In Vitro Susceptibility to Zoliflodacin and Solithromycin in *Neisseria gonorrhoeae* Isolated in the Republic of Korea from 2016 to 2018

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Acronyms

AMR	Antimicrobial resistance
AZTH	Azithromycin
CDC	Centers for Disease Control and Prevention
CFX	Cefixime
CIP	Ciprofloxacin
CRO	Ceftriaxone
ESBL	Extended-spectrumbeta-lactamase
FLMs	First-line managers
GM	Gentamycin
HAIs	Healthcare-associated infections
MDR	Multidrug Resistance
MIC	Minimum inhibitory concentrations
MLST	Multilocus sequence typing
MoH	Ministry of Health
NG-MLAST	Neisseria Gonorrhoea multi-antigen sequence typing
PCR	Polymerase chain reaction
PEN	Penicillin
SPT	Spectinomycin
STD	Sexually transmitted infection
TET	Tetracycline
UTI	Urinary Tract Infections
WHO	World Health Organization
XDR	Extensively Drug-Resistance

Abstract

Background: Zoliflodacin (topoisomerase II inhibitor) and solithromycin (first fluoroketolide) are new drugs that are in clinical development for the treatment of uncomplicated gonorrhea. In this study, we determined the *in vitro* activity of zoliflodacin and solithromycin in gonococcal isolates from the Republic of Korea.

Methods: A total of 250 isolates of *N. gonorrhoeae* collected throughout the Republic of Korea from 2016 to 2018 were tested to determine the MIC of therapeutic antimicrobials using the CLSI agar dilution method.

Results: Most isolates (86.4%, 234/250) were non-susceptible to penicillin G, tetracycline, and ciprofloxacin, but all isolates were susceptible to ceftriaxone and spectinomycin. The MIC range for zoliflodacin and solithromycin were ≤0.015–0.12 mg/L and ≤0.015–0.5 mg/L, respectively. MIC_{50} and MIC_{90} were 0.03 and 0.06 mg/L for zoliflodacin and 0.06 and 0.12 mg/L for solithromycin. All isolates belonged to the wild-type MIC distribution for zoliflodacin, and there were no cross-resistance between zoliflodacin and ciprofloxacin (also a topoisomerase II inhibitor). The azithromycin-resistant isolate with the highest MIC of azithromycin (32 mg/L) had the highest MIC of solithromycin (0.5 mg/L), illustrating cross-resistance between azithromycin and solithromycin at higher MICs.

Conclusion: Zoliflodacin and solithromycin showed potent antimicrobial activity against contemporary multidrug-resistant *N. gonorrhoeae* isolates in the Republic of Korea. However, the recently finished phase III clinical trial for solithromycin identified relatively

many treatment failures. Zoliflodacin remains more promising, and a multi-continental phase III clinical trial will be initiated in 2019.

Keywords: zoliflodacin, solithromycin, *N. gonorrhoeae*, South Korea

I. Introduction

1.1. Background

Neisseria gonorrhea is a species of Gram-negative diplococci bacteria that was isolated by Albert Neisser in 1879, also known as gonococcus (singular), or gonococci (plural). Gonorrhea is a sexually transmitted infection (STI) caused by *Neisseria gonorrhea* (gonococcus), one of the world's most chronic and intimidating sexually transmitted diseases, and is a significant public health concern worldwide.

For *Neisseria gonorrhea*, the sexually transmitted disease gonorrhea agent, humans are the only natural host. Gonorrhea is an acute pyogenic infection of the non-conciliated columnar and transitional epithelium; infection can be discovered wherever these cells are discovered. Gonococcal infections are mostly acquired through sexual contact and occur primarily in the urethra, endocervix, anal canal, pharynx, and conjunctiva [25]. The most common types of infection are acute urethritis in men and endocervicitis in women. Most untreated infections resolve spontaneously after several weeks, but serious complications such as pelvic inflammatory diseases and gonococcal infections can occur. People who often have unhealthy sex habits such as having multiple sex partners are at high risk of contracting gonorrhea. Besides, the bacteria that cause gonorrhea can directly infect open wounds in the oral mucosa [7]. Gonorrhea can be spread through everyday items such as towels, underwear. Moreover, gonorrhea can be transmitted through blood transfusions that cannot guarantee the principles of sterility and safety in the process, or through performing medical procedures that are not sterile, not sterilized [7] [25].

WHO announced a global action plan to control gonococcal AMR's spread and influence around the world in 2012 [53] [27]. Simultaneously, WHO Global Health Sector Strategy on Sexually Transmitted Infections was endorsed by the United Nations (UN) World Health Assembly, from 2016–2021, in which one of the significant targets is a 90% reduction in the incidence of gonorrhea globally [51].

In South Korea, There have been many studies carried out in South Korea since the 90s [22]; Moreover, gonorrhea has been prevalent among specific patient groups; findings from 2004 suggest that the disease is concentrated among young people in shelters. At the same time, the incidence of gonorrhea in college students with high sexual activity is 28% [23]. In Korea, antimicrobial resistance in *N. gonorrhea* in recent years has been a severe problem. However, no comprehensive antibiotic resistance data for cultured strains after 2006 has been published internationally [17]. Both spectinomycin and ceftriaxone have been used to treat gonococcal infections primarily as monotherapy before 2011. However, dual antimicrobial therapy (250 mg of ceftriaxone plus 1 g of azithromycin or 250 mg of ceftriaxone plus 100 mg of doxycycline twice daily for seven days) was implemented as the recommended first-line therapy for uncomplicated gonococcal infections in the 2011 Korean guideline [16]. The elevated selective pressure arising from extensive use can lead to resistance growth. Accordingly, quality-assured domestic and global monitoring of antimicrobial resistance is crucial to define the emerging resistance, resistance patterns, and provide adequate information to support timely revisions of treatment guidelines.

1.2. History and evidence of antibiotic-resistant *N. gonorrhoeae*

Not long after the first antimicrobials (sulfonamides) started to be used for the treatment of gonorrhea, AMR in *N. gonorrhoeae* was detected at the beginning of the 20th century. *N. gonorrhoeae* has continually presented an extreme capacity to develop resistance to all antimicrobials introduced for continuing the treatment.

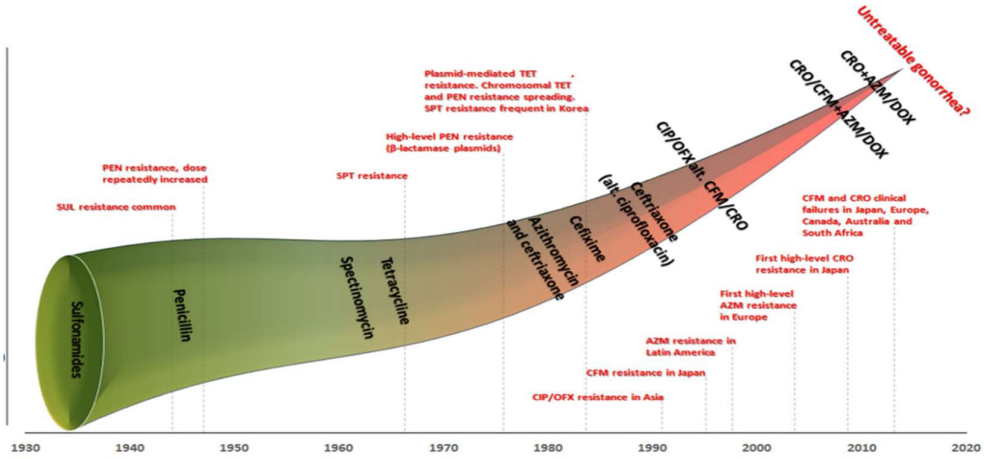


Figure 1: History of antimicrobial and resistance in *Neisseria gonorrhoea* [47]

Factors that contribute to increased resistance include suboptimal diagnostic and surveillance ability, simple antibiotic accessibility (including counterfeit drugs), and absence of drug quality control, which adds to the fast growth of resistance. Resistance has extended to include penicillin, tetracyclines, macrolides (including azithromycin), sulphonamides and combinations of trimethoprim, quinolones, and, more recently, a few isolated strains of cephalosporins [52]. Highly variable *N. gonorrhoeae* strains are widely antimicrobial-resistant, and gonorrhea has consistently jeopardized gonorrhea management

and control. Clinicians resort to empirical treatment for gonorrhea due to extensive AMR, the persistence of AMR determinants in gonococci, and the unavailability of diagnostic exams that provide AMR outcomes at the moment of therapy. Resistance to sulphonamides, penicillin, tetracyclines, macrolides, fluoroquinolones, and early-generation cephalosporins has appeared rapidly since antimicrobial therapy was introduced. Currently, ceftriaxone is the only surviving empirical monotherapy for gonorrhea injectable extended-spectrum cephalosporin (ESC) in most nations. Currently, in many countries were conducted gonococcal in vitro resistance and surveillance to treatment failures in the therapy of the last-line oral ESC cefixime and ceftriaxone have been checked but rarely meeting [53] [47] [38] [39] [45].

