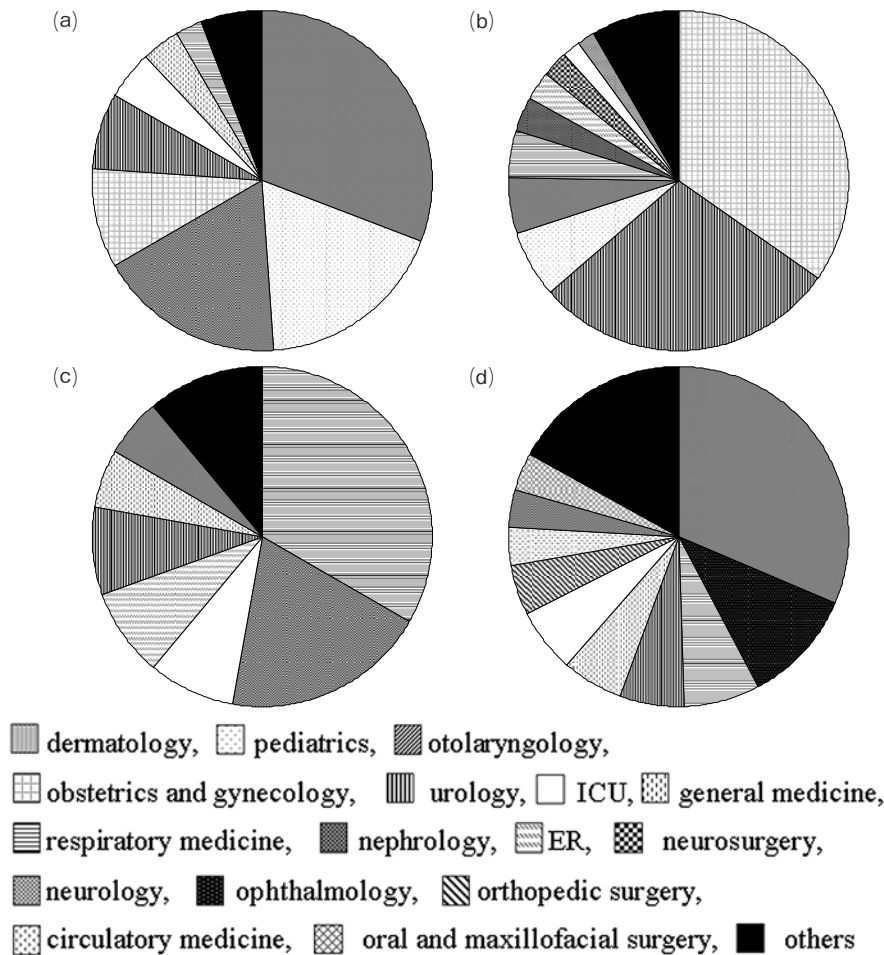
FIG. 4   Classification of clinical department where beta hemolytic streptococci were isolated
(a) group A streptococci, (b) group B streptococci, (c) group C streptococci, (d) group G streptococci

