

Susceptibility Pattern of *Neisseria gonorrhoeae* towards Cefixime and Ceftriaxone using Kirby-Bauer Method in Dr. Saiful Anwar General Hospital Malang

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ABSTRACT

Background: Gonorrhea remains the second most common sexually transmitted infection (STI) in the world with an increasing number of cases. Oral cefixime and IM ceftriaxone are still the mainstay therapy for gonorrhea in Indonesia. However, previous studies suggested possible resistance to ceftriaxone and cefixime, which are the first-line treatment of gonorrhea. To date, there are no data available regarding the susceptibility of these antibiotics for the treatment of gonorrhea in Dr. Saiful Anwar General Hospital (RSSA) Malang. **Purpose:** Determine susceptibility pattern of *Neisseria gonorrhoeae* towards cefixime and ceftriaxone in RSSA Malang. **Methods:** The samples were patients of the Outpatient Clinic of Dermatology and Venereology Department with a symptom of discharge which contained Gram-negative diplococcus after Gram staining and had a positive culture of *Neisseria gonorrhoeae*. Susceptibility testing for cefixime and ceftriaxone antibiotics were performed using the Kirby-Bauer method. The data are presented in percentages. **Result:** Antibiotic susceptibility test results showed that 80.77% of *Neisseria gonorrhoeae* isolates were still susceptible to cefixime, and 80.77% of isolates were still susceptible to ceftriaxone. **Conclusion:** Cefixime and ceftriaxone are still effective as gonorrhea therapy in RSSA Malang.

Keywords: susceptibility, resistance, cefixime, ceftriaxone.

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BACKGROUND

Gonorrhea is currently the second most common sexually transmitted infection (STI) in the world.^{1,2} According to the World Health Organization (WHO), there are a total of 106 million cases of gonorrhea infection globally each year.³ Incident of gonorrhea in the United States reached 98.1 cases per 100,000 population in 2009. During 2015 – 2016, the prevalence of gonorrhea cases increased by 48.6% compared to 2009.⁴ Incidence of gonorrhea in Southeast Asia is 16.2 per 1,000 females and 37 per 1,000 males, with a prevalence of 0.8% in females and 1.2% in males.⁵

Intramuscular (IM) ceftriaxone, a third-generation cephalosporin, is the first-line drug for the treatment of gonorrhea, while oral cefixime oral is no longer recommended by the Centers for Disease Control and Prevention (CDC) in 2015 as the primary choice of therapy for gonorrhea due to numerous reports of resistance.⁴ To date, there is no report from WHO regarding cephalosporin resistance in Indonesia. Several studies conducted in some cities of Indonesia (Semarang, Surabaya, and Denpasar) suggested a decreased susceptibility of cefixime and

ceftriaxone.^{6,7,8} Oral cefixime and IM ceftriaxone are still the mainstay therapy of gonorrhea in Indonesia.⁹

Cephalosporin resistance has been increasingly reported. Recent studies in Japan and France found two ceftriaxone resistant strains.¹⁰ Study by Hananta *et al.* collected isolates from multiple STI clinics in Jakarta, Yogyakarta, and Denpasar, and they found that the isolates were 100% susceptible to ceftriaxone and cefixime.⁶ In contrast, a study in Bali showed that 37.9% of isolates were susceptible to ceftriaxone, and 48.8% were susceptible to cefixime.⁷ Similar study in Semarang revealed that 77% of their samples were ceftriaxone-resistant, which means only 23% were susceptible to ceftriaxone.⁸ This study aims to determine susceptibility pattern of *Neisseria gonorrhoeae* towards cefixime and ceftriaxone in Dr. Saiful Anwar General Hospital (RSSA) Malang.

METHODS

The samples were patients who visited the Outpatient Clinic of Dermatology and Venereology Department at RSSA Malang. Inclusion criteria were

male and female patients with complaints of urethral, cervical, rectal, or conjunctival discharge which contained intracellular or extracellular Gram-negative diplococci upon Gram staining, and those who voluntarily participated as study subjects and signed written informed consent. Female patients who were menstruating, pregnant, or unmarried were excluded from this study.

The sample size of this study was determined to be 26. The data of the previous studies regarding the percentage of *Neisseria gonorrhoeae* susceptibility to cefixime and ceftriaxone suggested 23% and 48%, respectively, with 95% confidence interval, and 0.1 significance level. This study implemented consecutive sampling. This study has been reviewed and granted permission by the Ethics Committee of RSSA Malang.

