THE SUSCEPTIBILITY OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM PATIENTS WITH INVASIVE PNEUMOCOCCAL DISEASES TO FOSFOMYCIN DURING A 10-YEAR PERIOD AT A THAI HOSPITAL

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Abstract. We studied the minimum inhibitory concentration (MIC) of fosfomycin against 40 clinical isolates of *Streptococcus pneumoniae* obtained from patients with invasive pneumococcal disease admitted to Phramongkutklao Hospital, Thailand from January 2006 to December 2015. The MIC of fosfomycin for each *S. pneumoniae* isolate was determined using the Epsilometer test (E-test) supplemented with glucose-6-phosphate. The MIC range, MIC50 and MIC90 for fosfomycin were 6-64 μg/ml, 20 μg/ml and 48 μg/ml, respectively. Thirty-two out of 40 isolates (80%) were susceptible to fosfomycin (susceptibility breakpoint ≤32 μg/ml). Most *S. pneumoniae* isolates were sensitive to fosfomycin. Further studies are needed to evaluate treatment outcomes.

Keywords: *Streptococcus pneumoniae,* antibiotic susceptibility, pneumococcal disease, fosfomycin

INTRODUCTION

Streptococcus pneumoniae is a gram-positive diplococcal bacterium that is a major cause of community-acquired infections (Maraqa, 2014) ranging from mild upper respiratory tract infections to severe life-threatening infections, such as pneumonia, bacteremia and meninigitis (Blasi *et al,* 2012; Maraqa, 2014). In Thailand, the most common invasive pneumococcal diseases are pneumonia (50.7%), acute exacerbation of chronic obstructive pulmonary disease bronchopneumonia (21.0%), meningitis (14.6%) and bacteremia (8.3%). The mortality rate of invasive pneumococcal disease in Thailand during the first 7 days of hospitalization was 28.8% in one study (Leelarasamee *et al*, 1999). Early appropriate antibiotic treatment is vital to reduce unfavorable outcomes (Lujan *et al*, 2004).

Drug resistant *S. pneumoniae* (DRSP) has been reported worldwide; this in-

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cludes penicillin non-susceptible *S. pneumoniae* (PNSSP) and macrolide resistant *S. pneumoniae* (Richter *et al*, 2009; Kim *et al*, 2012). Cephalosporin resistant *S. pneumoniae* has also been reported (Kim *et al*, 2012; Lee *et al*, 2017). The World Health Organization (WHO, 2017) announced there is an urgent need for new antibiotics to treat DRSP. Development of new antibiotics is expensive and time consuming and there are few new antimicrobials under development, resulting in the re-evaluation of older antimicrobials to treat DRSP (Cassir *et al*, 2014).

Fosfomycin is a broad spectrum antibiotic with both of gram-positive cocci and gram-negative bacterial coverage that has been used in clinical practice for over 40 years. It penetrates into various organ due to its low molecular weight and low protein binding (Falagas *et al*, 2009). Previously *in vitro* studies using the disk diffusion method found good *S. pneumoniae* susceptibility (Falagas *et al*, 2010; Charfi *et al*, 2012). One study found fosfomycin to satisfactory for pneumococcal treatment (Falagas *et al*, 2009). However, only one previous study evaluated the minimum inhibitory concentration (MIC) of fosfomycin against *S. pneumoniae* (Kikuchi *et al*, 1995) showing it was sensitive and recommended it for treatment. We determined to reevaluate the MIC of fosfomycin against *S. pneumoniae* strains isolated from patients with invasive pneumococcal disease.

MATERIALS AND METHODS

Bacterial strain

All clinical *S. pneumoniae* isolates were obtained from in-patients at Phramongkutklao Hospital, a university hospital with 1,200 beds in Bangkok, Thailand, between January 2006 and December 2015. Patients included in the study had a diagnosis of invasive pneumococcal diseases confirmed by a positive *S. pneumoniae* culture from the cerebrospinal fluid (CSF) or blood culture. All *S. pneumoniae* isolates were kept in tryptic soy broth containing 20% glycerol at -80°C until used. The protocol was approved by the institutional review board, Royal Thai Army Medical Department and Phramongkutklao Hospital Bangkok, Thailand (approval number Q015h/59).

Determination of antimicrobial susceptibility

Antimicrobial susceptibility was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI), version 2017 (CLSI, 2017) and British Society for Antimicrobial Chemotherapy (BSAC), version 8 (Andrews and BSAC Working Party on Susceptibility Testing, 2009). Isolates were tested for sensitivity to oxacillin (1 μg), ceftriaxone (30 μg), vancomycin (30 μg), erythromycin (15 μg), trimethoprim-sulphamethoxazole (1.25 μg/23.75 μg), tetracycline (30 μg), chloramphenicol (30 μg), linezolid (30 μg), and levofloxacin (5 μg). Penicillin resistant *S. pneumoniae* isolates were examined for sensitivity to oxacillin.

