Original Article

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The susceptibility of penicillin, ceftriaxone, and cefotaxime against *Streptococcus pneumoniae* isolated from patients with invasive pneumococcal diseases at a teaching hospital in Thailand

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ABSTRACT

Objective: Pneumococcal disease is a severe invasive infection causing morbidity and death, so the appropriate antimicrobials are greatly important. We aimed to determine the minimum inhibitory concentration (MIC) of penicillin, cefotaxime and ceftriaxone against S. pneumoniae.
Methods: The clinical isolates were obtained from patients with invasive pneumococcal disease admitted to Phramongkutklao Hospital, Thailand over a period of 10 years between 2006 and 2015. The in vitro antimicrobial susceptibility testing of antibiotics including penicillin, ceftriaxone and cefotaxime against S. pneumoniae isolates were determined by E-test. The MIC range, MIC50, and MIC90 (μg/ml) and percentage of susceptible isolates were recorded.
Results: Forty clinical isolates were collected, the MIC range, MIC50 and MIC90 for penicillin were: ≤0.016-1.5 μg/ml, 0.25 μg/ml and 1 μg/ml, respectively. Only 35% of them were penicillin susceptible strains. Even our studied S. pneumoniae isolates demonstrated lower trends of MICs to cefotaxime than to ceftriaxone but the MIC50/90 for cefotaxime and ceftriaxone were equal to 0.19/0.5 μg/ml, whereas the same MIC range for cefotaxime and ceftriaxone was ≤0.016-0.5 μg/ml.
Conclusions: Penicillin G may no longer be an appropriate empirical pneumococcal meningitis treatment, although ceftriaxone and cefotaxime remained good activity as first-line agents for community-acquired meningitis due to S. pneumoniae. However, the non-susceptible third-generation cephalosporin strains need to be closely monitored in Thailand.

Keywords: Pneumococcal diseases, antimicrobial susceptibility, and minimum inhibitory concentration

INTRODUCTION

Streptococcus pneumoniae, Gram-positive diplococcus bacteria, is the leading cause of upper and lower respiratory tract infections and invasive pneumococcal disease (IPD) such as bacteremia, meningitis, and meningoencephalitis.[1] In Thailand, the incidence of IPD was estimated to be around 17/100,000 persons/year.[2] Since IPD is a severe infection that is associated with morbidity and death, the appropriate antimicrobials use is important.[3] However, the treatment of *S. pneumoniae* infection has become a challenge because of multidrug-resistant *S. pneumoniae*.

According to the National Antimicrobial Resistance Surveillance Thailand data for 2017, which was collected from 74 hospitals, *S. pneumoniae* isolates obtained from blood specimens were found to be non-susceptible to tetracycline, cotrimoxazole, erythromycin, clindamycin, and penicillin (determined by oxacillin susceptibility test), with the rates of 71.9%, 52.4%, 30.8%, 27.4%, and 31.1%, respectively. However, the susceptibility profile of ceftriaxone and cefotaxime as mainstay choices for empirical therapy against *S. pneumoniae* was not reported in this database.[4]

Penicillin resistance in *S. pneumoniae* remains a serious concern worldwide, particularly in Asian countries. A large surveillance study from Asian Network for Surveillance of Resistant Pathogens investigated 2184 *S. pneumoniae* isolates in 11 Asian countries during 2008–2009. They found that the prevalence of intermediate resistant (minimum inhibitory concentration; MIC 0.12–1 μg/ml) and resistant (MIC ≥2 μg/ml) to penicillin was 44.9% and 18.8%, respectively. Unfortunately, ceftriaxone resistance was also reported with the prevalence of 3.7% and 0.1% in non-meningeal and meningeal *S. pneumoniae* isolates, respectively.[5] Nowadays, the World Health Organization has announced; there is an urgent need for new antibiotics to treat drug-resistant *S. pneumoniae*.[6]