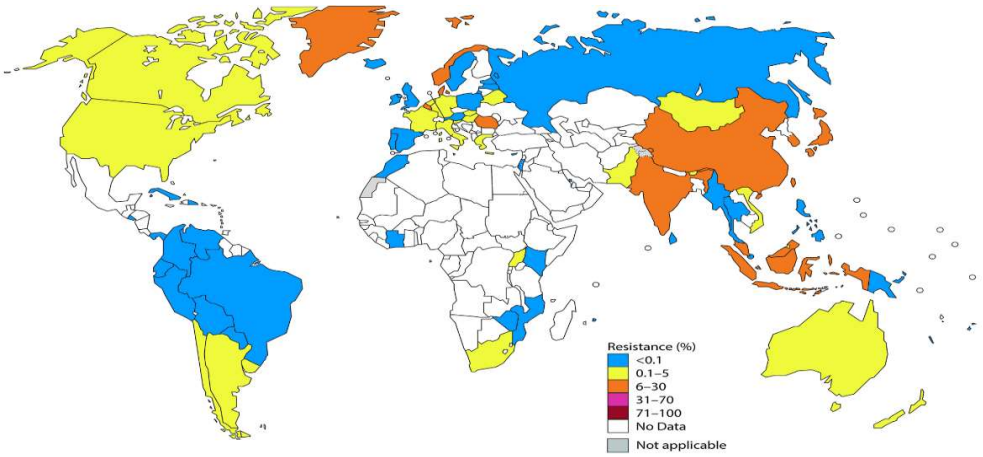


Figure 2: Countries reporting antimicrobial resistance to extended-spectrum cephalosporins [49]

Currently, the only first-line antimicrobials suggested for empirical treatment of uncomplicated gonorrhea in many nations are extended-spectrum cephalosporins. The

incidence of isolates with decreased susceptibility to cefixime (CFM) in the United States has led to dual therapy with ceftriaxone plus azithromycin or doxycycline being the only suggested therapy regimen by Centers for Disease Control and Prevention (CDC) [50]. So far, there have not been any new antibiotics approved to treat gonorrhoea for many years, and whereas the world, appearing in the first three cases of infection with extensively drug-resistance (XDR) *Neisseria gonorrhoeae* resistant to azithromycin and with ceftriaxone, would have a significant public health impact [2] [9] [15]. In the recent decades, several new antimicrobials were introduced with the intention of being patented as medications against gonorrhea in the context of gonorrhea becoming more resistant to medications currently being administered for treatment throughout the world. Some of them are solithromycin and zoliflodacin. In 2018, efficacy data on zoliflodacin, solithromycin, and gepotidacin. New medications for the treatment of uncomplicated gonorrhoea, were released [26] [35].

1.3. Aim of the study

This study aimed (1) to investigate in-vitro susceptibility to zoliflodacin and solithromycin against a collection of *N. gonorrhea* isolates. The susceptibility was compared to the susceptibility to antimicrobials previously and currently used for the treatment of gonorrhea (penicillin G, ceftriaxone, cefixime, spectinomycin, gentamycin, tetracycline, azithromycin, and ciprofloxacin). (2) to perform to molecular epidemiology *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and multi-locus sequence typing (MLST) in all isolates.

II. Literature Review

2.1. Epidemiology of *Neisseria gonorrhoeae* infection.

Considering that gonorrhoea is one of the most prevalent STIs globally, the World Health Organization (WHO), estimated 87 million fresh instances in 2016 [52], the absence of efficient therapy would result in a significant public health problem. It is the second most reported transmissible disease in the United States and the second most common sexually transmitted infection (STI).

WHO reported 106 million new cases of gonorrhea in adults worldwide in 2008, an alarming number of truly urgent risks worldwide. The situation was an increase of 21 per cent relative to 2005. The highest estimates are 42.0 million cases, 25.4 million cases, and 21.1 million cases, respectively, in the WHO Western Pacific Region, WHO Southeast Asia Region, WHO Africa Region. In 2012, the World Health Organization (WHO) continued to estimate that there were 78 million cases among adults worldwide, including 35.2 million, 11.4 million, 11.4 million, 11 million, 4.7 million cases, respectively, in the WHO Western Pacific Region, the African Region, the Americas Region, the European Region, and the Eastern Mediterranean Region [28]. *N. Gonorrhea* is caused by gonorrhea, which spreads mainly through sexual activities and penetrates through open wounds or thin skin and mucous membranes in the mouth. Most people who suffer from gonorrhea are often associated with people who have the disease. Especially the disease is extremely contagious when having unsafe sex; even if uses condom, the possibility of infection is still very high. This is explained by experts that when having sex; there will be rubbing due to

the copulatory movements, causing damage of the vital area. The enclosed area with many blood vessels and contains relatively thin mucosa, making it very susceptible to infection. In a published report of PLOS Medicine (2017), about 78 million people suffer from gonorrhea each year, including 35.2 million in the Western Pacific region, 11.4 million in Southeast Asia, 11.4 million in Africa, 11 million in the Americas, 4.7 million in Europe and 4.5 million in the Eastern Mediterranean region [48].

In 2017, CDC accounted for over 555,000 gonorrhea outbreaks, making it the second most common notifiable disease in the U.S. It is revealed that gonorrhea levels increased 75.2 per cent from the extraordinary low in 2009 and grew 18.6 per cent from 2016 onwards. Antimicrobial resistance in the treatment of gonorrhea remains a significant issue. Ccurrently, CDC has recommended a primary therapy for the disease, ceftriaxone and azithromycin therapy. Since 2008, the proportion of isolates with high minimum ceftriaxone inhibitory levels (MICs) has remained low and was only 0.2% in 2017. The number of isolates with elevated MICs of azithromycin increased from 2.5% to 4.4% during 2014–2017 [5].

South Korean’s neighbors also issued warnings about the country's incidence of gonorrhea. Every year, China reports about 115,000 fresh gonorrhea instances. Gonorrhea have risen in recent years and became one of the country's most surveilled infectious diseases [54].

Globalization is one of the reasons for the growth in infection rate related to travel-associated gonorrhoea accounting for a high proportion of gonorrhoea cases reported in Europe. The reporting showed that 25.5% of the recorded gonorrhea instances were from

overseas between 2008 and 2013, mostly in Thailand (31%), followed by the Philippines (8%) and Spain (7%) [3].

2.2. Epidemiology of antimicrobial resistance in *Neisseria gonorrhoeae*

In recent decades, *N. gonorrhoeae* has developed resistance to several antimicrobial classes such as sulphonamides, penicillin, tetracyclines, macrolides, fluoroquinolones and, more recently, the third-generation cephalosporins [47], Unfortunately, Japan also announced that treatment failure with cefixime was reported early [1] [34], similarly treatment failures to cefixime and azithromycin in England [15], resistance to cefixime in Austria [44], the first cases of *Neisseria gonorrhoeae* resistant to ceftriaxone in Spain [4], Sweden [12] [41], Australia [24] [32], Slovenia [43].

Unfortunately, multidrug-resistant (MDR) gonococcal strains have substantially increased resistance to high-level cefixime and ceftriaxone in France [42].

In some South-East Asian countries like Vietnam, there have also been reports of resistant to azithromycin, 5% to ceftriaxone, and 1% to cefixime [29], in China, the prevalence of resistance to azithromycin and decreased susceptibility to ceftriaxone increased significantly [55].

