

Antimicrobial Susceptibility of *Neisseria gonorrhoeae* in Adult Patients Seeking Care at Military Hospitals in Thailand From 2014 to 2020

Maneerat Somsri^{*}; Wilawan Oransathid[†]; Brian Vesely[†]; Mariusz Wojnarski[†];
 Samandra Demons[†]; Norman Waters[†]; Khunakorn Kana^{*}; Nithinart Chaitaveep^{*};
 Thanainit Chotanaphuti^{*}; Woradee Lurchachaiwong[†]

ABSTRACT

Introduction:

The effective dual antibiotics ceftriaxone (CRO) and azithromycin (AZM) have successfully treated *Neisseria gonorrhoeae* (GC) infection, however, the CRO- and AZM-resistant strains have been sporadically detected globally and in Thailand. Furthermore, there are no currently antimicrobial susceptibility profiles of the GC isolates obtained from soldiers reported in Thailand. Hence, this is the first study to describe the antimicrobial susceptibility profiles of GC isolates obtained from predominately soldiers who seeking care at Military Camp Hospitals, in Thailand from 2014 to 2020.

Materials and Methods:

A total of 624 symptomatic gonococcal samples were received from 10 military hospitals during 2014–2020. They were collected from urethral swabs and inoculated into selective media. The suspected GC isolates were subcultured and presumptively identified using conventional microbiology techniques. Antimicrobial susceptibility test was performed by Etest to determine minimal inhibitory concentration (μg/mL) against AZM, benzylpenicillin, cefepime, cefixime, ceftriaxone (CRO), ciprofloxacin, spectinomycin, and tetracycline using the criteria outlined in the Clinical and Laboratory Standards Institute guidelines. This study was approved by Institutional Review Board, Royal Thai Army Medical Department under protocol number S036b/56 and Walter Reed Army Institute of Research, and Silver Spring, MD under protocol number WR #2039.

Results:

A total of 624 samples were collected from symptomatic gonococcal infectious patients with 91.5% (571/624) of samples obtained from soldiers. Of those, 78% (488/624) were identified as GC and 92% (449/488) of them were isolated from soldiers. All GC samples collected were susceptible to CRO (first-line treatment) with only one GC isolate identified as non-susceptible to cefepime and three isolates identified as non-susceptible to AZM.

Conclusion:

The recommended dual treatment of GC infections with CRO and AZM is currently an effective empirical treatment for patients who are seeking care at military hospitals in Thailand. Nevertheless, cefepime is a fourth-generation cephalosporin with documented high activity against GC strains equal to other “third-generation” cephalosporins such as CRO. Due to the active duty of military personnel, they concerned about the confidentiality and frequently seek treatment at civilian clinics. Additionally, due to the availability of antibiotics over the counter in Thailand, many choose the option to self-medicate without a physician’s prescription. These could be subsequently driven the gradual increase of multidrug-resistant gonococcal strains throughout the country. Thus, the GC surveillance would be needed for further Force Health Protection and public health authorities in response to the drug-resistant GC threats.

^{*}Royal Thai Army, Armed Forces Research Institute of Medical Science (AFRIMS), Bangkok 10400, Thailand

[†]Bacterial and Parasitic Diseases Department, Armed Forces Research Institute of Medical Science (AFRIMS), Bangkok 10400, Thailand

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INTRODUCTION

More than 86 million new cases of gonococcal infections were reported globally in adults by the World Health Organization (WHO) in 2019¹ and the Centers for Disease Control (CDC) recently announced that Gonorrhea is the second-most common sexually transmitted disease (STD) behind Chlamydia in the USA². An increased rate of Gonorrhea infections was observed among males and females in 2010–2019; however, it was significantly greater among males.² With regards to vaccine development, there are literatures that suggested that a vaccine could cross-protect *Neisseria meningitidis* serogroup B against *Neisseria gonorrhoeae* (GC) infection.³ Nevertheless, it is a challenge due to the limited knowledge on immune evasion and understanding of what is required to induce a protective immune response.^{4,5} In the absence of

