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

Sudaluck Thunyaharn[1], Juthathip Suphanklang[2,3],

Wichai Santimaleeworagun[2,4*], Jantima Traipattanakul[5]

[1] Faculty of Medical Technology Nakhonratchasima College, Nakhonratchasima 30000 Thailand

[2] Department of Pharmacy, Faculty of Pharmacy, Silpakorn University, Nakorn Pathom 73000 Thailand

[3] College of Pharmacotherapy Thailand, Pharmacy Council, Nonthaburi 11000 Thailand

[4] Antibiotic Renaissance and Optimization Project and Antibiotic Optimization and Patient Care Project by Pharmaceutical Initiative for Resistant Bacteria and Infectious Diseases Working Group [PIRBIG]

[5] Division of Infectious Disease, Department of Medicine, Phramongkutklao Hospital, Bangkok 10400 Thailand

* Corresponding author:

Wichai Santimaleeworagun

Department of Pharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Phathom, Thailand Tel +6634 255 800, Fax +6634 255 801, Email address: swichai1234@gmail.com

Keywords: Antibiotic susceptibility, *Streptococcus pneumoniae*

Running title: Fosfomycin against clinical *S. pneumoniae* strains in Thailand

ABSTRACT

We studied the minimum inhibitory concentration (MIC) of fosfomycin against *Streptococcus pneumoniae*. We obtained clinical isolates of *S. pneumoniae* 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* isolates was determined using the Epsilometer test (E-test) supplemented with Glucose-6-phosphate. A total of 40 *S. pneumoniae* isolates were included in the study. The percentile at the fiftieth and ninetieth of MIC of fosfomycin were 20 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 studied are needed to evaluate treatment outcomes.

INTRODUCTION

Streptococcus pneumoniae is a Gram-positive diplococcal bacteria 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 found to be 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 includes 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 if 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 were identified 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 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). 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 supplemented with Glucose-6-phosphate (Liofilchem) was performed on the tested isolates on an agar plate. The plate was included at 35ºC for 24 hours in 5% CO2 (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. 31.8%, 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/mL, MIC50 of 20 μg/mL, and MIC90 of 48 μg/mL are similar to 8-64 μg/mL, 16 μg/mL and 20 μg/mL, respectively, reported by Kikachi et al. (1995) (Kikuchi *et al.*, 1995). Our finding of 80% of tested isolates sensitive to fosfomycin is similar to 70% reported by Falagas et al. (2010)(Falagas *et al.*, 2010).

Our finding 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

mg/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 studied are needed to evaluate treatment outcomes.

Acknowledgements

We would like to thank the Microbiology Laboratory Unit of Phramongkutklao Hospital for keeping the clinical *S. pneumoniae* isolates.

REFERENCES

Andrews JM. BSAC standardized disc susceptibility testing method (version 8). *J Antimicrob Chemother* 2009; 64: 454-89.

Asuphon O, Montakantikul P, Houngsaitong J, Kiratisin P, Sonthisombat P. Optimizing intravenous fosfomycin dosing in combination with carbapenems for treatment of Pseudomonas aeruginosa infections in critically ill patients based on pharmacokinetic/pharmacodynamic (PK/PD) simulation. *Int J Infect Dis* 2016; 50: 23-9.

Blasi F, Mantero M, Santus P, Tarsia P. Understanding the burden of pneumococcal disease in adults. *Clin Microbiol Infect* 2012; 18 (Suppl 5): 7-14.

Cassir N, Rolain JM, Brouqui P. A new strategy to fight antimicrobial resistance: the revival of old antibiotics. *Front Microbiol* 2014; 5: 551.