An urgent issue is thought to be serious with the emergence of MDR with high-level azithromycin resistance (azithromycin MIC of >256 mg/L), the latest case has been recorded in the UK from a heterosexual man who had a regular female sexual partner in the UK and also female sexual contact in South-East Asia [30].

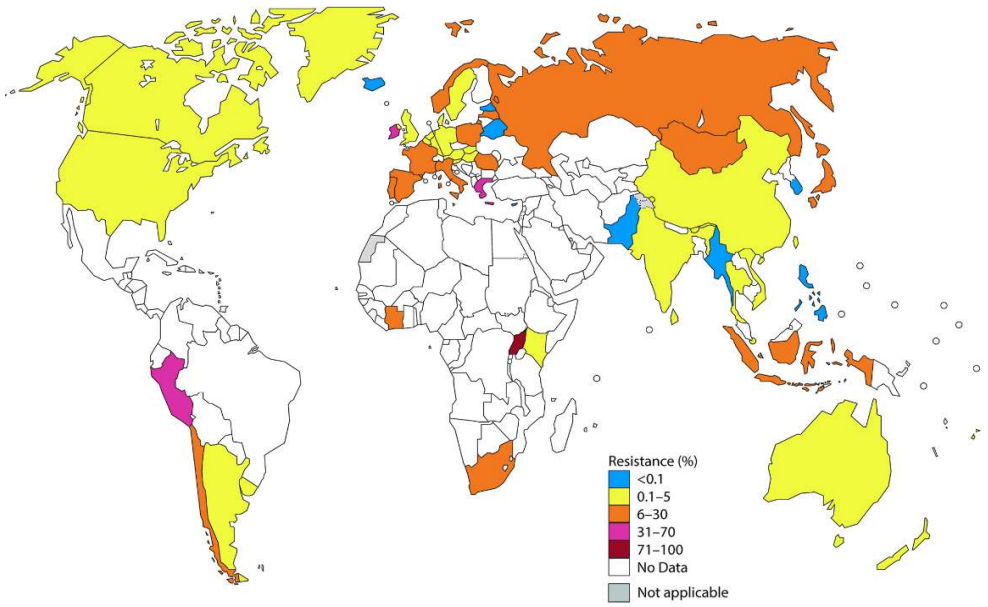


Figure 3: The percentage of isolates with resistance to azithromycin [48]

2.3. Solithromycin

Solithromycin (CEM-101): Solithromycin is a fourth-generation antibiotic of the macrolide, which has been considered to treat bacterial community-acquired pneumonia. Solithromycin has been studied in clinical isolates since 2009 in the United States and Europe [10], and also displayed antimicrobial activity against a diverse collection of Gram-positive and Gram-negative bacteria [31].

Solithromycin has shown a high in vitro activity against *N. gonorrhoeae* reference strains and clinical AMR isolates, that is, with MIC50 and MIC90 of 0.064–0.125 mg/L and 0.125–0.25 mg/L, respectively [11]. Moreover, currently, solithromycin is in phase 3 of clinical trials and has been shown to be active in the treatment of gonorrhea.

2.4. Zolifodacin

Zoliflodacin (ETX0914): As for zoliflodacin, it has a novel action mechanism by which it inhibits the Spiropyrimidinetrione topoisomerase. Zoliflodacin is a new Spiropyrimidinetrione bacterial DNA gyrase/topoisomerase inhibitor with potent in vitro antibacterial activity against key Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus agalactiae*), fastidious Gram-negative (*Haemophilus influenzae* and *Neisseria gonorrhoeae*), atypical (*Legionella pneumophila*), and anaerobic (*Clostridium difficile*) bacterial species, including isolates with known resistance to fluoroquinolones [14]. Currently, ETX0914 is in phase III RCT, now under final planning, in collaboration between the manufacturer Entasis, the Global Antibiotic Research and Development Partnerships (GARDP), and WHO.

III. Methodology

The methods used in this research consist of the following:

3.1. Collection of clinical *Neisseria gonorrhea* samples:

Specimens collected for the discovery of *Neisseria gonorrhea* were taken from the genital tract and other sites: the rectum, pharynx, and joint fluid. Samples were collected from different hospitals all over South Seoul, from January 2016 to December 2018. Moreover, patient identification information is hidden in the study according to ethical approval required for the present study.

3.2. Transporting *Neisseria gonorrhea*:

Samples were collected from the patient's urethra, cervical, rectal, and during the time of transport, media is best kept at room temperature and not refrigerated transport to Yonsei Severance Hospital [7].

3.3. Culture:

The specimens isolated to be modified by Thayer-Martin media (BBL, Becton Dickinson, Cockeysville, MD, USA) consisting of chocolate agar in addition to vancomycin antibiotics to the media used to inhibit the growth of Gram-positive bacteria [36].

3.4. Identification of *Neisseria gonorrhea*:

Inoculated plates incubated at 35±1°C in a 3% to 5% CO_2 atmosphere. Colonies of *Neisseria gonorrhea* on chocolate agar appear grey to tan in color, translucent, and raised after 24 to 48 hours of incubation, and bacteria grew into colonies with diameters of 1mm, opaque, greyish-white, glistening, and convex. At this time, the morphology can be observed under the microscope seen, and gram-negative dyeing is also carried out. The identification of suspected colonies is confirmed by MALDI-TOT mass spectrometry, by allowing for identification of a pathogen in a minute [7]. *Neisseria gonorrhea* isolates: All 250 isolates were collected, and samples were stored at Yonsei Severance Hospital to conduct similar studies in the future.

3.5. Antimicrobial susceptibility testing:

GC agar containing 1% growth supplement is used for disk diffusion testing of *Neisseria gonorrhea*, and also colony suspensions of isolates need to be adjusted to a 0.5 McFarland

standard before inoculation onto media [7]. Disk diffusion is one of the classic microbiology techniques, with convenience, efficiency, it is chosen as the method for determining antimicrobial resistance in this study. MICs of ceftriaxone, spectinomycin, penicillin, and ciprofloxacin were determined. Antimicrobial susceptibility testing was performed using the agar dilution method according to guidelines published by the CLSI [6]. The MIC of cefixime followed the European Committee on Antimicrobial Susceptibility Testing [8], in which gentamycin and azithromycin Antibiotic Susceptibility Testing was performed by the CDS method [37].

The plate was cultured at 36°C with 5% to 10% CO2 for 18 to 24 hours, and the growth of gonococci in different concentrations of antibiotics (0.016, 0.032, 0.064, 0.125, 0.25, 0.5, 1, 2,4, 8, 16, 32, 64 for all antibiotics) was observed and recorded.

3.6. Molecular epidemiological typing:

The DNA sequence of isolates was analyzed, to determine the molecular epidemiological relatedness, *N. gonorrhoeae* multiantigen (por and tbpB) sequence typing (NG-MAST) was carried out for all isolates (from 2016 to 2017). The sequences data of each por and tbpB gene were uploaded to the *N.gonorrhoea* multi-antigen sequence typing (NG-MAST) website (http://www.ng-mast.net/misc/info2.asp) to obtain the sequence type. The por and tbpB genes detected in the isolates, and multiplex polymerase chain reactions with two sets of primers for por *(*forward 5’ 350CAA GAA GAC CTC GGC AA366 3', reverse 5’ 1086CCG ACA ACC ACT TGG T1071 3'), and tbpB (forward 5’ 1098CGT TGT CGG CAG CGC GAA AAC 1118 3’, reverse 5’ 1686TTC ATC GGT GCG CTC GCC

TTG1666 3') were performed in 50µl volumes and are carried out in 96 well microtitre plates with QIAquick Gel Extraction Kit, using a PTC-200 DNA engine (MJ Research Inc) with an initial denaturation at 950C for 4 minutes, followed by 25 cycles of 95^0C for 30 seconds, 58^0C for 30 seconds for *por* and 69^0C for *tbpB*, 72^0C for 1 minute, followed by 72^0C for 10 minutes and cooled to 40^0C and held.

Multilocus sequence typing (MLST) performed for all isolates (from 2016 to 2018) that showed resistance to at least one of the ESCs examined, followed by guidelines outlined on the respective database website (http://pubmlst.org/neisseria/).