Minimum inhibitory concentration of fosfomycin against tested *Streptococcus pneumoniae* isolates

The minimum inhibitory concentration (MIC) of fosfomycin against tested *Streptococcus pneumoniae* isolates was determined with the Epsilometer test (E-test) plated on Müller-Hinton agar (MHA) with 5% sheep blood (Oxiod, Hamshire, UK). Briefly, a 0.5 McFarland colony suspension prepared using colonies from 18-20 hour subculture was spread on MHA with 5% sheep blood. The fosfomycin E-test

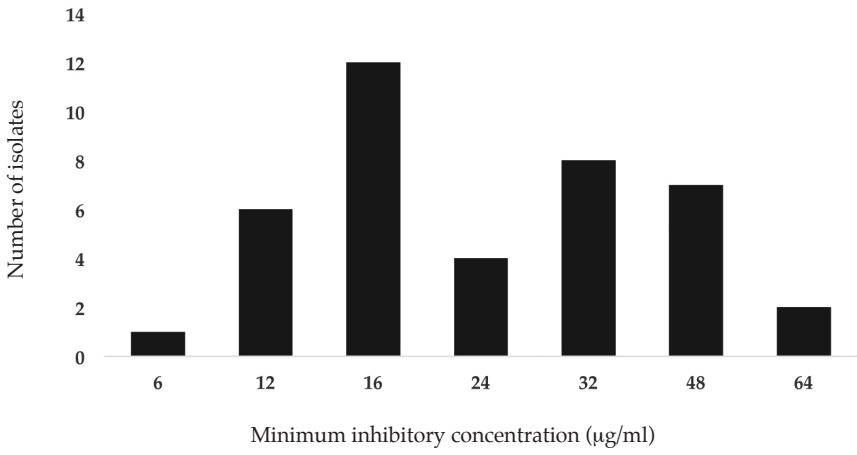

Fig 1–Minimum inhibitory concentration (MIC) of fosfomycin against studied *S. pneumoniae* isolates.

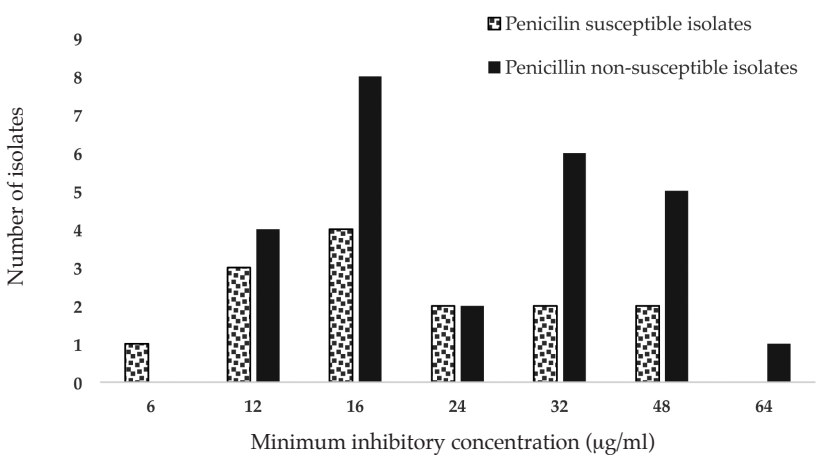

Fig 2–Minimum inhibitory concentration (MIC) of fosfomycin against *S. pneumoniae* isolates by penicillin susceptibility.

supplemented with glucose-6-phosphate [Liofilchem Resetodegli Abrerzz (Te), Italy] was performed on the tested isolates on an agar plate. The plate was incubated at 35°C for 24 hours in 5% CO_2 (CLSI, 2017). MIC range, MIC50 (Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms), MIC90 (Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms) (μg/ml) and percentage of susceptible isolates were recorded. A fosfomycin MIC ≤32 μg/ml was considered to be susceptible (The European Committee on Antimicrobial Susceptibility Testing, 2017).

RESULTS

Forty *S. pneumoniae* were used for the study. Ninety-five percent was obtained from the blood, 2.5% from CSF and 2.5% from pleural fluid. All *S. pneumoniae* isolates were susceptible to ceftriaxone, vancomycin, linezolid, and levofloxacin. Thirty-one point eight percent, 44%, 51.5%, and 87.9% of isolates were susceptible to tetracycline, trimethoprim-sulphamethoxazole, erythromycin and chloramphenicol, respectively. Sixty-five percent of tested isolates was PNSSP.

The MIC range, MIC50 and MIC90 for fosfomycin were: 6-64 μg/ml, 20 μg/ml and 48 μg/ml, respectively (Fig 1, 2). Eighty

percent of tested isolates were susceptible to fosfomycin.

DISCUSSION

In our study, 65% of studied isolates were PNSSP, similar to a previous study finding of 61.5% (National Antimicrobial Resistance Surveillance Center, 2016). Our results show *S. pneumoniae* should no longer be treated empirically with penicillin G.

Our finding of the MIC range of 6-64 μg/l, MIC50 of 20 μg/l, and MIC90 of 48 μg/l are similar to 8-64 μg/l, 16 μg/l and 20 μg/l, respectively, reported by Kikuchi *et al* (1995). Our finding of 80% of tested isolates sensitive to fosfomycin is similar to 70% reported by Falagas *et al* (2010).

Our findings suggest fosfomycin is a reasonable alternative drugs for empirical treatment of *S. pneumoniae* in penicillin allergic patients. High doses of fosfomycin have been shown effective in treating meningitis among susceptible strains of *S. pneumoniae* (MIC <32 μg/l) (Kuhnen *et al*, 1987). Fosfomycin has been safety used in humans at doses up to 24 gm/day and has been optimized for infections due to *S. pneumoniae* with a MIC of 64 of doses of 4 gm IV every 6 hours or in continuous drip of 16 gm per 24 hours (Asuphon *et al*, 2016).

Previously, ten of 12 patients were cured when fosfomycin was used with ampicillin or gentamicin (Sicilia *et al*, 1977) and 5 of 9 were cured when treating with fosfomycin and penicillin or ampicillin or chloramphenicol (Sicilia *et al*, 1981).

In our study, only 40 isolates were identified. With this low incidence at the study hospital, a multicenter study needs to be conducted to evaluate incidence and susceptibility pattern.

In conclusion, in our study, 80% of *S. pneumoniae* isolates were sensitive to fosfomycin. Further studies are needed to evaluate treatment outcomes.

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