highly efficacious GC vaccines, early diagnosis of GC in high-risk groups and effective antibiotics remains the cornerstone of treatment to help control the spread of the resistant strains in the community. The first-line treatment for GC in Thailand is ceftriaxone (CRO), however, CRO-resistant strains have been detected since 2018.¹ In 2016, the WHO recommended the use of CRO plus azithromycin (AZM) for GC treatment.⁶ There are sporadic reports of AZM-resistant gonococci as well as the emergence of multidrug resistance (MDR) or reduced susceptibility to CRO alone.^{7–10} With limited gonococcal antimicrobial resistance surveillance, the WHO has established the Gonococcal Antimicrobial Surveillance Program (GASP) through a worldwide laboratory network in Africa and Southeast Asia countries¹ and implemented the Enhanced Gonococcal Antimicrobial Surveillance Program (EGASP) in Thailand in 2015.¹¹ The study population from the previous GC antimicrobial susceptibility testing (AST) results were generated from civilian heterosexual men, bisexual men, transgender women (TGW) and men who have sex with men (MSM).¹¹ Additionally, sexually transmitted infections (STI) remain a force health protection (FHP) risk for joint military personnel and is repeatedly demonstrated to be an issue for military deployed. Military-related gonococcal resistance to penicillin was documented in soldiers returning to the USA from their deployments in Southeast Asia during the 1960s,¹² and STIs or venereal diseases were the second most common cause of soldiers' lost duty days in World War I.¹³ More recently in the U.S. military, GC has remained a commonly reported STI from 2000–2012.¹⁴ Thailand has been selected as an important study area for STDs such as gonococcal infection because of the growth of the sex industry leading to more antibiotic resistance.^{1,15} Nonetheless, STD data in soldiers is ambiguous due to them being classified as a vulnerable population and the information being sensitive and confidential. Moreover, the STDs research in Thailand mostly investigated the human immunodeficiency virus (HIV) rather than gonococcal infection. Hence, this is the first study to describe the AST profile of GC isolates obtained from soldiers in Thailand. The cohort in this study is predominately soldiers who are seeking care at military hospitals, in Thailand from 2014 to 2020, which captures before and after CRO was first reported in 2018.¹ Surveillance data obtained from this study provides useful data for effective antibiotic treatments, particularly in the military population.

MATERIALS AND METHODS

A total of 624 symptomatic gonococcal samples were received from 10 military hospitals during 2014–2020. The Institutional Review Board, Royal Thai Army Medical Department, and Walter Reed Army Institute of Research (WRAIR) as well as the hospital directors merely allowed us to collect the age, sex, and gonococcal samples. All of the samples were collected from urethral swabs and inoculated into selective media (InTray® GC, BioMed Diagnostics, San Jose,

Calif). Subsequently, the suspected GC isolates were subcultured and presumptively identified using growth characteristics on enrichment culture media, gram stain, oxidase, and catalase tests followed by API® NH (bioMérieux, Inc., Durham, NC, USA). Antimicrobial susceptibility test (AST) was performed by Etest (bioMérieux, Inc., Durham, NC, USA) to determine minimal inhibitory concentration (MIC) (µg/mL) against AZM, benzylpenicillin (PCG), cefepime (CPM), cefixime (CFM), CRO, ciprofloxacin (CIP), spectinomycin (SPT), and tetracycline (TET). Antimicrobial susceptibility patterns were interpreted using the criteria outlined in the Clinical and Laboratory Standards Institute guidelines 2021 (CLSI).¹⁶

RESULTS

Data was received from 624 symptomatic gonococcal samples, most of them were obtained from Phramongkutklo hospital (416 samples), followed by Anandamahidol hospital (133 samples), Fort Surasak Montri hospital (31 samples), Fort Nawamintharachini hospital (17 samples), Fort Mengrai hospital (11 samples), Fort Wachirawut hospital (5 samples), 4 Fort Kaiprajaksilaphakom hospital (5 samples), Fort Chiraprawat hospital (4 samples), and one each from Fort Kawila hospital and Fort Somdej Phranaresuan Maharaj hospital (Fig. 1). Among those samples, 91.5% (571/624) were confirmed to be obtained from soldiers. All patients enrolled in this study were males with age ranges between 13–71 years. Most of the patients were in the age range of 21–30 years (86%; 537/624), followed by <20 years (7.4%; 46/624), 31–40 years (4.5%; 28/624), >51 years (1.3%; 8/624), 41–50 years (0.5%; 3/624), and the remaining two patients were no record. Of all the samples received, 488 (78%) were identified as GC isolates and were subcultured for AST.