IV. Ethics statement

The research did not provide demographic information of the patients. Following internal sentinel surveillance processes, all gonococcal isolates were grown and stored. This research was also given an exemption from the ethics approval requirement because it was performed as one of the projects of the national surveillance program supported by the Korean Centers for Disease Control and Prevention.

V. Result

Based on historical data, the study isolated the result of increasing the quantity over three years. The chart provides the number of isolates in 250 samples from 2016 to 2018, in which the case by year is 59, 82, and 109, respectively (Figure 4). As can be seen, this result displays the situation of increasing gonorrhea in Korea, 2018 is twice as high as in 2016.

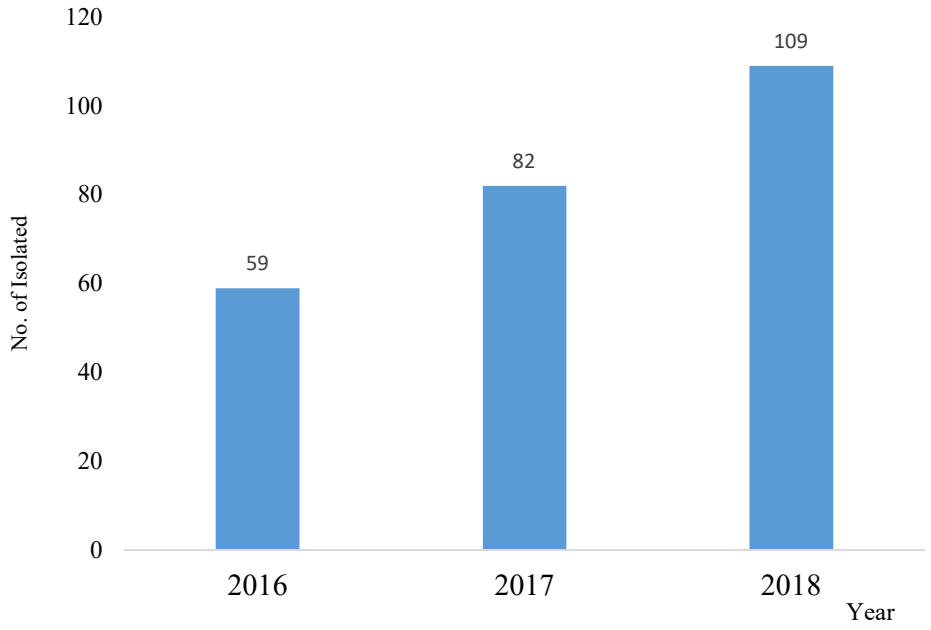


Figure 4: Distribution of *N.gonorrhea* isolated from 2016 – 2018 in South Korea

Table 1 illustrates the susceptibility to novel CEM-101, ETX0914, and antimicrobials previously and currently used for the treatment against *N.gonorrhea*. It can be clearly seen

that MIC of ETX0914 ranges from 0.014 to 0.12 mg/L, in which MIC_{50} and MIC_{90} are 0.03, 0.06 mg/L, respectively. In addition, MIC modal is 0.03 mg/L, from the results of this MIC, it can be stated that ETX0914's ability to inhibit bacteria is promising. Similarly CEM-101 displays MIC range from 014 - 0.5 mg/L, modal MIC is 0.06 mg/L and MIC_{50} and MIC_{90} are 0.06, 0.12 mg/L respectively.

Representative for ESCs, the figure of ceftriaxone MIC range is 0.007 - 0.5 mg/L, and MIC_{50} and MIC_{90} are 0.03, 0.12 mg/L respectively. In the same way, the MIC range of cefixime is the same as that ceftriaxone. There is a difference in concentration between MIC_{50} compared with MIC_{90} which are 0.06, 0.25 respectively.

While the illustration of azithromycin MIC range shows 0.05 – 32 mg/L, MIC_{50} and MIC_{90} are 0.25, 0.5 mg/L, respectively. Another result is that the value of the MIC range of spectinomycin is within the acceptable range 8 – 64 mg/L; wherein MIC_{50}, MIC_{90} had equal value (32 mg/L); this indicates that it can still be used for treatment against gonorrhoea.

The table also noted penicillin, tetracycline, and ciprofloxacin had antimicrobial resistance, it can be seen that particular: penicillin MIC range is 0.06 - 129 mg/L, and MIC_{50} and MIC_{90} are 2, 16 mg/L respectively. Tetracycline MIC range is 0.12 - 129 mg/L, and MIC_{50} and MIC_{90} are 4, 64 mg/L respectively. Similarly, the MIC of ciprofloxacin range is 0.05 - 64mg/L, and MIC_{50} and MIC_{90} are 8, 16 mg/L, respectively.

Table 1: Susceptibility to novel CEM-101, ETX0914, and antimicrobials previously and currently used for the treatment against NG in 250 isolates from 2016-2018 in Korea

Antimicrobial	MIC (mg/L)			MIC break-points (mg/L)	
	Range	50%	90%	I	R
ETX0914	0.014 - 0.12	0.03	0.06	ND	ND
CEM-101	0.014 - 0.5	0.06	0.12	ND	ND
PEN	0.06 - 129	2	16	0.12 - 1	≥ 2
CRO	0.007 - 0.5	0.03	0.12	-	-
CFX[1]	0.007 - 0.5	0.06	0.25	-	>0.125
SPT	8 - 64	32	32	64	≥ 128
GM[2]	0.25 - 32	4	8	8-16	>16
TET	0.12 - 129	4	64	0.5 - 1	≥ 2
CIP	0.05 - 64	8	16	0.12 - 0.5	≥ 1
AZTH[2]	0.05 - 32	0.25	0.5	-	≥ 1

ETX0914: Zoliflodacin; CEM-101: solithromycin; PEN: penicillin; CRO: ceftriaxone; CFX: cefixime;
SPT: spectinomycin; GM: gentamycin; TET: tetracycline; CIP: ciprofloxacin; AZTH: azithromycin.
1: European Committee on Antimicrobial Susceptibility Testing
2: Antibiotic Susceptibility Testing By The CDS method
ND: not determined

Table 2 showed the proportion of the difference between medicine susceptibility test results, high, and intermediate level resistance, among eight antibiotics done on 250 isolates. Over the years from 2016 – 2018, the high-proportion of resistance to both ciprofloxacin and tetracycline are 94.8%, 78.8%, respectively. Particular by year with ciprofloxacin, which are 98.3%, 98.8%, 89.9%, respectively. The same with tetracycline, which is 79.7%, 79.3%, and 78%%, respectively.

The results also displayed that ciprofloxacin and tetracycline susceptibility level was low, under 10% in all three years, that account for the non-use of these antibiotics in the treatment of gonorrhea. Intermediate level in penicillin accounted for exceeding 43% among the samples isolated from 2016 to 2018 were 44.1%, 43.9%, and 54%, respectively, without the appearance of medicine- susceptibility isolation in penicillin.

Susceptibility, according to table 2, shows no drug resistance observed for ceftriaxone, only 0.4% was intermediate level over three years, remaining the isolated samples remained susceptibility to ceftriaxone, the proportion in turn over the years was 100%, 98.8%, and 100%, respectively.

Similarly, the results also displayed that spectinomycin also has a 98.8% susceptibility level among the samples isolated through the years, in 2017 susceptibility level was 96.3%, no resistance in spectinomycin is isolated from 2016-2018.

It is noticeable that the proportion of resistance to cefixime exceeded 33% over three years, in which the proportion of resistance was 37.3%, 31.7%, and 25%, respectively. Meanwhile, for azithromycin, the sample isolated in 2017 did not detect drug resistance level, however, the year 2016 and 2018, the percentage of the isolated form of drug resistance level was slightly higher (3.4%) and 13.8%, respectively.

Besides, gentamycin did not have resistance levels in samples that are isolated. Although the trend, the proportion of gentamycin was high susceptibility, intermediate level, namely, 2016 year is 45.8%, expanding to 63% in 2018.

Table 2: Susceptibility to antimicrobials previously and currently used for the treatment of gonorrhea in 250 isolated in South Korea.