There were a few studies of penicillin and third-generation cephalosporins susceptibility *S. pneumoniae* in Thailand. Srifuengfung *et al*. reported *S. pneumoniae* from sterile site isolates at Siriraj Hospital in 2008 revealing 7.8% and 9.8% of which were penicillin and cefotaxime non-susceptible strains.[7] Whereas, Suwanpakdee *et al*. reported penicillin and cefotaxime susceptibilities for 30 isolates from patients with *S. pneumoniae* bacteremia from January 2004 through December 2008 at Phramongkutklao Hospital. They also found that 3.3% of studied isolates had intermediate susceptibility to penicillin and cefotaxime.[8] Although cephalosporin-resistant *S. pneumoniae* has also been reported in Thailand, only two studies evidenced this situation. Moreover, neither the study by Srifuengfung *et al*. nor Suwanpakdee *et al*. determined the ceftriaxone susceptibility. The facts are that divergent *in vitro* susceptibilities to ceftriaxone and cefotaxime have been published, especially PNSP stains.[9,10]

From the basis of the isolated strains from the past decade and the lack of penicillin and cephalosporin susceptibility studies, especially ceftriaxone determination, this study aimed to determine the MIC of penicillin, cefotaxime, and ceftriaxone against *S. pneumoniae* isolated from IPD patients.

METHODS

Study Design

This study was an *in vitro* activity of penicillin G, ceftriaxone, and cefotaxime against clinical *S. pneumoniae* isolates. The antimicrobial susceptibility of penicillin ceftriaxone, and cefotaxime was determined by the Epsilometer test. The other antibiotics including tetracycline, trimethoprim-sulfamethoxazole, erythromycin, and chloramphenicol were identified using the disk diffusion test. The protocol was approved with exempt review by the Institutional Review Board, Royal Thai Army Medical Department Bangkok, Thailand (approval number Q015h/59).

Bacterial Isolates

All clinical *S. pneumoniae* isolates were obtained from patients admitted at Phramongkutklao Hospital, a university hospital with 1200 beds in Bangkok, Thailand. Patients diagnosed with IPDs giving *S. pneumoniae* culture from blood culture or the sterile sites were included. Only 40 *S. pneumoniae* isolates in bacterial collection met our inclusion criteria during a 10-year period (between 2006 and 2015),[11] therefore, we had to gather all clinical *S. pneumoniae* isolates without sampling for antimicrobial susceptibility testing.

Measurement of Antibiotic Activity

The MIC of antibiotics including penicillin ceftriaxone, and cefotaxime against studied *S. pneumoniae* isolates was determined by the Epsilometer test (Liofilchem Resetodegli Abrerzz (Te), Italy) plated on Müeller-Hinton agar (MHA) with 5% sheep's blood (Oxoid, Hampshire, UK). Briefly, a 0.5 McFarland colony suspension was spread on MHA with 5% sheep's blood. The antibiotic E-test was performed on an agar plate spread with the tested isolates. The plate was incubated at 35°C for 24 h in 5% CO2 according to the Clinical and Laboratory Standards Institute.[12]

MIC range, MIC50, and MIC90 (μg/ml) and percentage of susceptible isolates were recorded. We used two interpretive susceptibility breakpoints for meningeal and non-meningeal infections to define penicillin, cefotaxime, and ceftriaxone resistance: MICs of ≥0.12 and ≥8 μg/ml for parenteral penicillin G, and ≥2 and ≥4 μg/ml for ceftriaxone and cefotaxime for meningeal and non-meningeal infections, respectively. Intermediate resistance to penicillin and to the third-generation cephalosporins (ceftriaxone and cefotaxime) for non-meningeal infections was 4 and 2 μg/ml, whereas MICs of 1 μg/ml were the criteria to interpret isolate as intermediate resistant ceftriaxone and cefotaxime for meningeal infections.[12]

Statistical Analysis

This study determined MIC range, MIC50, and MIC90 of penicillin, cefotaxime, and ceftriaxone against *S. pneumoniae* isolates. We also assessed the percentage of susceptible isolates for penicillin, cefotaxime, ceftriaxone, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, and chloramphenicol.

