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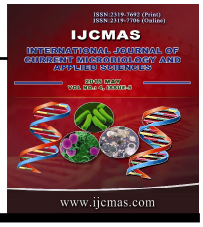

Original Research Article

Clinical Characteristics and Antimicrobial Susceptible Patterns of *Streptococcus dysgalactiae subsp, equisimilius* Isolates during 2009-2013 in Japan

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Keywords

Streptococcus dysgalactiae subsp, equisimilius. Clinicalcharacteristics, antimicrobial susceptibility

A B S T R A C T

Streptococcus dysgalactiae subsp. equisimilius cause various infectious diseases from acute pharyngitis to streptococcal toxic shock syndrome. Though *Streptococcus dysgalactiae subsp. equisimilius* infection has been increasing recently, the comprehensive characteristic investigation of the *Streptococcus dysgalactiae subsp. equisimilius* isolated in medical institution has not been performed in Japan. In this study, we investigated the clinical characteristics and antimicrobial susceptible patterns of 134 *Streptococcus dysgalactiae subsp. equisimilius* isolated at single medical institution during 2009 – 2013 in Japan. There was no significant difference between genders in *Streptococcus dysgalactiae subsp. equisimilius*. Half of *Streptococcus dysgalactiae subsp. equisimilius* were from over age 60. The clinical department from which *Streptococcus dysgalactiae subsp. equisimilius* was isolated most was pediatrics. *Streptococcus dysgalactiae subsp. equisimilius* in this study were completely susceptible to β-lactam antibiotics. In *Streptococcus dysgalactiae subsp. equisimilius*, clarithromycin, clindamycin, and minocycline non-susceptible rates were approximately 37%, 23% and 25%, respectively. Nonsusceptible rate of ciprofloxacin against *Streptococcus dysgalactiae subsp. equisimilius* was about 55%. Our results suggest the need for continuous antimicrobial susceptibility survey of *Streptococcus dysgalactiae subsp. equisimilius*.

Introduction

Most β-hemolytic streptococcus from human are identified as *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae subsp. equisimilius* (Facklam, 2002). In contrast to *Streptococcus pyogenes* and *Streptococcus agalactiae*, *Streptococcus dysgalactiae subsp. equisimilius* were long considered as commensal organisms that only rarely cause invasive infections as opportunistic pathogens (Brandt and Spellerberg, 2009). In 1996, *Streptococcus dysgalactiae subsp. equisimilius* was proposed to be a new streptococcal taxon (Vandamme *et al.*, 1996). It has also been reported to cause a wide variety of human infections such as pharyngitis, cellulitis, sepsis, meningitis, endocarditis, and streptococcal toxic shock syndrome (STSS) (Woo *et al.*, 2001; Hashikawa *et al.*, 2004).

Streptococcus dysgalactiae subsp. equisimilius possesses many virulence factors shared with *Streptococcus pyogenes*, such as M protein, streptolysin O, streptolysin S, streptokinase, and streptococcal inhibitory of complement lysis (Walter, 1989; Okumura, 1994; Schnizler, 1995; Humar, 2002; Minami, 2011). Although bacterial factors have been investigated precisely, little is known about the clinical characteristics of *Streptococcus dysgalactiae subsp. equisimilius* in Japan (Sunaoshi, 2010; Ichikawa, 2011). The present study was conducted to find out the clinical characteristics and antimicrobial susceptible patterns of *Streptococcus dysgalactiae subsp. equisimilius* isolated in Japan.

Materials and Methods

Strains and clinical data collection

A total of 134 *Streptococcus dysgalactiae subsp. equisimilius* isolates were obtained from various clinical specimens at Daido Hospital in Japan from 2009 to 2013. We used medical records appended to clinical species for the analysis of clinical feature at Daido 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 streptococci were identified by standard conventional biochemical methods or the VITEK2 system (bioMe'rieux, Durham NC, USA). Furthermore, we determined the identification of *Streptococcus dysgalactiae subsp. equisimilius* by 16S ribosomal RNA sequence methods (Woo *et al.*, 2001; Minami, 2011). Our experimental design was approved by the ethics committee at Daido hospital.

