A decennium of etiology and antimicrobial susceptibility patterns in patients with infective endocarditis at a university hospital, Thailand

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SUMMARY

Infective endocarditis is an infection with a high mortality rate. Antimicrobial therapy is important in treatment but data on antimicrobial susceptibilities are limited. This retrospective study analyzed the data of causative microorganisms and antimicrobial susceptibility patterns in patients with infective endocarditis who were aged 18 years or older and received inpatient care between 2006 and 2015 at King Chulalongkorn Memorial Hospital. A total of 213 patients fulfilled the inclusion criteria. Streptococcus spp. (54.5%) was the most common organism and Viridans streptococcus (46%) was the leading pathogen, followed by Group B streptococcus (27%). The majority of Streptococcus spp. was susceptible to penicillin (82.7%). Among Streptococcus spp., Streptococcus suis had the highest MIC$_{90}$ of penicillin and cefotaxime (1.65 µg/ml and 0.95 µg/ml). There was a statistically significant increase in the MICs of penicillin and cefotaxime for Streptococcus suis (p = 0.03 and 0.04). Only 45.5% of Streptococcus suis and 77.5% of Viridans streptococcus were susceptible to penicillin. All Enterococcus spp., Staphylococcus spp. were susceptible to vancomycin. In conclusion, Group B streptococcus isolates increased in patients with infective endocarditis in Thailand. Streptococcus suis had the highest MIC$_{90}$ and penicillin-nonsusceptible isolates. Rigorously restrict using of antimicrobial agents in animal feeds should more concern.

INTRODUCTION

Infective endocarditis (IE) is an important disease despite its rare occurrence in Thailand (1-3), as it is a systemic infection associated with a high mortality rate (3-5). In Thailand, the mortality rate ranges from 11% to 25% (1, 3). The identification of causative microorganisms and data of antimicrobial susceptibilities are helpful in the empirical therapy in patients with IE. The most common pathogens in IE are Gram positive bacteria:
Streptococcus spp., Staphylococcus spp. and Enterococcus spp. (3-5); however, up-to-date antimicrobial susceptibility patterns among Streptococcus spp., Staphylococcus spp. and Enterococcus spp. in patients with IE are limited. This research comprises a retrospective study to analyze the data of causative microorganisms and antimicrobial susceptibility patterns over a period of 10 years (2006 - 2015) in patients with IE at King Chulalongkorn Memorial Hospital.

MATERIALS AND METHODS

Patients and population: A retrospective study was conducted at King Chulalongkorn Memorial Hospital, a 1,479 bed, tertiary referral and teaching hospital for the Faculty of Medicine, Chulalongkorn University and the Thai Red Cross College of Nursing. Inclusion criteria were as follows: (1) infective endocarditis patients aged 18 years or older (2) who received inpatient care at King Chulalongkorn Memorial Hospital between 2006 and 2015. ICD 10 diagnostic code was used to identify the infective endocarditis condition. Research Affairs, Faculty of Medicine, Chulalongkorn University reviewed and approved the study (IRB No.613/58).

Data collection: Data were retrieved from medical records and collected, including baseline demographics, causative microorganisms and data of antimicrobial susceptibilities. Modified Duke criteria were used to define possible and definite cases of infective endocarditis. For bacterial identification, the 5-ml sample was inoculated into an aerobic bottle of blood culture broth (Versatrek Diagnostic Systems, Cleveland, OH, USA), and then was incubated at 37 °C for 7 days. The identification of Viridans streptococcus was performed by analytical profile index (API) test. For antimicrobial susceptibility testing, the Kirby-Bauer disk diffusion method was performed according to the Clinical and Laboratory
Standards Institute (CLSI) 2015. Minimal inhibitory concentration (MICs) were measured by E-test and interpreted in accordance with CLSI 2015 (6).

**Statistical analysis:** Data were analyzed by SPSS version 20.0 for Windows (SPSS Co., Ltd. Bangkok, Thailand). Mean ± standard deviation (SD) and frequencies were used to describe patients’ baseline characteristics. For frequencies, the 50th and 90th percentiles were used to describe antimicrobial susceptibilities. Chi-square test was used for categorical variables. MICs were compared using the Wilcoxon Rank sum test. Variables were found to be significant at p < 0.05.