MIC range was defined as the smallest versus the largest MIC values. MIC50 and MIC90 values meant the MIC of antibiotics inhibiting 50% and 90% of the studied population isolates.

RESULTS

Forty *S. pneumoniae* clinical isolates were included. Of them, 38 isolates were obtained from the blood and the remaining two isolates from CSF and pleural fluid, respectively. The studied *S. pneumoniae* isolates were susceptible to tetracycline, trimethoprim-sulfamethoxazole, erythromycin, and chloramphenicol at 31.8%, 44%, 51.5%, and 87.9%, respectively.

The MIC range, MIC50, and MIC90 for penicillin were: ≤0.016–1.5 μg/mL, 0.25 μg/mL, and 1 μg/mL,

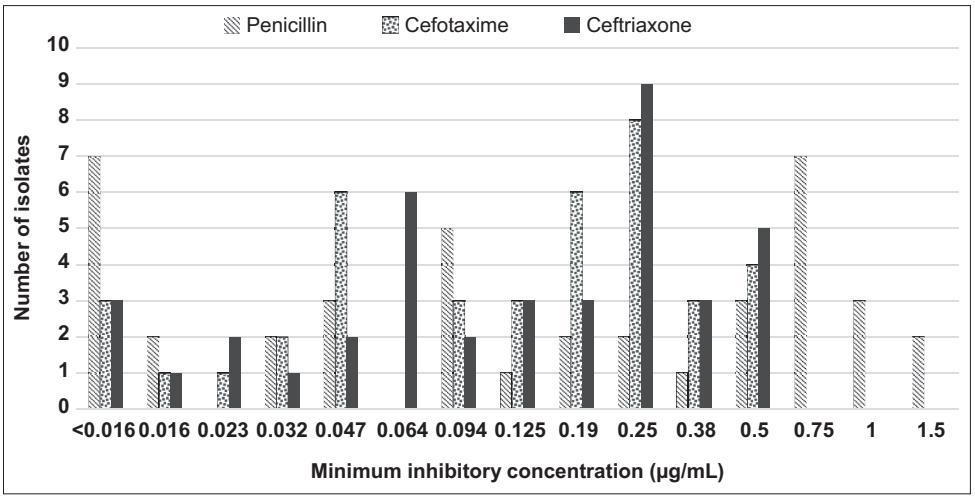

Figure 1: Minimum inhibitory concentration of penicillin (line bar), cefotaxime (dotted bar), and ceftriaxone (black bar) against *Streptococcus pneumoniae* isolates (n=40)