Antimicrobials	S/I/R (%)			
	2016	**2017**	**2018**	**Total**
PEN	0/44.1/**55.9**	0/43.9/**56.1**	0.9/54.1/**45**	0.4/48.4/**51.2**
CRO	100/0/0	98.8/1.2/0	100/0/0	99.6/0.4/**0**
CFX[1]	62.7/0/**37.3**	68.3/0/**31.7**	67/0/**23**	66.4/0/**33.6**
SPT	100/0/**0**	96.3/3.7/**0**	100/0/**0**	98.8/1.2/**0**
GM[2]	54.2/45.8/**0**	73.2/26.8/**0**	36.1/63/**0.9**	52.6/47/**0.4**
TET	0/20.3/**79.7**	6.1/14.6/**79.3**	1.8/20.2/**78**	2.8/18.4/**78.8**
CIP	1.7/0/**98.3**	1.2/0/**98.8**	3.7/6.4/**89.9**	2.4/2.8/**94.8**
AZI[2]	96.6/0/**3.4**	100/0/**0**	86.2/0/**13.8**	93.2/0/**6.8**

ETX0914: zoliflodacin; CEM-101: solithromycin; PEN: penicillin; CRO: ceftriaxone; CFX: cefixime; SPT: spectinomycin; GM: gentamycin; TET: tetracycline; CIP: ciprofloxacin; AZTH: azithromycin.
1: European Committee on Antimicrobial Susceptibility Testing
2: Antibiotic Susceptibility Testing By The CDS method
S: susceptibility, I: intermediate, R: resistance

The Table 3 showed the MIC distributions for solithromycin, zoliflodacin, the penicillin, ceftriaxone, cefixime, spectinomycin, gentamycin, tetracycline, ciprofloxacin, azithromycin (Figure 5 and Table 3), of which penicillin (51.2%), tetracycline (78.8%) and ciprofloxacin (94.8%) are fully resistant to the treatment of gonorrhea, besides azithromycin has also developed resistance (17 cases), similar to cefixime. More than 1/3 (84 cases) of isolated samples also developed resistance.

In this study, the antibiotics currently used to treat gonorrhea are still susceptible to the gonorrhea bacteria, particular: azithromycin (93.2%), spectinomycin (100%), ceftriaxone (100%), cefixime (66.4%). Only ciprofloxacin (94.8%), tetracycline (78.8%), penicillin (51.2%) were resistant to *N. gonorrhea.*

The MIC distribution for zoliflodacin is less than 0.25 mg/L, of which less than 78% or equal to 0.032 m /L, the result is close to 0. Similarly, most of MICs ceftriaxone is less than or equal to 0.125 mg/L while distributive cefixime is discrete. A slight difference in MIC distribution for solithromycin was around over 2.8% samples higher than 0.125mg/L. Overall, the MIC range of antimicrobials (ETX, SOL, CRO, and CFX) had from 0.016-0.125 mg/L.

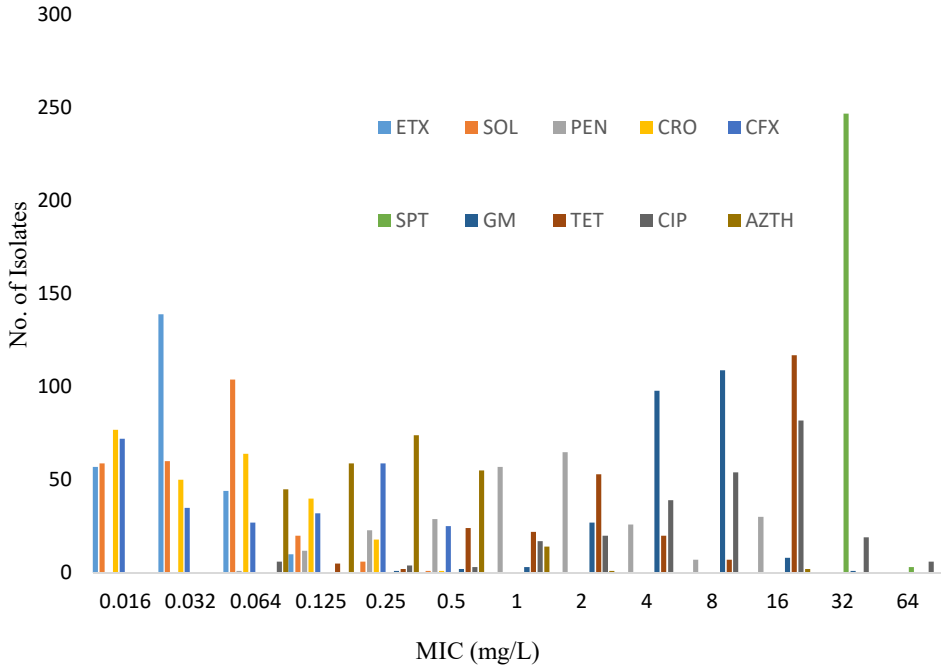
Table 3: MIC (mg/liter) distributions for ETX0914, AZTH, CFX, CIP, CRO, GM, SOL, SPT, TET in 250 isolated in South Korea

Anti-micro-bials	**MIC (mg/L)**												
	0.016	**0.032**	**0.064**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
ETX	57	139	44	10									
SOL	59	60	104	20	6	1							
PEN			1	12	23	29	57	65	26	7	30		
CRO	77	50	64	40	18	1							
CFX	72	35	27	32	59	25							
SPT												247	3
GM					1	2	3	27	98	109	8	1	
TET				5	2	24	22	53	20	7	117		
CIP			6		4	3	17	20	39	54	82	19	6
AZTH			45	59	74	55	14	1			2		

ETX0914: zoliflodacin; CEM-101: solithromycin; PEN: penicillin; CRO: ceftriaxone; CFX: cefixime; SPT: spectinomycin; GM: gentamycin; TET: tetracycline; CIP: ciprofloxacin; AZTH: azithromycin.

Herein, both zoliflodacin and solithromycin were diluted in cell culture medium and tested at concentrations ranging from 0.016 to 16mg/L, the inhibitory concentration of zoliflodacin and solithromycin is nearly zero value, which suggests that the effectiveness of testing isolates is extremely susceptible to both antibiotics. The correlation coefficient between the MICs of zoliflodacin and ciprofloxacin in 250 isolates was - Coefficient of correlation Spearman's: 0.437 ($p<0.05$). This means there are no associations between the MICs of zoliflodacin and the previously used DNA gyrase/topoisomerase IV inhibitor fluoroquinolone ciprofloxacin or there were no cross-resistance between zoliflodacin and ciprofloxacin.

On the by hand, between the two drug groups of the same generation, experiments on isolated samples also showed that the inhibitory concentration of solithromycin is much less than that of azithromycin, the coefficient of correlation was – Coefficient of correlation Spearman's: 0.745 ($p<0.05$). This correlation may be related to the gonorrhea strains circulating in Seoul, mainly when travel and mutant circulation have occurred.



ETX0914: zoliflodacin; CEM-101: solithromycin; PEN: penicillin; CRO: ceftriaxone; CFX: cefixime;
SPT: spectinomycin; GM: gentamycin; TET: tetracycline; CIP: ciprofloxacin; AZTH: azithromycin

Figure 5: MIC (mg/liter) distributions for ETX0914, AZTH, CFX, DIP, CRO, GM, SOL, SPT, TET in 250 isolated in South Korea

Figure 6 illustrates of the molecular epidemiology of the 240 *N.gonorrhoea* isolates sequence type detected from 2016 to 2018, the majority of MLST are ST1901 (71), ST1588 (68), ST7823 (21), ST1759(9) and a few other sequence types are detected but in small numbers, and three new strains NEW-1(8), NEW-2(1), NEW-3(1) were also identified.

An analysis of *N.gonorrhoea* isolates MLST STs over the years observed eight STs (1901, 1588, 7363, NEW-1, 1579, 7822, 1596, 11431) , eleven STs (1901, 1588, 7823, 7363,

NEW-1, 1579, 1583, 9362, 8780, 1596, 7367, 8143), and twenty-five STs (didn't show in Table 4), respectively (Table 4). In which the number MLST ST1588 increased more than two times in 2016. The remaining strains did not show any abnormal increase, cover newly discovered strain.