**RESULTS**

A total of 213 patients with infective endocarditis (IE) were diagnosed at King Chulalongkorn Memorial Hospital between January 2006 and December 2015. The mean patient age was 50.7 ± 17.7 years. 129 (60.6%) patients were male. 149 patients were categorized as definite IE according to modified Duke criteria. Most IE episodes occurred in the native valve (81.7%). Thirty-nine (18.3%) patients had diabetes mellitus. Ceftriaxone was predominant empiric therapeutic regimens (28.2%). Penicillin G plus aminoglycosides regimen (64.4%) and cloxacillin (40.7%) were predominant documented therapeutic regimens for *Streptococcus* spp. and *Staphylococcus* spp., respectively. The overall in-hospital mortality rate was 17.4 %. For *Streptococcus* spp. and *Staphylococcus* spp., In-hospital mortality rate were 24.3% and 40.5%, respectively. Among 212 hemoculture results, the causative microorganisms were identified in 174 (82%) patients. Baseline characteristics are shown in Table 1. The most common causative microorganisms were gram positive organisms. From the total gram positive bacterial isolates, *Streptococcus* spp. were ranked first 90 (54.5%), followed by *Staphylococcus* spp. 54 (32.7%) and *Enterococcus* spp. 11 (6.7%). For *Streptococcus* spp., Viridans streptococcus (46%) was the leading causative
microorganisms, followed by Group B streptococcus (27%). The species distribution among Streptococcus spp. is presented in Figure 1. Among Viridans streptococcus, Streptococcus oralis (17%) were the most frequent isolates. For Staphylococcus spp., the most common pathogens were Staphylococcus aureus (75.9%) and 6 (14.6%) isolates were methicillin resistant Staphylococcus aureus.

**Data of antimicrobial susceptibilities over 10 years (2006-2015)**

Seventy two (82.7%) isolates of Streptococcus spp. were susceptible to penicillin, and all Streptococcus spp. were susceptible to cefotaxime and vancomycin. The MIC$_{50}$ and MIC$_{90}$ of penicillin were 0.094 and 0.25 µg/ml; MIC$_{50}$ and MIC$_{90}$ of cefotaxime were 0.125 and 0.464 µg/ml and for vancomycin were 0.5 and 1 µg/ml. Among Streptococcus spp., Streptococcus suis had the highest MIC$_{90}$ of penicillin (1.65 µg/ml) and cefotaxime (0.95 µg/ml) followed by Viridans streptococcus (MIC$_{90}$ of penicillin and cefotaxime were 0.25 µg/ml and 0.38 µg/ml). Beta-hemolytic streptococcus and Streptococcus bovis had lower MIC$_{90}$ of penicillin (0.094 µg/ml) and cefotaxime (0.132 and 0.25 µg/ml). Only 5 isolates (45.5%) of Streptococcus suis were susceptible to penicillin. Thirty-one (77.5%) isolates of Viridans streptococcus were susceptible to penicillin. All Beta-hemolytic streptococcus and Streptococcus bovis were susceptible to penicillin. All Staphylococcus spp. were susceptible to vancomycin. The MIC$_{50}$ and MIC$_{90}$ of vancomycin were 1.5 and 2 µg/ml. All Enterococcus spp. were susceptible to ampicillin and vancomycin. The 3 isolates of Enterococcus spp. were intrinsically resistant to high levels of aminoglycoside antibiotics (gentamicin 2 isolates and streptomycin 1 isolate). The antimicrobial susceptibilities of causative microorganisms over 10 years are presented in Table 2.
MICs comparison between the first five-year-period (2006-2010) and second five-year-period (2011-2015) of *Streptococcus* spp., *Viridans streptococcus* and *Streptococcus suis*

There was a statistically significant increase in the MICs of penicillin and cefotaxime for *Streptococcus suis* during the second five-year-period (*p* = 0.03 for penicillin, *p* = 0.04 for cefotaxime) (Figure 2A) but no statistically significant difference in MICs for *Viridans streptococcus* over the 2 periods. (Figure 2B). The MICs of penicillin, cefotaxime and vancomycin for *streptococcus* spp. were not statistically significant different over the two periods (Figure 2C).