Antimicrobial susceptibility analysis

Streptococcus dysgalactiae subsp. equisimilius isolates were examined for 15 antibiotic susceptibilities as follows; piperacillin, amoxicillin, cefotiam, flomoxef, ceftazidime, ceftriaxone, panipenem, vancomycin, fosfomycin, minocycline, clarithromycin, clindamycin, amikacin, isepamicin, and ciprofloxacin. Antimicrobial susceptibility was determined by the disc diffusion technique (Kirby-Bauer method). These results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) criteria (Clinical and Laboratory Standards Institute, 2014).

Statistical analysis of the data

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.

Results and Discussion

First of all, we evaluated the relationship between clinical patients' features and

Streptococcus dysgalactiae subsp. equisimilius.

The number of *Streptococcus dysgalactiae subsp. equisimilius* isolates tended to increase with each passing year (Figure 1). The number of *Streptococcus dysgalactiae subsp. equisimilius* isolates in2011 was about two times as same as that in 2009.The number of *Streptococcus dysgalactiae subsp. equisimilius* isolates in both 2012 and 2013 was about four times as same as that in 2009.

The total number of female patients was same as that of males for 5 years (Figure 1). Although the number of male patients was larger than that of female patients in 2011, the number of male patients was smaller than that of female patients in 2012. In short, there was no significant difference between genders in *Streptococcus dysgalactiae subsp. equisimilius*.

The age range was categorized every 10 years old except under one-year in figure 2. The number of 1–10 years patients was largest in this study ($p< 0.05$). The number of 10–60 years patients was low for 5 years. Totally, about Fifty-three percent of *Streptococcus dysgalactiae subsp. equisimilius* were isolated from patients of over 60 years.

The relationship between clinical department and *Streptococcus dysgalactiae subsp. equisimilius* revealed that pediatrics (35.1%) was the most frequent clinical department from five years($p<0.05$) (Figure 3). Approximate thirteen percent of *Streptococcus dysgalactiae subsp. equisimilius* were isolated from respiratory medicine and general medicine, respectively.

We also represent that the relationship between biological sources and *Streptococcus dysgalactiae subsp. equisimilius* (Figure 4). The biological sources of *Streptococcus dysgalactiae subsp. equisimilius* isolated most was sputum (27%) for five years ($p<0.05$). Twenty-one *Streptococcus dysgalactiae subsp. equisimilius* was isolated from nasal discharge in five years. Approximately nine percent of *Streptococcus dysgalactiae subsp. equisimilius* were isolated from blood, pharyngeal mucus, urine, and tonsil, respectively in five years. Twenty-three *Streptococcus dysgalactiae subsp. equisimilius* (17.1%) were isolated from aseptic site in five years. The number of *Streptococcus dysgalactiae subsp. equisimilius* isolates from sputum tended to increase with each passing year. The number of *Streptococcus dysgalactiae subsp. equisimilius* isolates from nasal discharge tended to increase after 2012.

Finally, we analyzed the antimicrobial susceptibility of *Streptococcus dysgalactiae subsp. equisimilius* in this study. All β-lactam, carbapenem and glycopeptide were susceptible against *Streptococcus dysgalactiae subsp. equisimilius*. However, all aminoglycosides such as amikacin and isepamicin were resistant against *Streptococcus dysgalactiae subsp. equisimilius*. Figure 5(A) showed the susceptibility of clarithromycin against *Streptococcus dysgalactiae subsp. equisimilius*. The highest non-susceptible rate of clarithromycin was about 52% in 2011 and the lowest non-susceptible rate of clarithromycin was about 23% in 2013. The total non-susceptible rate was about 37% for 5 years. Especially, the total resistant rate of clarithromycin was about 20% for 5 years. Figure 5(B) showed the susceptibility of clindamycin against *Streptococcus dysgalactiae subsp. equisimilius*. The highest non-susceptible rate of clindamycin was 30% in 2009 and the lowest non-