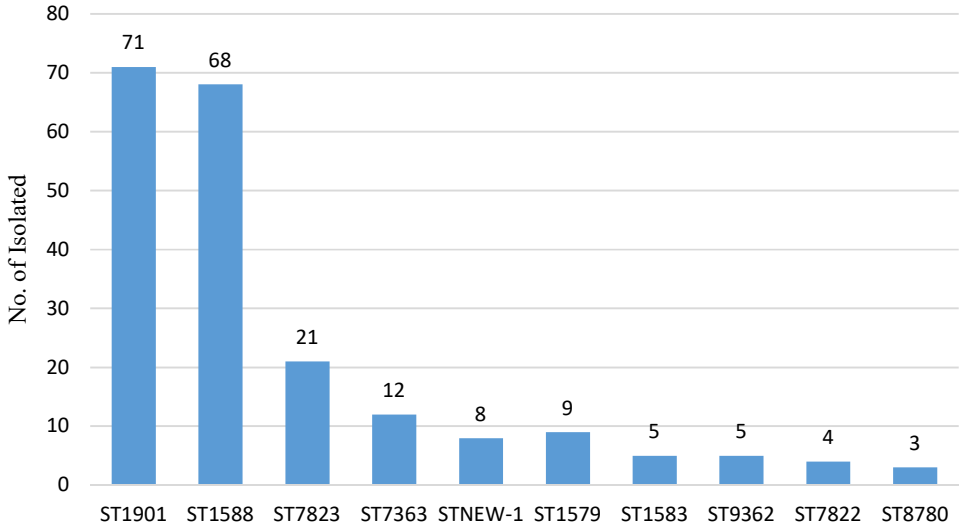


Figure 6: Distribution of isolates by sequence type of MLST from 2016 – 2018 in South Korea

The six strains found in 2016 were observed again in 2017, 2018 (Table 4). Given that these strains are still circulating in the community, some other strains were found to have no overlap that could indicate the circulation from different places, is possible. This evidence study showed that ST1901, ST1588, ST7823, ST7363 are the most common and genetically closely related ST for three years. A new strain is inherited for more than two years, and some others appear scattered but not genetically related (Table 4).

Table 4: Comparison of molecular typing MLST and NG-MAST of specimens positive with Neisseria Gonorrhoeae by year, South Korea.

Year	MLST ST (No. specimens)	NG-MAST ST (No. specimens)
2016	1901 (28)	***10668 (7), 11497 (4),*** 2958 (2), 14678 (2), 14673 (1), 14671 (1), 11495 (1), 7309 (1), 14672 (1), 14676 (1), 14675 (1), 15097 (1), 7307 (1), 14662 (1), 15098 (1), 14666 (1), 14667 (1)
	1588 (8)	3611 (2), 14668 (1), 14661 (1), 5576 (1), 14674 (1), 6696 (1), 12399 (1)
	7363 (3)	6910 (2), 10216 (1)
	NEW-1 (3)	7304 (2), 14664 (1)
	1579 (1)	8044 (1)
	7822 (3)	14663 (1), 14670 (1), 14665 (1)
	1596 (1)	6696 (1)
	11431 (1)	14669 (1)
2017	1901 (24)	***15014 (6), 14678 (5)***, 15019 (1), 4500 (1), 16244 (1), 7307 (1), 15016 (1), 7309 (1), 15866 (1), 11497 (1), 16247 (1), 16270 (1), 16271 (1), 4431 (1), 2958 (1)
	1588 (21)	***15024 (8), 14668 (3)***, 16267 (1), 13411 (1), 16262 (1), 16263 (1), 16264 (1), 16265 (1), 16266 (1), 16255 (1), 3611 (1), 16261 (1)
	7823 (11)	***16250 (5), NEW (2)***, 16274 (1), 16275 (1), 16245 (1), 16251 (1)
	7363 (3)	5308 (1), 16272 (1), 6910 (1)
	NEW-1 (4)	7304 (2), 16280 (1), 16243 (1)
	1579 (3)	8044 (1), 16254 (1), 16203 (1)
	1583 (1)	12535 (1)
	9362 (1)	16278 (1)
	8780 (1)	16277 (1), 15070 (1)
	1596 (1)	16268 (1)
	7367 (1)	16248 (1)
	8143 (2)	9918 (2)

These circulating strains have a high proportion of antibiotic resistance (Table 5), particularly such as ST1901, which is resistant to ciprofloxacin (28.4%), tetracycline (21.2%), penicillin (16.8%), cefixime (12.4%), and also azithromycin (3.6%). A second common strain ST1588 also had a very high resistance rate, namely ciprofloxacin resistance (26.4%), tetracycline (26.4%), penicillin (10.8%), cefixime (13.2%). The NEW-1 strain was resistant to cefixime and azithromycin, while the NEW-2 strain and NEW-3 were not (Table 5).

Table 5: MLST and resistant isolates in South Korea, 2016-2017

ST	No. of isolates	No. of resistant isolates							
		PEN	CRO	CFX	SPT	GM	TET	CIP	AZTH
1901	71	42	0	**31**	0	1	53	71	9
1588	68	27	0	**33**	0	0	66	67	0
7823	21	14	0	0	0	0	18	18	0
7363	12	10	0	8	0	0	8	12	1
NEW-1	8	2	0	**1**	0	0	7	8	1
1579	9	1	0	5	0	0	8	9	3
1583	5	0	0	0	0	0	1	2	0
9362	5	1	0	1	0	0	1	3	0
7822	4	1	0	0	0	0	3	4	0
8780	3	3	0	0	0	0	0	3	0
1596	2	0	0	0	0	0	2	2	0
1600	2	1	0	0	0	0	2	2	1
7367	2	0	0	0	0	0	2	0	0
7371	2	0	0	0	0	0	2	2	1

ST	No. of isolates	No. of resistant isolates							
		PEN	CRO	CFX	SPT	GM	TET	CIP	AZTH
8143	2	2	0	0	0	0	0	2	0
8774	2	0	0	0	0	0	2	2	0
11431	2	2	0	0	0	0	0	2	0
NEW-2	1	0	0	0	0	0	1	1	0
NEW-3	1	1	0	0	0	0	0	1	0

PEN: penicillin; CRO: ceftriaxone; CFX: cefixime; SPT: spectinomycin; GM: gentamycin; TET: tetracycline; CIP: ciprofloxacin; AZTH: azithromycin

VI. Discussion

N. Gonorrhoeae has consistently shown extreme ability to create resistance to all antimicrobials introduced for therapy, and remains one of the world's most prevalent sexually transmitted diseases (STDs). Emerging multi-drug resistant *N. Gonorrhoea* affects global therapy for gonorrhea, a significant public health concern. Dual antibacterial therapy for treatment is currently used in many nations. The suggested dual treatment has a high rate of success but may have contributed to the international reduction of ESC resistance [13] [21] [40]. This study observed that, penicillin, tetracycline, ciprofloxacin was resistant and, in the world today, these three drugs are entirely resistant to gonorrhea, and it has been eliminated from the gonorrhea treatment program by WHO.

On the by hand, our isolates identified and conducted MIC with ESCs, in which ceftriaxone resistance was not detected, but there were some resistant strains of ESCs - cefixime (33.6%) and azithromycin (6.8%) – this cutoff is considered to have started a resistance. In Canada, the government's announcement that antibiotics should not be used to treat gonorrhea if the prevalence of resistance to that antibiotic exceeds 3% [33]. Among ESCs, oral cefixime and injected ceftriaxone is recommended for the treatment of gonorrhea internationally, but most likely caused a treatment failure with cefixime was reported first in Japan [1] [34] thereafter cefixime was no longer recommended as a treatment option in Japan, and cefixime resistance was subsequently reported in many countries around the globe such as France [42], and Austria [44]. Currently, new research in Japan also accounted for the proportion of gonococci that reduce susceptibility or resistance to

ceftriaxone has increased, from 2.5% to 4.0%; besides an increase in azithromycin from 0% to 14.5% was also detected, however, this increase was not significant [34]. A significant increase in resistance of cefixime from 18.0% in 1996-2005 to 46.0% in 2008-2016 in Japan has also been reported [34]. In Korea, there were also studies on isolated samples from 2000 - 2006 where ESCs - cefixime resistant strains also appeared but not significant, with only 1/977 isolates [17], resistance to cefixime has also been reported in subsequent years but in small percentage [20]. The warning of ESCs resistance in South Korea has also been early, in-depth studies of molecules and mutations have been conducted [18] [20] [19]. The discovery of the resistance mechanism of *N. gonorrhoeae* has helped the world to have a better understanding view of the cause of *N. gonorrhoeae* resistance, helping to invent new drugs in the future that can better treat gonorrhea (*Appendix 1*).