Antibiotic susceptibility is based on the concept of disruption of microbial life due to an antibiotic. An organism is considered susceptible if the diameter of the inhibition zone is ≥ 35 mm for ceftriaxone (disc contains 30 μg of drug) and ≥ 31 mm for cefixime (disc contains 5 μg of drug).¹¹ Antibiotic resistance is based on the concept of no disturbance of microbial life due to an antibiotic. An organism is considered resistant if the diameter of inhibition zone is < 35 mm for ceftriaxone (disc contains 30 μg of drug) and < 31 mm for cefixime (disc contains 5 μg of drug).¹¹

Kirby-Bauer method is an antibiotic susceptibility testing method utilizing diffusion technique by placing antibiotic-imbued discs placed in a bacterial colony growing in a culture medium. The diameter of zone of inhibition is then measured using a ruler or calliper. This method was chosen as it was readily available and feasible.

History and physical examinations were performed as the first step of the study. Swabbing of the discharge was then performed. Swab samples from endocervix were obtained using sterile charcoal Amies swab. The swab was rotated for 3 seconds or 3 times. Swab samples in males were obtained by swabbing the urethra from meatal part up to navicular fossa or swabbing the rectum using charcoal Amies swab. Swab samples in charcoal Amies transport medium were stored using ice gel, taken to the Centre of Health Laboratory (*Balai Besar Laboratorium Kesehatan [BBLK]*) Surabaya in less than 24 hours, and were immediately inoculated in modified Thayer Martin media.

Colonies which grew on modified Thayer Martin media were then examined using Gram staining, catalase, peroxidase, glucose, sucrose, lactose, and maltose tests to identify *Neisseria gonorrhoeae* bacteria. Colonies confirmed to be *Neisseria gonorrhoeae* were then re-inoculated in a second

modified Thayer Martin media. Colonies which grew in these media were then examined to determine their susceptibility toward cefixime and ceftriaxone using Kirby-Bauer method. The resulting data were collected in forms and were subsequently inputted into the computer. Obtained data were then presented in tables and graphs.

RESULT

During July 2018 – October 2018, there were 70 patients with complaints of discharge visited the Outpatient Clinic of Dermatology and Venereology Department at RSSA Malang. A total of 31 patients fulfilled the study inclusion and exclusion criteria. Out of 31 swab samples from patients' discharge inoculated into the first batch of modified Thayer Martin media. There were 26 positive colony isolates, which were examined using Gram staining. The 26 positive colony isolates were confirmed to be *Neisseria gonorrhoeae*. These positive isolates were subsequently inoculated into the second batch of modified Thayer Martin media, and all of them underwent susceptibility testing for cefixime and ceftriaxone.

Baseline characteristics of study subjects can be seen in Table 1. The proportion of gonorrhea patients who seek treatment in the clinic were dominated by males (96.15%), the most of the patients were aged 17 – 25 years old (65.38%) and unmarried (73.07%).

Most subjects were residents of Malang city (42.31%). The most common sexual orientation were heterosexuals (57.69%), followed by male homosexuals (30.76%), and bisexuals (11.53%). Human Immunodeficiency Virus (HIV) infection status has been examined on the majority of the study subject, and most were non-reactive (80.77%).

Clinical manifestations of the subjects are shown in Table 2. The discharge was commonly originated from the urethra (92.31%), followed by anal fistula and cervix (each was 3.85%). History of suspected intercourse occurred mostly in < 1 week before illness (57.69%). A history of previous gonorrhea complaints was found in 30.76% of the subjects. Co-existing syphilis and condyloma acuminata infection were found in 11.54% and 3.85% subjects, respectively.

Table 3 shows the history of previous treatment, 38.46% subjects already received treatment, and 100% of those subjects consumed unprescribed oral drugs which they bought from pharmacy or drugstores. No subject had a history of treatment by the injection-type drug. Antibiotics were used by 90% of subjects, and the remaining 10% were using herbal drugs. Type of drugs used was amoxicillin (11.54%), ciprofloxacin (11.54%), thiamphenicol (3.58%), doxycycline

(3.85%), ampicillin (3.85%), pipemidic acid (3.85%), and herbal drugs (3.85%).