susceptible rate of clindamycin was 20% in 2012. The total non-susceptible rate was about 23% for 5 years. Especially, the total resistant rate of clarithromycin was about 19% for 5 years. Figure 5(C) showed the susceptibility of minocycline against *Streptococcus dysgalactiae subsp. equisimilius*. The highest non-susceptible rate of minocycline was 40% in 2009 and the lowest non-susceptible rate of minocycline was about 9% in 2013. The total non-susceptible rate was about 25% for 5 years. However, the total resistant rate of minocycline was about 7% for 5 years. Figure 5(D) showed the susceptibility of ciprofloxacin against *Streptococcus dysgalactiae subsp. equisimilius*. The highest non-susceptible rate of ciprofloxacin was 81% in 2011 and the lowest non-susceptible rate of ciprofloxacin was about 10% in 2009. The total non-susceptible rate was about 55% for 5 years. However, the total resistant rate of ciprofloxacin was about 12% for 5 years.

Although many previous studies represented the data of only invasive streptococcal diseases (Hashikawa *et al.*, 2004; Brandt and Spellerberg, 2009; Takahashi, 2010a; Takahashi, 2010b), the comprehensive analysis of recent *Streptococcus dysgalactiae subsp. equisimilius* in Japanese general hospital have been seldom performed (Sunaoshi, 2010; Ichikawa, 2011). In this study, we described the clinical characteristics and antimicrobial susceptible patterns of all *Streptococcus dysgalactiae subsp. equisimilius* isolated from general hospital in Japan among recent 5 years. Although we have little interest of *Streptococcus dysgalactiae subsp. equisimilius* as compared with *Streptococcus pyogenes* and *Streptococcus agalactiae* before, this study may imply that *Streptococcus dysgalactiae subsp. equisimilius* will increase gradually from now on.

With respect to gender group, the number of isolation in female patients was same as that in male patients. This result is almost consistent with previous report (Ichikawa, 2011). We clarified *Streptococcus dysgalactiae subsp. equisimilius* with age distribution. Young patients under 10-years frequently caused *Streptococcus dysgalactiae subsp. equisimilius* infection. *Streptococcus dysgalactiae subsp. equisimilius* was hardly isolated from patients ranging from 20 to 60 years. However, the large numbers of *Streptococcus dysgalactiae subsp. equisimilius* were isolated from over 60s-patients. Previous report also showed that elder people were infected with severe *Streptococcus dysgalactiae subsp. equisimilius* more often (Takahashi, 2010a; Ichikawa, 2011). Some studies revealed that more *Streptococcus dysgalactiae subsp. equisimilius* recently caused invasive infection (Hashikawa *et al.*, 2004; Brandt and Spellerberg, 2009; Takahashi, 2010a; Takahashi, 2010b). This hypothesis may be supported by the fact that 13 (about 10%) *Streptococcus dysgalactiae subsp. equisimilius* was isolated from blood sample in our studies. Although we did not confirmed streptococcal toxic shock syndrome cases in this study, the number of streptococcal toxic shock syndrome caused by *Streptococcus dysgalactiae subsp. equisimilius* may be increasing. In the analysis of clinical departments, the department where most patients with *Streptococcus dysgalactiae subsp. equisimilius* was detected, were pediatrics. Acute pharyngitis and tonsillitis are usually popular as pediatric or otolaryngology diseases. Although *Streptococcus pyogenes* is the main pathogenic bacteria which cause pharyngitis and tonsillitis (Caparon, 2001) and the number of *Streptococcus dysgalactiae subsp. equisimilius* causing pharyngitis and tonsillitis may also be increasing.