All GC isolates were susceptible to CFM with MICs lower than 0.5 µg/mL, however, one GC isolate had intermediate susceptibility to SPT with a MIC of 64 µg/mL. The clinical effectiveness of SPT for treating infections caused by organisms that produce intermediate drug susceptibility results is unknown.¹⁶ Most of the GC isolates (89%) were resistant to CIP, followed by 86.9% and 86.3% of isolates being resistant to TET and PCG, respectively. All GC isolates collected were sensitive to CRO and 0.8% and 0.2% of the GC isolates presented as non-susceptible to AZM and CPM, respectively (Table I). Similar to what was previously reported in South-East Asia and the Western Pacific Region, the highest resistance to an antibiotic we found in GC was against CIP.^{11,17,18} The most common MDR pattern in our study was PCG-CIP-TET (69%; 338/488). Moreover, three GC isolates were non-susceptible to AZM with an MIC value greater than 1.5 µg/mL. Two isolates were obtained from a 23-year-old soldier and a 29-year-old civilian at Bangkok province and presented with the MDR pattern against PCG-CIP-AZM. In addition, the MDR patterns against the fourth-generation cephalosporin have also been observed in this surveillance

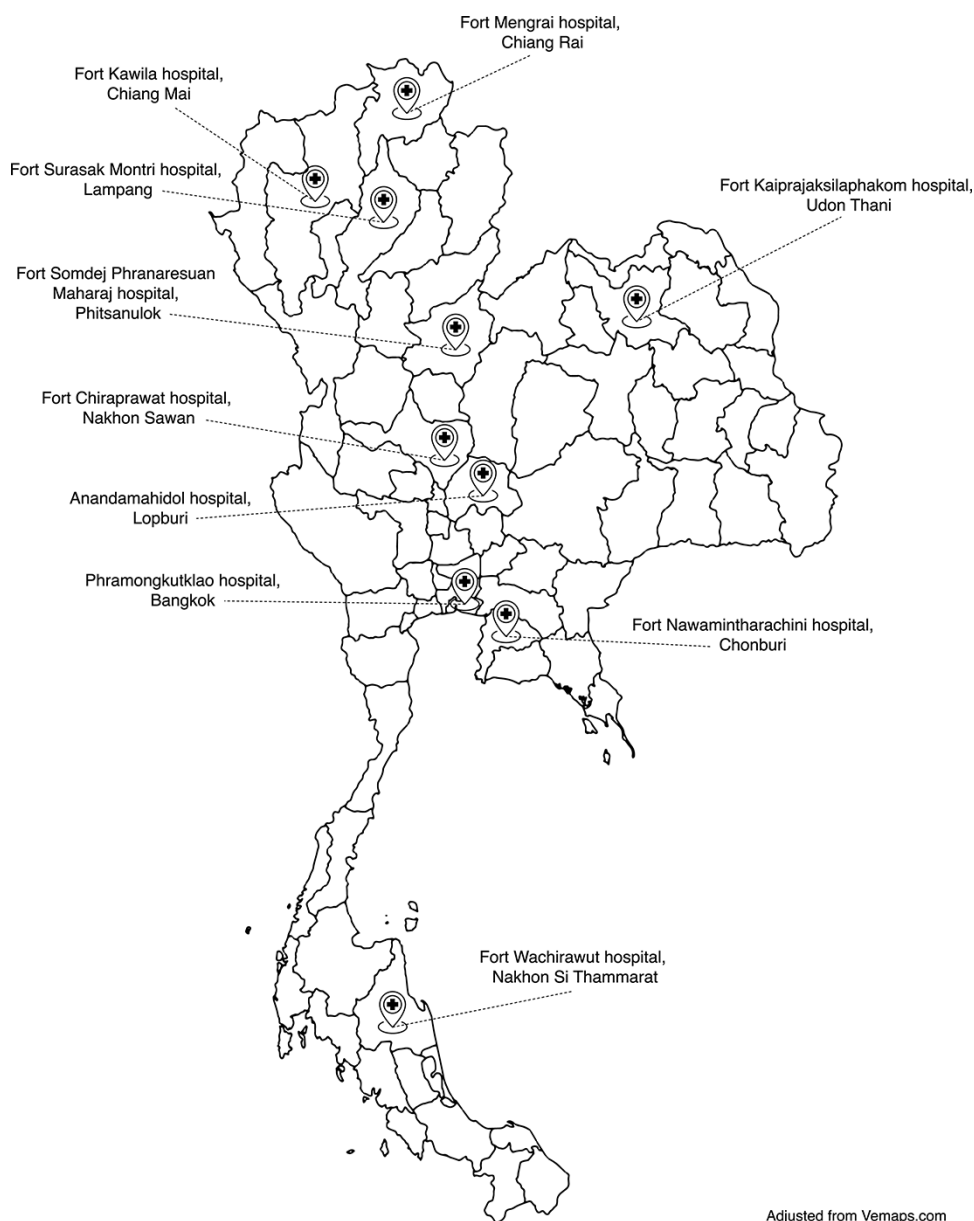


FIGURE 1. The location of 10 military hospitals (Phramongkutklao hospital, Anandamahidol hospital, Fort Surasak Montri hospital, Fort Nawamintharachini hospital, Fort Mengrai hospital, Fort Wachirawut hospital, Fort Kaiprajaksilaphakom hospital, Fort Chiraprawat hospital, Fort Kawila hospital, and Fort Somdej Phranaresuan Maharaj hospital) through Thailand.

study. One isolate obtained from a 22-year-old soldier at Bangkok province showed an MDR pattern against PCG-CIP-CPM. In regards to the dual therapy with CRO plus AZM, the emergence dual resistance to them was first reported in South-east Asia in 2018.^{1,19} No surveillance data generated from our study showed isolates that are dual resistant to these first-line antibiotic treatments but, we observed non-susceptibility to AZM with MIC 4 µg/mL and CPM with MIC > 0.75 µg/mL from different GC isolates.