Charfi F, Smaoui H, Kechrid A. Non-susceptibility trends and serotype coverage by conjugate pneumococcal vaccines in a Tunisian paediatric population: a 10-year study. *Vaccine*. 2012; 30 (Suppl 6): G18-24.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty-Seventh informational supplement. Wayne: CLSI, 2017.
Falagas ME, Roussos N, Gkegkes ID, Rafailidis PI, Karageorgopoulos DE. Fosfomycin for the treatment of infections caused by Gram-positive cocci with advanced antimicrobial drug resistance: a review of microbiological, animal and clinical studies. *Expert Opin Investig Drugs* 2009; 18: 921-44.
Falagas ME, Maraki S, Karageorgopoulos DE, Kastoris AC, Kapaskelis A, Samonis G. Antimicrobial susceptibility of Gram-positive non-urinary isolates to fosfomycin. *Int J Antimicrob Agents* 2010; 35: 497-9.
Kikuchi K, Totsuka K, Shimizu K, Ishii T, Yoshida T, Orikasa Y. Effects of combination of benzylpenicillin and fosfomycin on penicillin-resistant Streptococcus pneumoniae. *Microb Drug Resist*. 1995; 1: 185-9.
Kim SH, Song JH, Chung DR, *et al*. Changing trends in antimicrobial resistance and serotypes of Streptococcus pneumoniae isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrob Agents Chemother* 2012; 56: 1418-26.
Kuhnen E, Pfeifer G, Frenkel C. Penetration of fosfomycin into cerebrospinal fluid across non-inflamed and inflamed meninges. *Infection* 1987; 15: 422-4.
Lee HY, Wu TL, Su LH, et al. Invasive pneumococcal disease caused by ceftriaxone-resistant Streptococcus pneumoniae in Taiwan. *J Microbiol Immunol Infect*. 2017 (2017). doi: 10.1016/j.jmii.2016.12.004.
Leelarasamee A, Dhiraputra C, Hunnangkul S. Severe pneumococcal infection at a Thai hospital. *Int J Infect Dis* 1999; 3: 147-52.
Lujan M, Gallego M, Fontanals D, Mariscal D, Rello J. Prospective observational study of bacteremic pneumococcal pneumonia: Effect of discordant therapy on mortality. *Crit Care Med* 2004; 32: 625-31.
Maraqa NF. Pneumococcal infections. *Pediatr Rev* 2014; 35: 299-310.
National Antimicrobial Resistance Surveillance Center Thailand (NARST). Antibiograms 2016. Nonthaburi: National Antimicrobial Resistance Surveillance Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. [Cited 2017 February 27]. Available from: http://narst.dmsc.moph.go.th/antibiograms.html
Richter SS, Heilmann KP, Dohrn CL, Riahi F, Beekmann SE, Doern GV. Changing epidemiology of antimicrobial-resistant Streptococcus pneumoniae in the United States, 2004-2005. *Clin Infect Dis* 2009; 48: e23-33.
Sicilia T, Fadon A, Rodriguez A, Soto J. Fosfomycin in pneumococcal meningitis. *Chemotherapy* 1977; 23 (Suppl 1): 429-40.
Sicilia T, Estevez E, Rodriguez A. Fosfomycin penetration into the cerebrospinal fluid of patients with bacterial meningitis. *Chemotherapy* 1981; 27: 405-13.
The European Committee on Antimicrobial Susceptibility Testing. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 7.1, 2017; [cited 2017 February 27]. Available from: http://www.eucast.org/clinical_breakpoints/.

World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed: WHO Department of Communications; 2017 [cited 2017 February 27]. Available from: http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/.

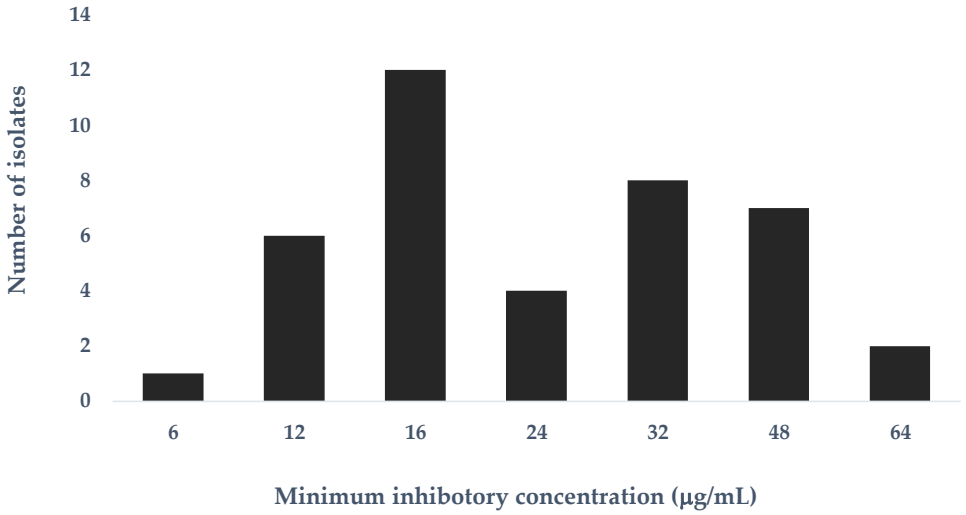

Fig. 1 Minimum inhibitory concentration (MIC) of fosfomycin against studied *S. pneumoniae* isolates.

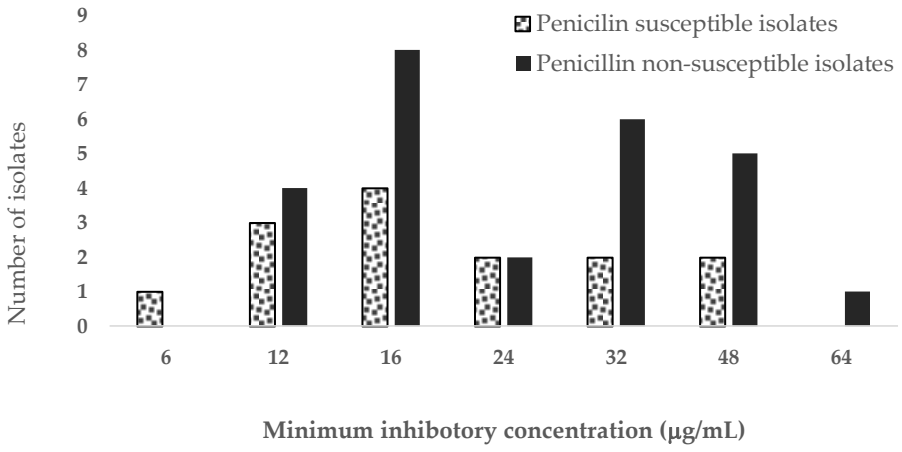

Fig. 2 Minimum inhibitory concentration (MIC) of fosfomycin against *S. pneumoniae* isolates by penicillin susceptibi