Figure.1 Gender distribution of *Streptococcus dysgalactiae subsp. equisimilius*

Figure.2 Age distribution of *Streptococcus dysgalactiae subsp. equisimilius*

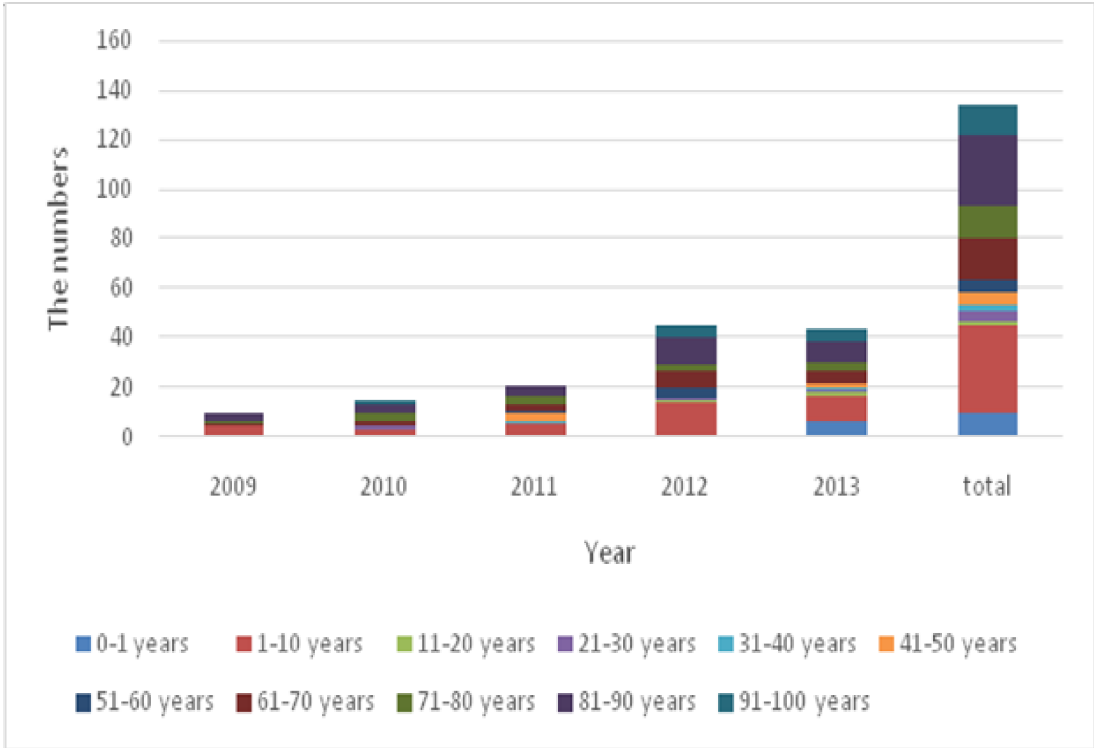


Figure.3 Biological source distribution of *Streptococcus dysgalactiae subsp. equisimilius*