ceptibility against ABPC in GCS than other BHS. The MIC 90 of TC, CP and LVFX in GCS were>4, 8, and 2, respectively (Table 3). The susceptibility of GGS against LVFX was effective. The MIC 90 of EM, CLDM and TC in GGS showed>2, >1, and 4, respectively (Table 4). Furthermore we evaluated the antimicrobial resistant rate with antibiogram (Figure 7). Antibiogram of GAS shows that EM, CLDM, and TC resistant rates were 33%, 13%, and 53%, respectively. It also shows that LVFX intermediate resistant pattern was detected in 13% of GAS (Fig 7(a)). Antibiogram of GBS shows that EM, CLDM, TC, and LVFX resistant rates were 15%, 9%, 50%, and 23%, respectively (Fig. 7(b)). Antibiogram of GCS shows that ABPC and TC resistant rates were 11%, and 45%, respectively (Fig. 7(c)). Al-
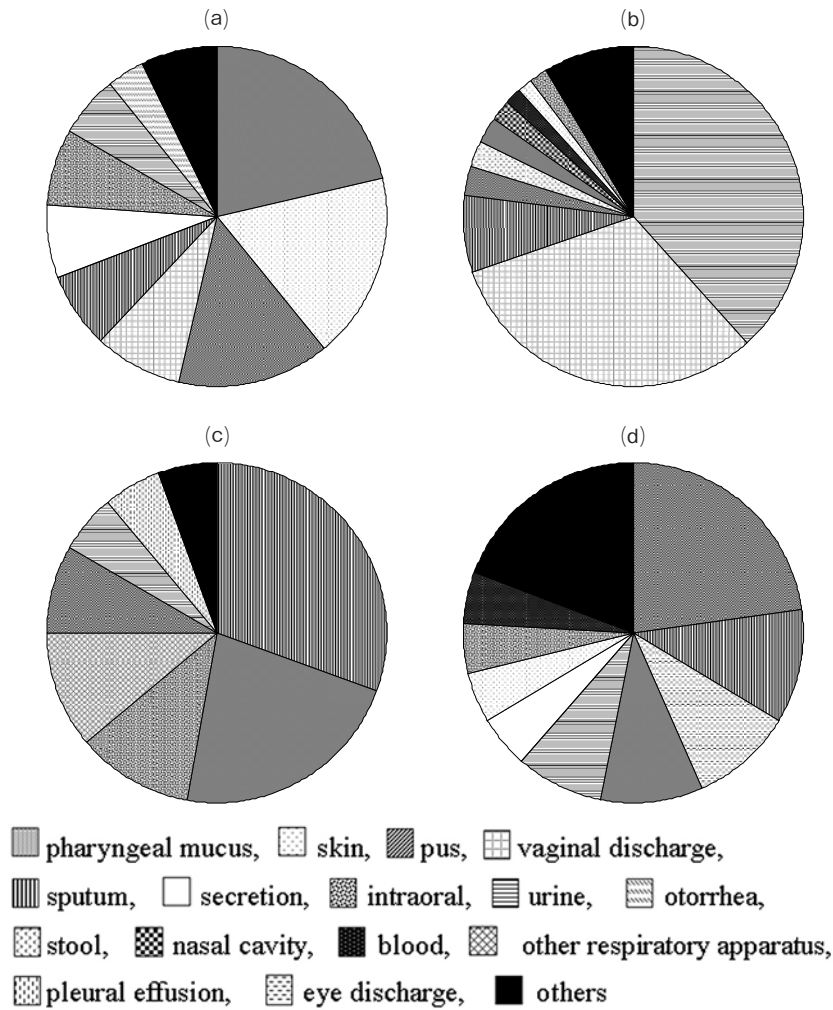
FIG. 5 Prevalence of isolation sites of beta hemolytic streptococci
（a）group A streptococci, （b）group B streptococci, （c）group C streptococci （d）group G streptococci

though we found no LVFX-resistant GCS in this study, our results revealed that 25％ of GCS had LVFX-intermediate resistant pattern. Antibiogram of GGS shows that EM, CLDM, and TC resistant were 30％, 16％, and 46％, respectively. All GGS had LVFX-susceptible pattern in this study （Fig. 7（d））.