**DISCUSSION**

The characteristics of infective endocarditis patients in this study were similar to other studies (4,5,7). Most patients were male and most IE episodes occurred in the native valve. When comparing the first five-year-period (2006-2010) and second five-year-period (2011-2015), there was statistically significant increasing age of infective endocarditis patients. The proportion of diabetes mellitus patients was increasing from 15.6% to 20.8% but no statistically significant difference. The three most causative microorganisms were *Streptococcus* spp., *Staphylococcus* spp. and *Enterococcus* spp., which is similar to the finding of a previous study in Thailand and Japan (1-3, 8, 9). *Streptococcus* spp. was also found the most causative microorganism in Laos and China (10, 11). The causative microorganisms of infective endocarditis vary according to region. Developed countries have observed an increasing trend for infective endocarditis caused by *Staphylococcus* spp. (7,12,13). This could be attributed to invasive medical technology, high population of intravenous drug injection and *Staphylococcus aureus* nasal carriage rates were higher in Europe and USA than Southeast Asia (14). *Staphylococcus* spp. was one of the risk factors for mortality in patients with infective endocarditis. Overall in-hospital mortality rate in this
study was 17.4% which was lower than other studies (4,5). This study also showed higher mortality rate in *Staphylococcus* spp. infection than *Streptococcus* spp. infection. Beta-hemolytic streptococcus and *Streptococcus suis* were uncommon causative microorganisms in infective endocarditis (2, 3, 15). A study conducted in the United States also showed a decreasing incidence of Group B streptococcus among infants but an increasing incidence of Group B streptococcus in adults. Furthermore, all isolates were susceptible to penicillin, which is similar to the present study (16). In Thailand, there was an increasing trend for Group B streptococcus infection particularly blood stream infection and meningitis (17). Ongoing research need to be explored the cause of increasing of Group B streptococcus infection. This study also showed an increasing trend for infective endocarditis caused by Group B streptococcus when comparing the first five-year-period (2006-2010) and the second five-year-period (2011-2015) from 11.6% to 15.9%, respectively. The explanation from this study may be an increasing age and higher proportion of diabetes mellitus in patients with infective endocarditis during the second five-year-period. Previous study from Thailand showed that bacteremia followed by bone and joint infection was the most common clinical manifestation (18). Sixty percent of patients in this study had coinfection particularly septic arthritis and hematogenous seeding could be the predominant mechanism of infection (19). The incidence of Group B streptococcus infective endocarditis in this study was higher than the other studies (3, 17). Many studies in Brazil and Belgium showed only 1.6% and 0.9% of Group B streptococcus infective endocarditis, respectively (4, 20). For *Streptococcus suis*, a meta-analysis showed that the prevalence of *Streptococcus suis* infection was the highest in Asia, particularly in Thailand (36%) (21). There were different data for antimicrobial susceptibilities among *Streptococcus* spp., while *Streptococcus suis* had the highest MIC$_{90}$. One explanation for increasing MICs of *Streptococcus suis* could be the usage of antimicrobial agents in animal feeds with decreasing penicillin susceptibility (62%) in
Thailand (22). All *Streptococcus suis* isolated from patients with meningitis from Vietnam were susceptible to penicillin, ceftriaxone and vancomycin. MIC$_{90}$ of penicillin and ceftriaxone were 0.047 and 0.125 µg/ml, respectively which was lower than our study (23). The same trend of antimicrobial susceptibilities for invasive *Streptococcus suis* infection was found in Poland and Hong Kong (24, 25). A retrospective study at Chiang Mai University Hospital, Thailand from 2000 to 2002 showed that all *Streptococcus suis* were susceptible to penicillin (mean MIC$_{90} = 0.028$ µg/ml), whereas only 45.5% of *Streptococcus suis* in the present study were susceptible to penicillin, and MIC$_{90}$ of penicillin was 1.65 µg/ml (26). The present study also showed an elevated MIC$_{90}$ of penicillin in both Viridans streptococcus and Hemolytic streptococcus when compared to a previous study (27). For *Staphylococcus* spp., many previous studies found that *Staphylococcus aureus* were more common than coagulase negative *staphylococcus* spp. (2, 4, 5). The same trend was found in this study. The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in the current study was 14.6%. Most MRSA patients had a prostatic valve and been admitted to hospital within three months. All *staphylococcus* spp. were susceptible to vancomycin. *Enterococcus faecalis* were a more common pathogen than *Enterococcus faecium* (4, 5). All *Enterococcus* spp. in the present study were *Enterococcus faecalis*, and all isolates were susceptible to ampicillin and vancomycin, which is similar to the finding of a study conducted in Laos and Turkey (5, 10). According to the 2015 ESC Guidelines for the management of infective endocarditis, penicillin G was the preferred antimicrobial agent for oral *Streptococcus, Streptococcus bovis* and Hemolytic streptococcus. The appropriate dose of penicillin G depends on MICs of penicillin for *Streptococcus* spp. Due to an increasing MIC$_{90}$ but decreasing susceptibility to penicillin for *Streptococcus suis* and Viridans streptococcus in the present study, a high dose of penicillin G (24 million Units/day) or ceftriaxone should be more appropriate empiric therapeutic regimens than low dose penicillin G (12–18 million Units/day) (28). There are
some limitations of our study. First, this research was a retrospective study and, thus, some data were not available. Second, some infective endocarditis patients were referred from another hospital, and so some data for antimicrobial susceptibilities were lacking (12%). Third, species identification of Viridans streptococcus for the first period was not available.