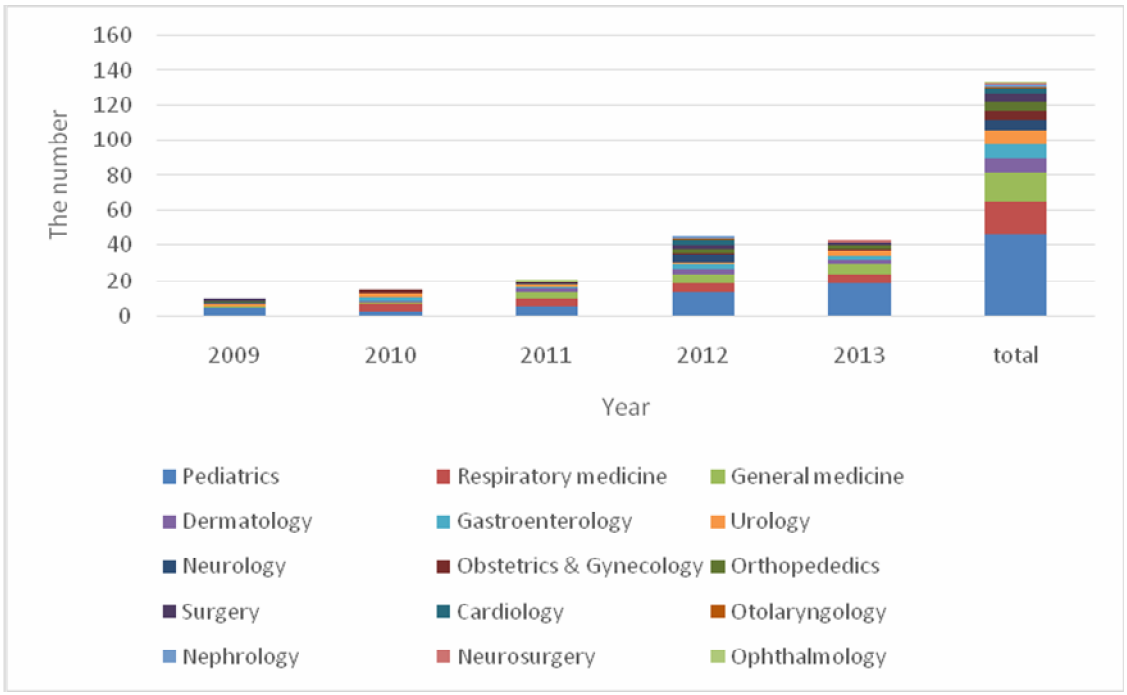


Figure.4 Clinical department distribution of *Streptococcus dysgalactiae subsp. equisimilius*