In response to this need, we evaluated the antimicrobial activity of CEM-101 and ETX0914 against *N. gonorrhoea*. Herein, the study showed that zoliflodacin and solithromycin are significantly more active against *N. gonorrhoea* isolates and superior to that of other antimicrobials currently or previously recommended for gonorrhea treatment. The MIC distribution for ETX0914 appeared to represent mainly a wild-type MIC distribution, as well as no cross-resistance between ETX0914 and ciprofloxacin (also a topoisomerase II inhibitor). In comparison, all MIC of isolates were less than 0.25 mg/L and displayed high potency against isolates.

It is related to ETX0914 of MIC distribution of CEM-101 <0.5mg/L is also much lower than the other antibiotics tested with it. Only one isolate (0.4%) MIC of solithromycin (0.5 mg/mL) has been confirmed by MLST (ST1600), which is resistant to azithromycin. In short, in vitro antimicrobial susceptibility results for both CEM-101 and ETX0914 demonstrate better activity compared with azithromycin, ceftriaxone, which first-line treatment of *N. gonorrhoeae*.

For all isolates with in vitro resistance to cefixime (n=84; 33.6%), and azithromycin (n=17; 6.8%) the MIC50, MIC90 of solithromycin were 0.06 mg/L, 0.12mg/L, respectively. Similarly zoliflodacin were 0.03mg/L, 0.06mg/L, respectively. The findings of this research were consistent with that of earlier smaller studies that examined the susceptibility of ETX0914 and CEM-101 among *N. gonorrhoeae* isolates [46] [26], the breakpoint was suggested with both MICs <0.25mg/L susceptible in this study. Asserting that, evidence from previous clinical trials and in vitro isolates has shown that both zoliflodacin and solithromycin might be an effective treatment option for gonorrhea in the future, specifically solithromycin is more potent than azithromycin against gonococcal strains.

Multi-locus sequence typing investigation and relationship between MLST and cephalosporin resistance: Molecular typing of gonococci circulating in South Korea were also found in the isolates of this study [21]. Genetic strains such as ST1901 and ST1588 displayed resistance to cefixime of 12.4% and 13.2%, respectively. Discover the prevalence of antibiotic-resistant gonococci, and molecular mechanisms of ESC resistance, antibiotic susceptibility and molecular characteristics of *N. gonorrhoeae* strains showed a significant

increase in susceptibility or resistance to cefixime (33.6%) and resistance to ciprofloxacin (94.4%) and azithromycin (14.5%) detected among gonococcal strains obtained from 2016 2018. Failure of ceftriaxone treatment has also been identified worldwide, with evidence of MLST ST1901 reported in Sweden [41] [12], Australia [24] [32], Slovenia [43], although we have not observed it in vitro in this time.

N. gonorrhoeae multiantigen sequence typing analysis and relationship between NG-MAST and MLST: In recent years, NG-MAST ST15024, ST10668, ST14678, ST15014, ST11497, ST16250 have been a prevalent ST in South Korea, and this ST has also accounted for a substantial proportion of the decreased susceptibility and resistance to ESCs in South Korea [21]. In this study, a total of 131 NG-MAST STs were identified among the 250 isolates from 2016 to 2018 and three new strains were detected. ST15024, ST10668, ST14678 were the most prevalent NG-MAST ST while the first NEW strain is related to por8724, tbpB445, the second NEW strain is related to por1494, tbpB110. Both strains are still susceptible to cefixime and ceftriaxone, and there was not any difference between por alleles, tpbB alleles, and MLST ST among the most common NG-MAST.

Antimicrobial resistance (AMR) in bacterial pathogens has become one of the most critical public health threats worldwide, not for gonorrhea but for all other infectious diseases. Morbidity and mortality rates are high. A comprehensive antibiotic resistance surveillance program will help reduce morbidity, mortality, prolong life, and reduce treatment costs, reducing the burden of disease in the country. In the scope of this study, the following are recommended:

- Continue to maintain essential surveillance for gonorrhea as a response to global health security goals.
- Currently approved treatments of double antibacterial therapy (250 mg of ceftriaxone plus 1g of azithromycin or 250 mg of ceftriaxone plus 100 mg of doxycycline twice daily for 7 days) can still respond well to treatment for patients. However, azithromycin resistance will be a potential challenge in the future

VII. Conclusion

The in vitro susceptibility to zoliflodacin and solithromycin were examined *N. gonorrhoeae* isolates (n=250) from 2016 to 2018. These in vitro outcomes indicate that for close future gonorrhea therapy, zoliflodacin and solithromycin may be efficient antimicrobials. Results of the MICs indicated a wild-type distribution primarily and compared in vitro activity against gonococcal isolates that are prone and resistant to ciprofloxacin, ceftriaxone, cefixime and azithromycin, the first line of which is gonorrhea therapy. There were no cross-resistance between zoliflodacin and ciprofloxacin (also a topoisomerase II inhibitor) or ones previously used for the treatment of gonorrhea. However, cross-resistance between azithromycin and solithromycin has been illustrated. Additional research on *N. gonorrhea* also confirms the results of the recently completed phase 3 trial in vitro/pharmacokinetics in humans. Nevertheless, it should be noted that the recently completed Phase III clinical trial for solithromycin has identified relatively many treatment failures. Zoliflodacin is more promising, and a multi-continental Phase III clinical trial will begin in 2019, which would be highly valuable.

ESC's resistance is also a focus on the treatment of gonorrhea. Although ceftriaxone-resistant *N. gonorrhoea* isolates have not yet appeared, more than one-third of the isolates were resistant to cefixime. These strains have a very close genetic relationship with the current common strains. Three new strains were detected in *N. gonorrhoea* isolates, of which one was resistant to azithromycin. With the current first-line treatment regimen, it is likely that in the future, there will be more strains resistant to azithromycin, and the need to find new alternatives is urgent.

Appendix 1: Antimicrobial resistance determinants in *Neisseria gonorrhoeae* for antimicrobials used for treatment of gonorrhea [47]

Antimicrobial	Resistance determinants/mechanisms
Ceftriaxone, cefixime	– **Mosaic penA alleles:** encode mosaic PBP2s with decreased PBP2 acylation rate. Mosaic PBP2s amino acid substitutions confirmed to contribute to resistance are A311V, I312M, V316T, V316P, T483S, A501P, A501V, N512Y, and G545S – **penA SNPs**: A501Vand A501T in nonmosaic penA alleles can increase the MICs of ESCs. Also G542S, P551S, and P551L have been statistically associated with elevated MICs ofESCs; however, their effects on resistance have not been proven with, for example, site-directed penA mutants into isogenic backgrounds – **Mosaic mtr locus or mtrR mutations**, in promoter (mainly a single nucleotide (A) deletion in the 13-bp inverted repeat sequence) or coding sequence (most common being a G45D amino acid substitution), that cause an overexpression and enhanced efflux of the MtrCDE efflux pump – **porB1b SNPs**: for example, G120Kand G120D/A121D in loop 3 ofPorB1b that decrease influx (penB resistance determinant). The penB phenotype appears only expressed in gonococcal strains that express also the mtrR resistance determinant – **"Factor X"**: unknown nontransformable penicillin and ESC resistance determinant
Azithromycin	– **SNPs in the 23S rRNA gene** (in 1–4 ofthe four alleles) that encodes 23S rRNA (peptidyltransferase loop ofdomain V) with decreased affinity to the 50S ribosomal target for azithromycin. The SNPs C2611T and A2059G cause low-level and high-level resistance, respectively; however, the number of mutated alleles is correlated with the MICs of azithromycin – **mtrR mutations**: see above – **erm genes (ermB, ermC, and ermF):** acquired from other bacterial species and encode rRNA methylases that can methylate nucleotides in the 23S rRNA azithromycin target – **MacAB efflux pump**: overexpression can elevate the MICs of azithromycin – **mef-encoded efflux pump**: acquired from other bacterial species and export macrolides out of the bacterial cell and elevate the MICs of macrolides
Spectinomycin	– **16S rRNA SNP**: C1192U in the spectinomycin-binding region of helix 34 that decreases affinity to ribosomal spectinomycin target – **rpsE mutations** (encoding the 30S ribosomal protein S5): resulting in amino acid alterations such as T24P, deletion of V25, and K26E, which disrupt the spectinomycin binding to ribosomal target
Ciprofloxacin, ofloxacin	– **gyrA SNPs**: for example, S91F, D95N, and D95G in the QRDRthat decrease the fluoroquinolone binding to the GyrA subcomponent of DNA gyrase – **parCSNPs**: for example, D86N, S88P, and E91K in the QRDRthat decrease the fluoroquinolone binding to the ParC subcomponent of topoisomerase IV