DISCUSSION

Although many previous studies represented the data of only invasive streptococcal diseases （6-8）, the analyses of total BHS have not been performed in Japan before. In this study, we described the characteristics of all BHS isolated from tertiary hospital in Japan among re-
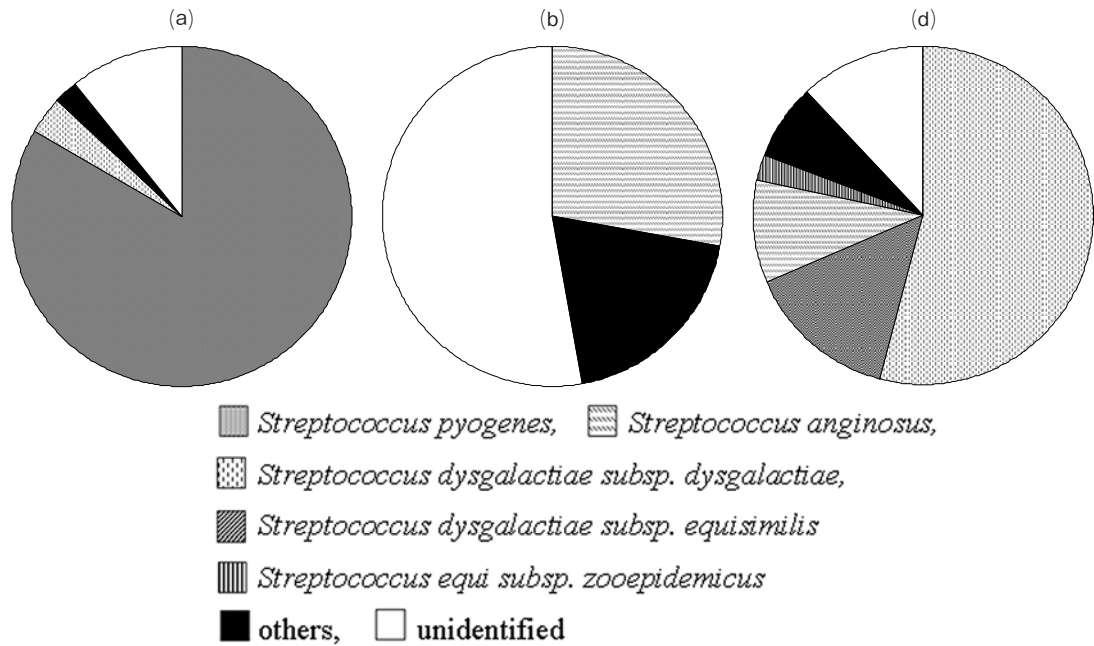
(a) (b) (d)

Streptococcus pyogenes, Streptococcus anginosus,

Streptococcus dysgalactiae subsp. dysgalactiae,

Streptococcus dysgalactiae subsp. equisimilis

Streptococcus equi subsp. zooepidemicus

others, unidentified

FIG. 6 Bacterial classification of beta hemolytic streptococcal nomenclature
（a）group A streptococci,（b）group C streptococci,（c）group G streptococci

Table 1 Antimicrobial susceptibility of group A streptococci against 14 antimicrobial agents

Antimicrobial Agent	MIC (ug/mL)		
	Range	50%	90%
PCG	<=0.03	<=0.03	<=0.03
ABPC	<=0.06	<=0.06	<=0.06
SBT/ABPC	<=0.25	<=0.25	<=0.25
CVA/AMPC	<=1	<=1	<=1
CTX	<=0.06	<=0.06	<=0.06
CFPM	<=0.5	<=0.5	<=0.5
MEPM	<=0.12	<=0.12	<=0.12
VCM	0.25-1	0.5	0.5
EM	<=0.12->2	<=0.12	>2
CLDM	<=0.12->1	<=0.12	>1
TC	<=0.5->4	1	>4
CP	<=4	<=4	<=4
LVFX	<=0.25-2	0.5	2
ST	<=0.5->4	<=0.5	<=0.5

Antimicrobial Agent
PCG, benzylpenicillin; ABPC, ampicillin; SBT/ABPC, ampicillin-sulbactam; CVA/AMPC, amoxicillin-clavulanate; CTX, cefotaxime; CFPM, cefepime; MEPM, meropenem; VCM, vancomycin; EM, erythromycin; CLDM, clindamycin; TC, tetracycline; CP, chloramphenicol; LVFX, levofloxacin; ST, sulfamethoxazole-trimethoprim

Table 2　Antimicrobial susceptibility of group B streptococci against 14 antimicrobial agents

Antimicrobial Agent	MIC (ug/mL)		
	Range	50%	90%
PCG	<=0.03-2	0.06	0.12
ABPC	<=0.06-2	0.12	0.25
SBT/ABPC	<=0.25-4	<=0.25	<=0.25
CVA/AMPC	<=1-4	<=1	<=1
CTX	<=0.06->2	<=0.06	0.12
CFPM	<=0.5->2	<=0.5	<=0.5
MEPM	<=0.12->2	<=0.12	<=0.12
VCM	<=0.12->1	0.5	0.5
EM	<=0.12->2	<=0.12	2
CLDM	<=0.12->1	<=0.12	<=0.25
TC	<=0.5->=16	1	>4
CP	<=4->16	<=4	<=4
LVFX	<=0.25->8	1	>8
ST	<=0.5->4	<=0.5	<=0.5