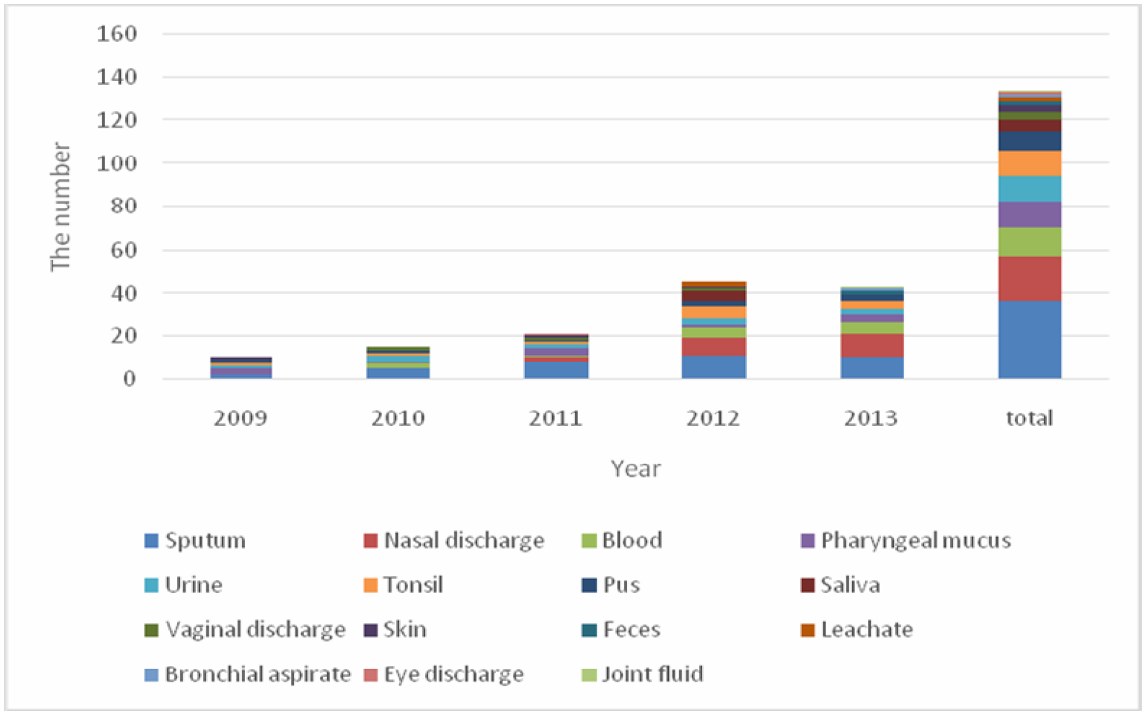
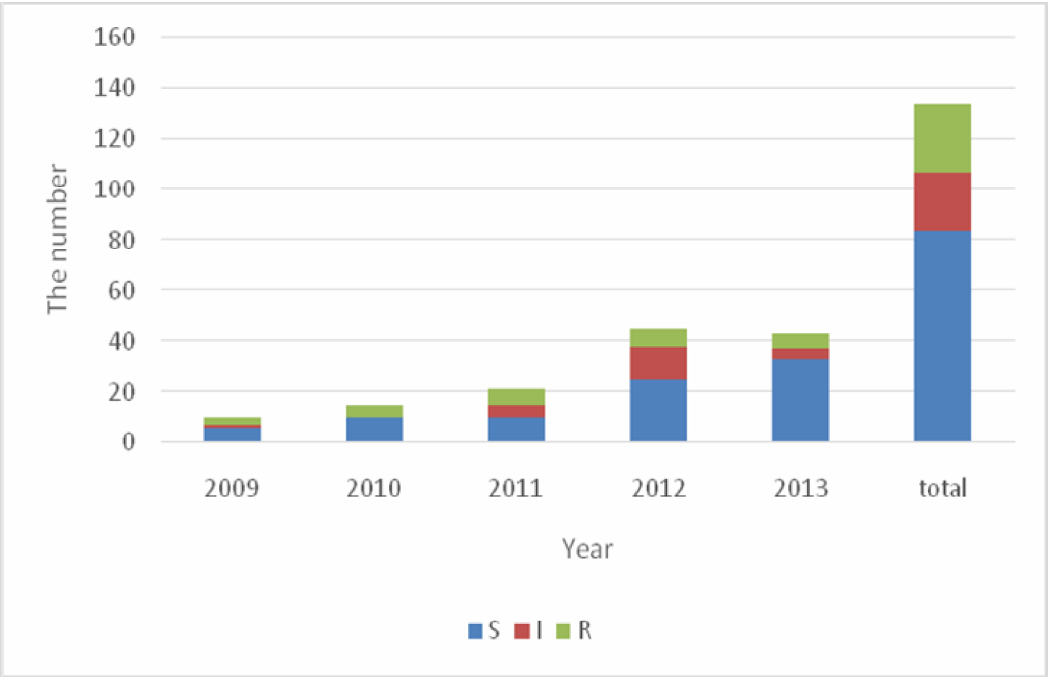
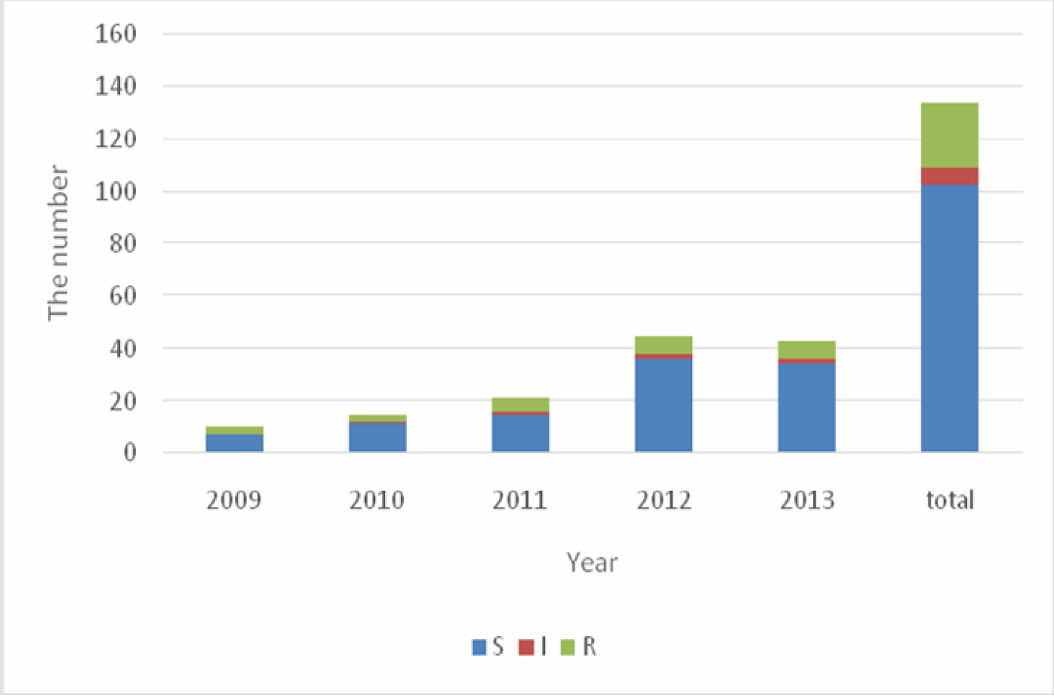