In conclusion, in Thailand, *Streptococcus* spp. was the most causative microorganisms, and the incidence of Group B streptococcus isolates showed an increase in patients with infective endocarditis. There were elevated MICs and penicillin-non-susceptible isolates among *Streptococcus suis* and Viridans streptococcus. *Streptococcus suis* had the highest MIC$_{90}$ and penicillin–non-susceptible isolates, therefore rigorously restrict using of antimicrobial agents in animal feeds should more concern. High dose penicillin G or ceftriaxone appeared to be more appropriate empiric therapeutic regimens. It is recommended to measure MICs of penicillin for *Streptococcus* spp. to determine the most appropriate antimicrobial regimen.

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**CONFLICT OF INTEREST:** None to declare

**REFERENCES**


Table 1 Characteristics of 213 patients with infective endocarditis between January 2006 and December 2015

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value (%)</th>
<th>Characteristics</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>129 (60.6)</td>
<td>Streptococcus spp.</td>
<td>90 (54.5)</td>
</tr>
<tr>
<td>Age (years); mean±SD</td>
<td>50.7±17.7</td>
<td>-Viridans streptococcus*</td>
<td>41 (46)</td>
</tr>
<tr>
<td>Clinical characteristic</td>
<td></td>
<td>Staphylococcus spp.</td>
<td>54 (32.7)</td>
</tr>
<tr>
<td>Definite case</td>
<td>149 (70)</td>
<td>-Staphylococcus aureus</td>
<td>41 (75.9)</td>
</tr>
<tr>
<td>Possible case</td>
<td>64 (30)</td>
<td>- Methicillin resistant Staphylococcus aureus</td>
<td>6 (14.6)</td>
</tr>
<tr>
<td>Valve type</td>
<td></td>
<td>-Coagulase negative Staphylococcus spp. (CoNS)</td>
<td>13 (24.1)</td>
</tr>
<tr>
<td>Native valve</td>
<td>174 (81.7)</td>
<td>-Methicillin resistant CoNS</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td>Prostatic valve</td>
<td>39 (18.3)</td>
<td>Enterococcus faecalis</td>
<td>11 (6.7)</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>37 (17.4)</td>
<td>Other Gram positive organisms**</td>
<td>10 (6.1)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>9 (24.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>15 (40.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microorganisms</td>
<td></td>
<td>Gram negative organisms***</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Culture positive</td>
<td>174 (82)</td>
<td>Fungal (Aspergillus flavus)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Gram positive organisms</td>
<td>165 (94.8)</td>
<td>Zoonosis (Bartonella henselae)</td>
<td>1 (0.6)</td>
</tr>
</tbody>
</table>