Table 3　Antimicrobial susceptibility of group C streptococci against 14 antimicrobial agents

Antimicrobial Agent	MIC (ug/mL)		
	Range	50%	90%
PCG	<=0.03	<=0.03	<=0.03
ABPC	<=0.06->8	<=0.06	>8
SBT/ABPC	<=0.25	<=0.25	<=0.25
CVA/AMPC	<=1	<=1	<=1
CTX	<=0.06-0.25	<=0.06	0.25
CFPM	<=0.5	<=0.5	<=0.5
MEPM	<=0.12	<=0.12	<=0.12
VCM	0.25-1	0.5	1
EM	<=0.12	<=0.12	<=0.12
CLDM	<=0.12	<=0.12	<=0.12
TC	<=0.5->4	1	>4
CP	<=4-8	<=4	8
LVFX	<=0.25-2	1	2
ST	<=0.5	<=0.5	<=0.5

cent 4 years. The proportion of BHS revealed that GCS was the smallest population of BHS. This result was coincident with the previous report （9）. Our result showed that there were no significant differences of isolates numbers between GAS and GGS. Although we have little interest of GGS as compared with GAS before, this study may imply that GGS will increase gradually from now on.

　　With respect to gender group, GBS were isolated more from female patients than male patients. This result is consistent with the fact that GBS usually colonize in genitals （10）. It

Table 4　Antimicrobial susceptibility of group G streptococci against 14 antimicrobial agents

Antimicrobial Agent	MIC (ug/mL)		
	Range	50%	90%
PCG	<=0.03-0.5	<=0.03	0.06
ABPC	<=0.06-0.5	<=0.06	0.12
SBT/ABPC	<=0.25-0.5	<=0.25	<=0.25
CVA/AMPC	<=1	<=1	<=1
CTX	<=0.06-2	<=0.06	0.25
CFPM	<=0.5->2	<=0.5	<=0.5
MEPM	<=0.12-1	<=0.12	<=0.12
VCM	<=0.12->1	0.5	1
EM	<=0.12->2	<=0.12	>2
CLDM	<=0.12->1	<=0.12	>1
TC	<=0.5->4	1	>4
CP	<=4	<=4	<=4
LVFX	<=0.25-1	0.5	1
ST	<=0.5	<=0.5	<=0.5