DISCUSSION

In Thailand, symptomatic patients are treated with CRO 500 mg intramuscular injection or CFM 400 mg by oral

route.²⁰ There have been no resistant CFM strains identified in this study of predominantly active duty soldiers. This is consistent with prior reports by EGASP with no resistant GC isolates against CFM and other cephalosporins.¹¹ Nevertheless, the GC isolates were mostly resistant to CIP.¹¹ This is similar to reports from other countries in the Southeast Asia, Western Pacific regions and our study at military hospitals but they were higher than other parts of the world.^{17,18} The GC isolates obtained from civilian patients presented the AST profiles against PCG (86.3%) and TET (86.9%) (Table 1) but all of them but all of them susceptible to SPT, CRO, and CFM have been reported elsewhere²¹ with similarity to the AST profiles reported in our study. Hence, CRO and CFM remain

TABLE I. Antimicrobial Susceptibility Data of GC Isolates from 10 Military Hospitals (Phramongkutklao Hospital, Anandamahidol Hospital, Fort Surasak Montri Hospital, Fort Nawamintharachini Hospital, Fort Mengrai Hospital, Fort Wachirawut Hospital, Fort Kaiprajaksilaphakom Hospital, Fort Chiraprawat Hospital, Fort Kawila Hospital, Fort Somdej, and Phranaresuan Maharaj Hospital) during 2014-2020 ($N = 488$)

Antimicrobial agents	MIC results range (Φ 0EXg/mL)	Percent of isolates which met interpretative criteria			
		Non-susceptible (NS) ^a	Susceptible (S)	Intermediate (I)	Resistant (R)
Azithromycin (AZM)	0.016–256	0.6% (3/488)	99.4% (485/488)		
Cefepime (CPM)	0.016–256	0.2% (1/488)	99.8% (487/488)		
Cefixime (CFM)	0.016–256	0	100% (488/488)		
Ceftriaxone (CRO)	0.002–32	0	100% (488/488)		
Benzylpenicillin (PCG)	0.016–256		2.9% (14/488)	10.8% (53/488)	86.3% (421/488)
Ciprofloxacin (CIP)	0.002–32		3% (15/488)	8% (39/488)	89% (434/488)
Spectinomycin (SPT)	0.064–1,024		99.8% (487/488)	0.2% (1/488)	0
Tetracycline (TET)	0.016–256		5.9% (29/488)	7.2% (35/488)	86.9% (424/488)