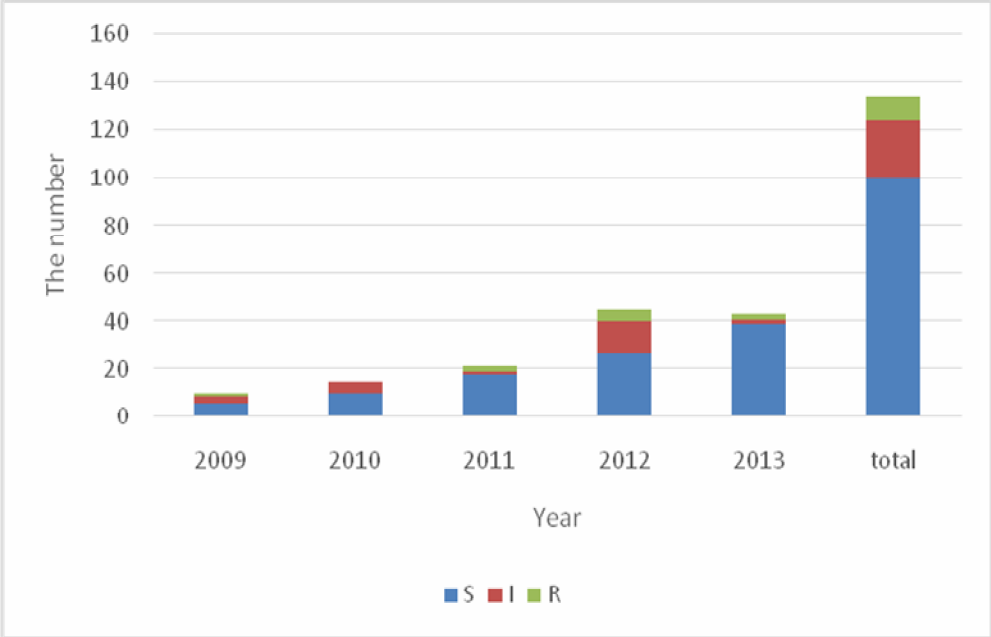
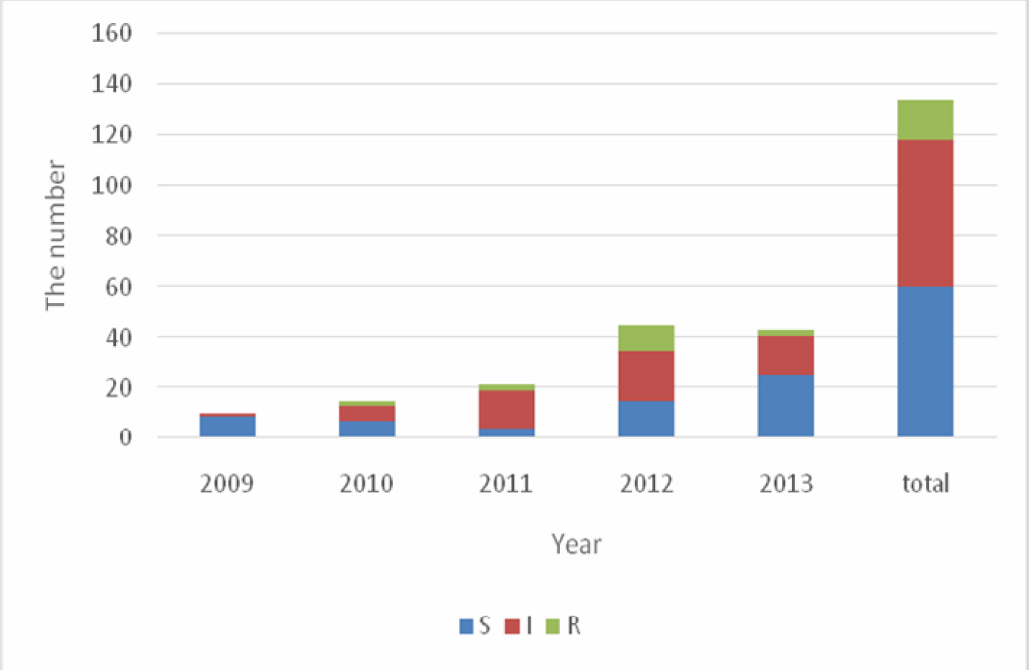