may also explain that GBS is one pathogen of birth canal infection（11）. We clarified BHS with age distribution. Young patients under 30 years frequently caused GAS infection. Many GBS were isolated from patients ranging from 20 to 40 years old. However, the large numbers of BHS except GAS were isolated from over 60s-patients. Previous report showed that elder people were infected with severe BHS more often（9）. The number of BHS isolated from inpatients, especially GAS, was significant less than that of outpatients. We speculate that patients of severe invasive BHS infection are rarely admitted in Nagoya City University Hospital. This hypothesis may be supported by the fact that BHS was rarely isolated from blood sample. Actually, we found no STSS cases in this hospital during this study. In the analysis of clinical departments, we found that department where most patients with GAS were detected was dermatology. GAS infection, especially acute pharyngitis and tonsillitis, was usually popular as pediatric or otolaryngology diseases. Our result implies two reasons. One is that patients infected by GAS usually do not consult at tertiary hospital but consult outpatient clinic nearby. Another is that bacterial culture method was not used but rapid ELISA kit for detection of GAS was used in pediatrics and otolaryngology departments. The sample for ELISA kit is usually pharyngeal mucus（13）. ELISA kit is faster and more convenient than bacterial culture（13）. For dermatologic infectious disease, clinical samples must be examined by bacterial culture, but not for pediatric or otolaryngology disease. Similar results of clinical department were seen from GGS cases. As GGS is often colonized at skin（1）and possesses the same kind of virulent factors of GAS; streptolysin O, streptolysin S, nga, streptokinase, and sic（12）（14）, GGS may often cause infectious dermatological dis-
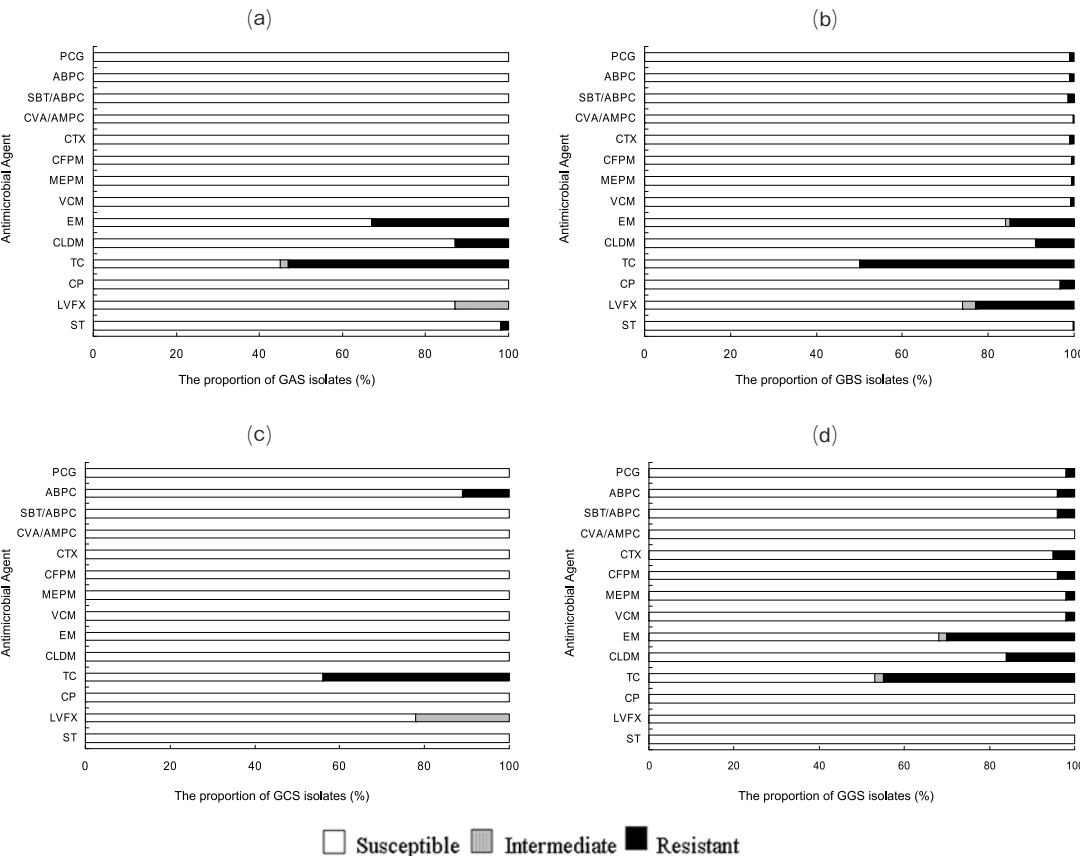
FIG. 7　Antibiogram of beta hemolytic streptococci against 14 antimicrobial agents
（a）group A streptococci, （b）group B streptococci, （c）group C streptococci, （d）group G streptococci
PCG, benzylpenicillin；ABPC, ampicillin；SBT／ABPC, ampicillin-sulbactam；CVA／AMPC, amoxicillin-clavulanate; CTX, cefotaxime; CFPM, cefepime; MEPM, meropenem; VCM, vancomycin; EM, erythromycin; CLDM, clindamycin；TC, tetracycline；CP, chloramphenicol；LVFX, levofloxacin；ST, sulfamethoxazole-trimethoprim.

eases such as GAS.　Our study revealed that patients with GBS may cause genitourinary system disorders and those with GCS may cause respiratory system disorders same as the previous report（6）.　Most isolation sites of BHS were strongly related with the result of clinical department.　Especially, the large numbers of BHS obtained from inflammation site（i.e. pus）were GAS and GGS.　This result may also support that GGS possesses the same kind of virulent factors of GAS（7）,（12）.