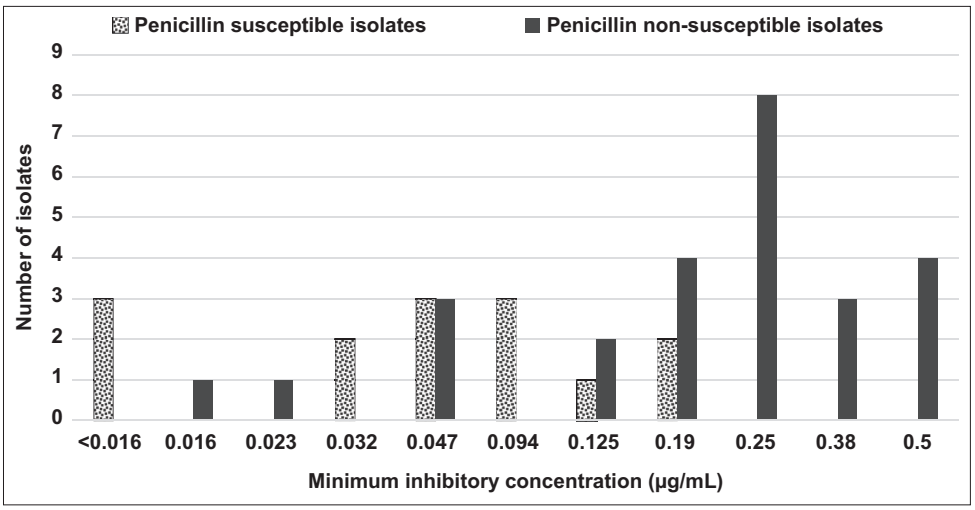

Figure 2: Minimum inhibitory concentration (MIC) of cefotaxime against *Streptococcus pneumoniae* isolates by penicillin susceptible isolates (MIC breakpoint ≤0.06 μg/mL; dotted bar) and penicillin non-susceptible isolates (MIC breakpoint >0.06 μg/mL; black bar)

respectively [Figure 1]. About 35% and 100% of tested isolates were susceptible to penicillin if considered by breakpoints for meningeal and non-meningeal infections, respectively. Focusing on isolates having MIC ≤0.06 µg/mL (meningeal breakpoint), the MIC range, MIC50, and MIC90 for penicillin were ≤0.06–0.047 µg/mL, 0.32 µg/mL, and 0.047 µg/mL, respectively. Furthermore, the MIC range, MIC50, and MIC90 of *S. pneumoniae* isolates with MIC >0.06 µg/mL for penicillin were 0.094–1.5 µg/mL, 0.5 µg/mL, and 1.0 µg/mL, respectively.

The MIC50/90 for cefotaxime and ceftriaxone was equal to 0.19/0.5 µg/mL, whereas the MIC range for cefotaxime and ceftriaxone was ≤0.016–0.5 µg/mL [Figure 1]. All tested isolates were susceptible to cefotaxime and ceftriaxone regardless of breakpoints for meningeal or non-meningeal infections. The cefotaxime and ceftriaxone MIC categorized by penicillin breakpoint (at ≤0.06 µg/mL) seemed to increase in penicillin non-susceptible isolates [Figures 2 and 3]. Focusing on isolates having MIC ≤0.06 µg/mL (meningeal breakpoint), the MIC range/MIC50/MIC90 for ceftriaxone and cefotaxime was ≤0.016–0.25/0.064/0.25µg/mLand≤0.016–0.19/0.094/0.19µg/mL, respectively. The MIC range/MIC50/MIC90 of *S. pneumoniae* isolates with MIC >0.06 µg/mL for ceftriaxone and cefotaxime was 0.016–0.5/0.25/0.5 µg/mL and 0.016–0.5/0.25/0.5 µg/mL, respectively.

DISCUSSION

In our study, penicillin G should no longer be a therapeutic choice for empirical meningitis due to *S. pneumoniae*. About 65% of studied isolates were penicillin non-susceptible *S. pneumoniae* (PNSSP), similar to a previous study finding of 61.5%.[4] This increase of MIC of penicillin might be from the antibiotic exposure. Dejthevapor *et al*. performed the study to identify risk factors for acquisition of PNSSP in patients in Bangkok. They found that only previous antibiotic use was a risk factor for related acquisition of PNSSP.[13] However, the increase of penicillin MIC did not affect the treatment of non-meningitis infections from all studied *S. pneumoniae* isolates with MIC below 2 µg/mL.