*S.oralis (7), S. sanguinis (6), S.mitis (6), S.constellatus (3), S.melleri (1), S.intermedius (1), S.parasanguinis (1), S.angiosus (1), Other viridans streptococcus (21),

**Micrococcus luteus (1), Micrococcus spp. (2), Gemella morbillo (3), Abiotrophia defectiva (1),
Aerococcus spp. (1), Granulicatella adiacens (1), Gamella haemolysans (1)

*** Escherichia coli (2), Klebsiella pneumoniae (1), Acinetobacter baumannii (1), Moraxella spp. (1),
Burkholderia cepacia (1), Haemophilus influenzae (1)
Table 2 The antimicrobial susceptibilities of causative microorganisms over 10 years (2006-2015)

<table>
<thead>
<tr>
<th>Microorganisms (total)</th>
<th>Penicillin&lt;sup&gt;N (%)&lt;/sup&gt;</th>
<th>Cefotaxime&lt;sup&gt;N (%)&lt;/sup&gt;</th>
<th>Vancomycin&lt;sup&gt;e,N (%)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>Range</td>
<td>S&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Streptococcus spp. (87)</td>
<td>0.094/0.25</td>
<td>0.003-2</td>
<td>72 (82.7)</td>
</tr>
<tr>
<td>Viridans Streptococcus (40)</td>
<td>0.094/0.25</td>
<td>0.003-0.380</td>
<td>31 (77.5)</td>
</tr>
<tr>
<td>Beta-hemolytic Streptococcus (26)</td>
<td>0.064/0.094</td>
<td>0.016-0.125</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Streptococcus suis (11)</td>
<td>0.190/1.65</td>
<td>0.032-2</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Streptococcus bovis (11)</td>
<td>0.094/0.094</td>
<td>0.064-0.094</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Staphylococcus spp.&lt;sup&gt;f&lt;/sup&gt; (54)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Enterococcus spp.&lt;sup&gt;g&lt;/sup&gt; (8)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviation: S: susceptible, I: intermediate, R: resistant, NA: not applicable
<sup>b</sup> CLSI breakpoints: Streptococcus spp.: Penicillin, S ≤ 0.12 µg/ml, I 0.25-2 µg/ml, R > 4µg/ml
<sup>c</sup> CLSI breakpoints: Streptococcus spp.: Cefotaxime, S ≤ 1 µg/ml, I 2 µg/ml, R ≥ 4 µg/ml
<sup>d</sup> CLSI breakpoints: Streptococcus spp.: Vancomycin, S ≤ 1 µg/ml
<sup>e</sup> CLSI breakpoints: Staphylococcus spp.: Vancomycin, S ≤ 2 µg/ml, I 4-8 µg/ml, R ≥ 16 µg/ml
<sup>f</sup> MIC<sub>50</sub> of Ampicillin for Enterococcus spp. is 1.5 µg/ml, and all Enterococcus spp. are sensitive to Ampicillin
<sup>g</sup> The 3 isolates were intrinsically resistance to high levels of aminoglycoside antibiotics (gentamicin 2 isolates and streptomycin 1 isolate)
Figure 1 Species distribution among *Streptococcus* spp.
2A (Streptococcus suis)

![MIC (µg/ml) vs Years for Penicillin, Cefotaxime, and Vancomycin for Streptococcus suis]

2B (Viridans streptococcus)

![MIC (µg/ml) vs Years for Penicillin, Cefotaxime, and Vancomycin for Viridans streptococcus]
Figure 2  The MICs comparison between the first five-year-period (2006-2010) and the second five-year-period (2011-2015)