^aInterpretative criteria for macrolide (AZM) and cepheims (CPM, CFM, and CRO) follow the interpretative guideline as recommended by CLSI2021.¹⁶

effective treatment options for GC in military populations in Thailand. The recommended co-treatment of GC infections with CRO and AZM is currently an effective empirical treatment for patients who are seeking care at military hospitals in Thailand. Nonetheless, we found the non-susceptibility to CPM which is a fourth-generation cephalosporin with documented high activity against GC strains equal to other “third-generation” cephalosporins. The mode of actions of CPM and CRO is the disruption of synthesis of the peptidoglycan layer in bacterial cell walls causing defective cell walls and bacterial cell death.¹³ CPM was confirmed as being very active against GC strains that have high resistance to β -lactam.^{22,23} Historically, the highest CPM MIC was reported at 0.5 μ g/mL.²⁴ The non-susceptibility of GC isolates against the third- and fourth-generation cephalosporins is uncommonly reported.¹⁶ Additionally, there were the first two GC isolates with a decreased CRO susceptibility but remained susceptible to AZM which have just been identified in heterosexual civilian men in Thailand.²⁵ This may compromise early diagnosis and treatment of MDR GC infection with appropriate antibiotics, as well as to initiate possible new strategies to treat the GC cephalosporin resistant strains in the national treatment guideline.

According to the MSMR report from 2013 in five countries, GC resistant profiles were found to be significantly more diverse in samples collected from civilian hospital-based referral clinics rather than military clinics.¹² For concerns about confidentiality, military personnel frequently seek treatment for their STIs at civilian clinics. Additionally, due to a perceived stigma for STIs and the availability of antibiotics over the counter in Thailand, many choose the option to self-medicate without a physician’s prescription. There is limited data available on the proportion of patients who fail treatment, but our data suggest that treatment failures are likely low for standard first-line antimicrobials. According to Thailand’s guidelines,²⁰ hospitals regularly diagnose and follow the standard of care to treat patients who are identified with gonococcal infections from their urogenital swabs by gram stain or culture methods. Physicians empirically treat symptomatic

patients without waiting for confirmation of the AST results. Furthermore, some antibiotics, like AZM, the drug of choice for enteric bacterial infection caused by traveler’s diarrhea in Thailand are used routinely to treat eye problems and soft-skin infections²⁶ and can be purchased without a prescription. As a result of these reasons, there has been a gradual increase of MDR gonococcal strains throughout the country. These factors combined create the prerequisites for the emergence and spread of GC-resistant strains. Another challenge is that GC is classified as a fastidious bacteria, which requires an appropriate media for growth and prevention of loss of bacterial viability. Limitations of GC microbiological procedures, as well as the specimen collection processes, are the major issues faced across resource-limited settings. Additionally, there is limited AST data derived from patients receiving inappropriate treatments or from treatment failures leading to emerging of resistant strains.

In summary, STDs caused by antimicrobial-resistant GC strains are becoming a public health concern. The global antimicrobial surveillance program assists with the appropriate public and FHP measures to improve appropriate treatment for drug-resistant cases. Due to the limited GC surveillance data and self-antibiotic treatment in Thailand, the GC surveillance program has been widely supported by the government and military leadership since these factors would be associated with the spread of antimicrobial-resistant GC strains in the community. Moreover, advanced genome characterization of MDR isolates from military treatment facilities will be prioritized as one of our main future research objectives, to fill this surveillance gap when compared to civilian and/or military databases of isolate genomes. The desired outcome is to provide data to inform FHP decision-making and public health authorities’ response to the drug-resistant GC threats.

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CONFLICT OF INTEREST STATEMENT

None declared.

ETHICAL APPROVAL

The study protocol was approved by Institutional Review Board, Royal Thai Army Medical Department under protocol number S036b/56 and Walter Reed Army Institute of Research (WRAIR), and Silver Spring, MD under protocol number WR #2039.

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