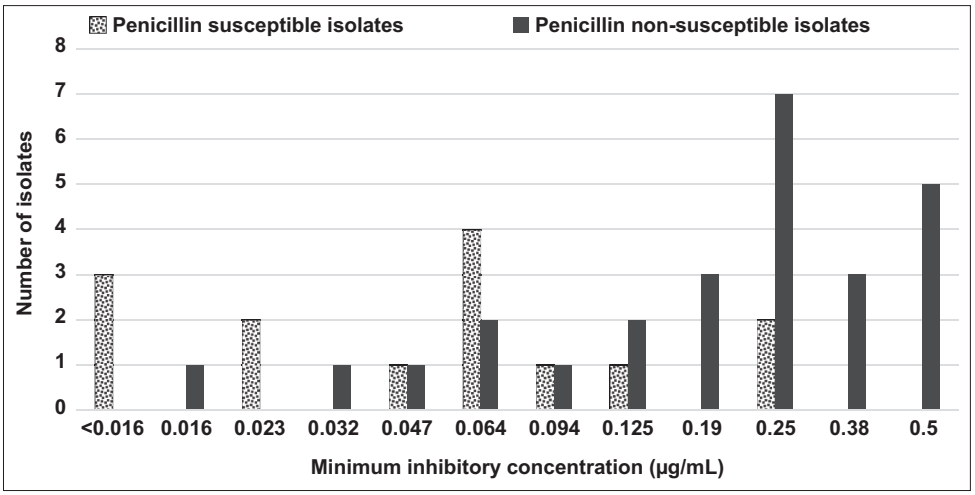

Figure 3: : Minimum inhibitory concentration (MIC) of ceftriaxone against Streptococcus pneumoniae isolates by penicillin susceptible isolates (MIC breakpoint ≤0.06 μg/mL; dotted bar) and penicillin non-susceptible isolates (MIC breakpoint >0.06 μg/mL; black bar) *S. pneumoniae* isolates by penicillin susceptibility (MIC breakpoint ≥ 0.06 μg/mL)

In terms of the third-generation cephalosporins, all studied *S. pneumoniae* isolates either penicillin susceptible or non-susceptible isolates were susceptible to ceftriaxone and cefotaxime regardless of any penicillin susceptibility breakpoints of *S. pneumoniae*. This finding was similar to our previous study revealing all *S. pneumoniae* isolated from patients with meningitis were susceptible to cefotaxime and ceftriaxone.[14] In contrast, Srifuengfung *et al*. reported that approximately 10% of PNSSP strains isolated from patients with IPDs were resistant to ceftriaxone, mostly isolates which were non-susceptible to penicillin.[15] Therefore, due to discordant results of the third-generation cephalosporin-resistant isolates, a multicenter study conducted in hospitals across Thailand is needed to further investigate the true prevalence of these strains. It is fact that MIC determination might be useful for empirical therapy selection, between the third-generation cephalosporin monotherapy and third-generation cephalosporin combined with vancomycin, in cases of suspected pneumococcal meningitis in Thailand.

Focusing on MIC values between ceftriaxone and cefotaxime, the previous study by Karlowsky *et al*., indicated that some isolates of penicillin-resistant pneumococci were less susceptible to cefotaxime than to ceftriaxone[9]. Conversely, our studied *S. pneumoniae* isolates demonstrated lower trends of MICs to cefotaxime than to ceftriaxone, but neither MIC50 nor MIC90 were different. However, published studies on different susceptibilities for cefotaxime and ceftriaxone are currently limited. Further studies are required to confirm our findings.

Our study has some limitations, the studied isolates were from a medical school hospital which might be dissimilar when taken from other types of hospital. Second, although we gathered data covering 10 years at a single super tertiary hospital, only 40 isolates from sterile site were identified.

Generalizability, our finding could not detect the cephalosporin-resistant *S. pneumoniae* isolates whereas the previous two studies reported the third-generation cephalosporin-resistant *S. pneumoniae* in Thailand.[7,8] Therefore, larger sample size studies or multicenter studies are needed to determine the real situation of antimicrobial-resistant *S. pneumoniae*.

CONCLUSION

Penicillin G may no longer be an appropriate empirical pneumococcal meningitis treatment, although both the third-generation cephalosporins, ceftriaxone, and cefotaxime remained good efficacy as the first-line agents for community-acquired meningitis due to *S. pneumoniae* and even our studied isolates demonstrated lower trends of MICs to cefotaxime than to ceftriaxone.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

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