PBP2 penicillin-binding protein 2 (PBP2), MIC minimum inhibitory concentration, ESCs extended-spectrum cephalosporins, SNP single nucleotide polymorphism, QRDR quinolone resistance determining region.

Appendix 2: Poster International Interscience Conference on Infection and Chemotherapy and International Symposium on Antimicrobial Agents and Resistance

P1-GN21

In Vitro Susceptibility to Zoliflodacin and Solithromycin in *Neisseria gonorrhoeae* Isolated in Korea from 2016 to 2018

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Abstract

Background: Zoliflodacin (topoisomerase II inhibitor) and solithromycin (first fluoroketolide) are new drugs which are in clinical development for treatment of uncomplicated gonorrhea. In this study, we determined the in vitro activity of zoliflodacin and solithromycin in gonococcal isolates from Republic of Korea.

Methods: A total of 250 isolates of *N. gonorrhoeae* collected throughout Republic of Korea from 2016 to 2018 were tested to determine MIC of therapeutic antimicrobials using CLSI agar dilution method.

Results: Most isolates (86.4%, 234/250) were non-susceptible to penicillin G, tetracycline and ciprofloxacin, but all isolates were susceptible to ceftriaxone and spectinomycin. The MIC range for zoliflodacin and solithromycin were ≤0.015–0.12 mg/L and ≤0.015–0.5 mg/L, respectively. MIC_{50} and MIC_{90} were 0.03 and 0.06 mg/L for zoliflodacin and 0.06 and 0.12 mg/L for solithromycin. All isolates belonged to the wild-type MIC distribution for zoliflodacin and there were no cross-resistance between zoliflodacin and ciprofloxacin (also a topoisomerase II inhibitor). The azithromycin-resistant isolate with the highest MIC of azithromycin (32 mg/L) had the highest MIC of solithromycin (0.5 mg/L), illustrating cross-resistance between azithromycin and solithromycin at higher MICs.

Conclusion: Zoliflodacin and solithromycin showed potent antimicrobial activity against contemporary multidrug-resistant *N. gonorrhoeae* isolates in Republic of Korea. However, the recently finished phase III clinical trial for solithromycin identified relatively many treatment failures. Zoliflodacin remains more promising and a multi-continental phase III clinical trial will be initiated in 2019.

Introduction

In recent decades, several antimicrobials with the intention of being able to patent out certain medications against gonorrhea in the context of medications currently being administered treatment for gonorrhea is becoming more resistant throughout the world. Zoliflodacin (topoisomerase II inhibitor) and solithromycin (first fluoroketolide) are new drugs which are in clinical development for treatment of uncomplicated gonorrhea. In this study, we aimed to investigate in-vitro susceptibility to zoliflodacin (ETX0914) and solithromycin (CEM-101) against a collection of *N. gonorrhoeae* from 2016 to 2018 in Republic of Korea.

Materials and Methods

- Bacterial strains
  - A total of 250 *N. gonorrhoeae* isolates were collected from patients with urethritis from 2016 to 2018. Most of *N. gonorrhoeae* were isolated from specimens delivered from primary-care hospitals. Thirty-five hospitals located all over the country were enrolled.
  - The specimen was inoculated to modified Thayer-Martin media (BBL, Becton Dickinson, Cockeysville, MD, USA) and identification was performed by MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA, USAGerman) and biochemical methods using Vitek NHI card (bioMerieux, Marcy E'toile, France).
- Antimicrobial susceptibility testing
  - Antimicrobial susceptibility was tested by agar dilution method with GC II agar base plus 1% IsoVitaleX and interpreted by CLSI, EUCAST, and Australian guideline (CDS).
  - Antimicrobial agents were used as following: Penicillin G, ceftriaxone, cefixime, spectinomycin, gentamicin, tetracycline, ciprofloxacin and azithromycin.
  - Zoliflodacin and solithromycin were tested by manufacturer's guidance using agar dilution method. Zoliflodacin and solithromycin were resolved using 100% DMSO and diluted.

Results

- Most isolates (86.4%, 234/250) were non-susceptible to penicillin G, tetracycline and ciprofloxacin, but all isolates were susceptible to ceftriaxone and spectinomycin (Table 1).
- The MIC range for zoliflodacin and solithromycin were ≤0.015–0.12 mg/L and ≤0.015–0.5 mg/L, respectively. MIC_{50} and MIC_{90} were 0.03 and 0.06 mg/L for zoliflodacin and 0.06 and 0.12 mg/L for solithromycin (Table 2).
- All isolates belonged to the wild-type MIC distribution for zoliflodacin and there were no cross-resistance between zoliflodacin and ciprofloxacin (also a topoisomerase II inhibitor) (Figure 1) (Table 2).
- The azithromycin-resistant isolate with the highest MIC of azithromycin (16 mg/L) had the highest MIC of solithromycin (0.5 mg/L), illustrating cross-resistance between azithromycin and solithromycin at higher MICs (Table 2).
- The MIC distribution for zoliflodacin appeared to represent mainly a wild-type MIC distribution, all MICs of isolates were less than 0.25 mg/L. similar with zoliflodacin, the MIC distribution of solithromycin <0.5mg/L. The breakpoint was suggested with both MICs <0.25mg/L susceptible in this study.

Table 1. Antimicrobial susceptibility of *N. gonorrhoeae* isolated from Korea during 2016 and 2018

Antibiotics	2016		2017		2018		Total
	S	R	S	R	S	R	R
Penicillin	0	56	0	56	0	45	51
Ceftriaxone	100	0	100	0	100	0	0
Cefixime	63	37	68	32	67	23	34
Spectinomycin	100	0	96	0	100	0	0
Gentamicin[1]	54	0	73	0	36	1	<1
Tetracycline	0	80	6	79	2	78	79
Ciprofloxacin	2	98	1	88	4	80	95
Azithromycin[2]	81	3	87	0	57	11	5

1. Gentamicin was interpreted with CDS guideline by Australia
2. Azithromycin was interpreted with EUCAST guideline (2018)

Table 2. MIC (mg/liter) distributions for zoliflodacin, ciprofloxacin, solithromycin and azithromycin in 250 *N. gonorrhoeae* isolates collected from 2016-2018 in South Korea (Red color means resistant and orange color means intermediate)

Antibiotics	No. isolates with MIC (mg/L) of										
	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	≥16
Zoliflodacin	57	139	44	10							
Ciprofloxacin			6		4	3	17	10	36	64	110
Solithromycin	59	68	104	20	6	1					
Azithromycin			45	59	74	55	16	1			1

Figure 1. MIC (mg/L) distributions for zoliflodacin (ETX), solithromycin (SOL), ciprofloxacin (CIP) and azithromycin (AZTH) in 250 *N. gonorrhoeae* isolates collected from 2016-2018 in South Korea.

Conclusions

- Zoliflodacin and solithromycin showed potent antimicrobial activity against contemporary multidrug-resistant *N. gonorrhoeae* isolates in Republic of Korea.
- The recently finished phase III clinical trial for solithromycin identified relatively many treatment failures. Zoliflodacin remains more promising and a multi-continental phase III clinical trial will be initiated in 2019

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