Figure.5 Antimicrobial susceptibility of *Streptococcus dysgalactiae subsp. equisimilius* 5(A): Clarithromycin, 5(B): Clindamycin, 5(C): Minocycline, 5(D): Ciprofloxacin. S: sensitive, I: intermediate, R: resistant.









Except pediatrics, *Streptococcus dysgalactiae subsp. equisimilius* frequently was isolated from respiratory medicine and general medicine. This may be the reason why elder people often consult those departments because of respiratory symptom. Most biological sources of *Streptococcus dysgalactiae subsp. equisimilius* were strongly related with the result of clinical department. Especially, the large number of *Streptococcus dysgalactiae subsp. equisimilius* was obtained from

respiratory system as follows; sputum, nasal discharge, pharyngeal mucus and tonsil in our studies. This result may also support that *Streptococcus dysgalactiae subsp. equisimilius* possesses the characteristics of air-infectious pathogen. Antimicrobial susceptible analysis of *Streptococcus dysgalactiae subsp. equisimilius* revealed that fluoroquinolone was no longer significant effective. Because ciprofloxacin non-susceptible rate of *Streptococcus dysgalactiae subsp. equisimilius* was over 50%in our studies. As fluoroquinolone has been widely used genitourinary disease, digestive disease and respiratory disease (Davis, 1994). We assume that the change of fluoroquinolone resistance among *Streptococcus dysgalactiae subsp. equisimilius* spread worldwide in future. The total macrolide non-susceptible rate of *Streptococcus dysgalactiae subsp. equisimilius* was about 40% in our study. Previous report showed that the macrolide non-susceptible rates of *Streptococcus dysgalactiae subsp. equisimilius* were about 30% (Ichikawa, 2011). Recently clarithromycin and azithromycin have been used frequently against respiratory tract infectious disease and the emergence of macrolide-resistant *Streptococcus pneumonia* has increased (Ubukata, 2003; Minami, 2014). This fact may apply to *Streptococcus dysgalactiae subsp. equisimilius* cases from our results. Thus, we need further antimicrobial surveillance to prevent the spread of macrolide resistant *Streptococcus dysgalactiae subsp. equisimilius*. However, the total lincosamide and tetracycline non-susceptible rate of *Streptococcus dysgalactiae subsp. equisimilius* ranged from 23 to 25 % in our study. Previous report showed that the lincosamide and tetracycline non-susceptible rates of *Streptococcus dysgalactiae subsp. equisimilius* were about 20% and 50%, respectively (Ichikawa, 2011). Compared to ciprofloxacin and clarithromycin, clindamycin and minocycline may be effective against *Streptococcus dysgalactiae subsp. equisimilius* for a short while. From these views, we considered the further necessity of the analysis of *Streptococcus dysgalactiae subsp. equisimilius* strains.

In summary, we clarified the characteristics of *Streptococcus dysgalactiae subsp. equisimilius* in mid-Japan. Our data suggest that we need pay attention to the emergence of antimicrobial-resistant *Streptococcus dysgalactiae subsp. equisimilius* hereafter.

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