All GAS have been believed as *Streptococcus pyogenes* until quite recently（15）, our result also revealed that one group of GAS was *Streptococcus dysgalactiae* as the other researcher reported（6）.　*Streptococcus anginosus* was involved in both GCS group and GGS group（16）.　Although it often causes deep abscess, most virulent factors of *Streptococcus*

anginosus have not been elucidated before（16）. *Streptococcus dysgalactiae subsp. dysgalactiae, Streptococcus dysgalactiae subsp. equisimilis,* and *Streptococcus equi subsp. zooepidemicus* cause streptococcal toxic shock syndrome frequently（7）. As GBS has been consistent with *Streptococcus agalactiae*（10）, we did not further investigate the bacterial nomenclature of GBS. We did not confirm several bacterial nomenclatures precisely because we could not recover those bacteria at our lab after identification of them at Nagoya City University Hospital.

Antimicrobial susceptible analysis of BHS revealed that TC was no longer effective against these bacteria because TC resistant rates of BHS were about 50%. BHS except GCS were resistant to macrolide and lincosamide. Previous report showed that the EM resistant rates of GAS ranged from 10 to 20% during 1995 to 2004 in Japan（17）. However, EM resistant rates of both GAS and GGS in our study were over 30%. Recently clarithromycin and azithromycin, which we call new macrolide, have been used frequently against respiratory tract infectious disease and the emergence of macrolide-resistant *Streptococcus pneumoniae* has increased（18）. This fact may apply to BHS cases from our results. Furthermore, our previous investigation revealed that CLDM, which functions by similar mechanism as macrolide, induced virulent exoproteins from CLDM-resistant GAS（19）. Thus, we need further antimicrobial surveillance to prevent the spread of EM resistant GAS and GGS. GBS had most patterns of antimicrobial resistance; macrolide, lincosamide, tetracycline and fluoroquinolone（FQ）. GAS and GCS had FQ intermediate susceptible pattern and GGS had no FQ resistant pattern. As FQ has been widely used genitourinary disease, digestive disease and respiratory disease（20）, we assume that the change of FQ resistance among different streptococci represents the progress of streptococcal evolution against antimicrobial agent in future. BHS except GAS had small beta-lactam resistant rates in our study. As the emergence of penicillin resistant- GBS and GGS is one of the antimicrobial resistant problems in the world（21）, our result may also supports this serious situation.

We chose the EUCAST（European Committee on Antimicrobial Susceptibility Testing）（http://www.eucast.org/）for the evaluation of antimicrobial resistance instead of CLSI. An increasing accumulation of clinical data implies that the clinical success of antimicrobial agent therapy is related more to the minimum inhibitory concentration in Europe（22）. Although the breakpoints recommended by CLSI may be determined by *in vitro* assay, we need consider the discrepancy between *in vitro* and *in vivo* strictly. EUCAST was based on the accumulation of European clinical data and available for free instead of CLSI. The criteria of breakpoints in EUCAST were stricter than those previously recommended by many breakpoint committees including CLSI（23）. In the USA, the CLSI recently voted to adopt similar EUCAST guidelines（22）. As our results were available for streptococcal infection therapy

in Nagoya City University Hospital in future, we have adopted EUCAST criteria.

We mention the limitation of our study. As we used medical records appended to clinical species in this study, we could analyze neither the final clinical outcomes nor clinical laboratory data. Though we also could not distinguish infection from colonization about isolated bacteria in this study, several reports suggested that host-colonized streptococci, especially GGS, may invade elder host patient directly to cause severe infectious disease（6）,（24）. From these views, we considered the necessity of the analysis of total BHS strains and performed this study. However, further investigation is desired to accumulate the evidence of the true role of streptococcal colonization against severe infection disease.

In summary, we clarified the characteristics of BHS in Nagoya City University Hospital. Our data suggest that we need pay attention to the emergence of antimicrobial resistant BHS hereafter.

ACKNOWLEDGMENTS

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