In this study, 26 isolates which were grown in culture media were obtained. Antibiotic susceptibility testing was subsequently carried out using the Kirby-Bauer method. The results of antibiotic susceptibility test for cefixime and ceftriaxone against *Neisseria*

gonorrhoeae showed that 80.77% of isolates were susceptible to cefixime, and 80.77% of isolates were susceptible to ceftriaxone. Meanwhile, the remaining 19.23% of isolates were resistant to cefixime, and 19.23% were resistant to ceftriaxone. The percentages of cefixime and ceftriaxone resistance are presented in graphic 1.

Table 1. Baseline characteristics of subjects visiting outpatient clinic of Dermatology and Venereology Department at RSSA Malang during July 2018 – October 2018

Variable	Number (%)
Sex	
Male	25 (96.15%)
Female	1 (3.85%)
Age Group	
17 – 25 years old	17 (65.38%)
26 – 35 years old	6 (23.08%)
36 – 45 years old	3 (11.54%)
Place of Residence	
Malang city	11 (42.31%)
Malang district	2 (7.69%)
Outside Malang	13 (50%)
Occupation	
College student	6 (23.07%)
Private sector employee	5 (19.23%)
Salesperson	4 (15.38%)
Teacher	2 (7.69%)
Security officer	2 (7.69%)
Karaoke staff	1 (3.85%)
Bartender	1 (3.85%)
Construction worker	1 (3.85%)
Staff of Religious Affairs Office	1 (3.85%)
Farmer	1 (3.85%)
Singer	1 (3.85%)
Unemployed	1 (3.85%)
Education level	
Senior high school	17 (65.38%)
Junior high school	4 (15.38%)
Diploma 1	1 (3.85%)
Bachelor	3 (11.54%)
Master	1 (3.85%)
Marital status	
Married	7 (26.92%)
Unmarried	19 (73.07%)
Sexual orientation	
Heterosexual	15 (57.69%)
Homosexual male	8 (30.76%)
Bisexual	3 (11.53%)
HIV status	
Non-reactive	21 (80.77%)
Reactive	3 (11.54%)
Unchecked	2 (7.69%)

Table 2. Clinical manifestations of subjects visiting outpatient clinic of Dermatology and Venereology Department at RSSA Malang during July 2018 – October 2018

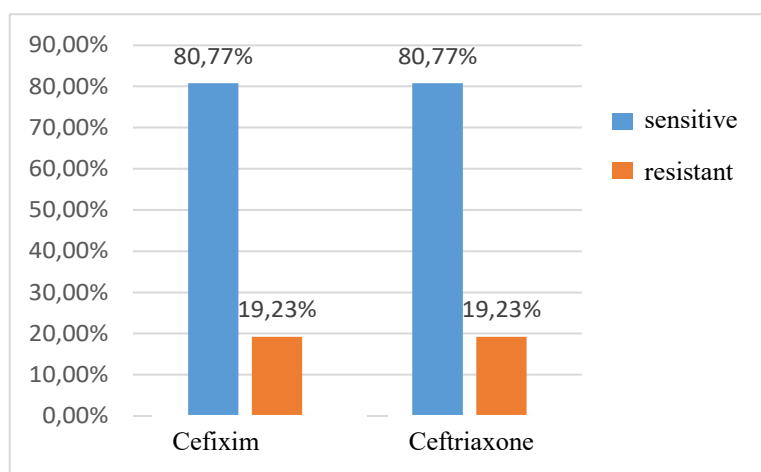
Clinical Manifestations	Number (%)
Discharge origin	
Urethra	24 (92.31%)
Anal fistula	1 (3.85%)
Cervical	1 (3.85%)
Discharge consistency	
Mucopurulent	25 (96.15%)
Seropurulent	1 (3.85%)
External urethral orifice	
Ectropion, erythema, and edema	24 (92.31%)
Duration of symptoms	
<1 week	16 (61.54%)
1 week	5 (19.23%)
>1 week	5 (19.23%)
History of the suspected intercourse	
<1 week	15 (57.69%)
1 week	7 (26.92%)
>1 week	4 (15.38%)
Previous history of gonorrhoea	
Yes	8 (30.76%)
No	18 (69.24%)
Other co-existing STI	
Syphilis	3 (11.54%)
Condyloma acuminata	1 (3.85%)
Absent	22 (84.61%)

Susceptibility to ceftriaxone and cefixime was assessed based on clinical manifestations and risk factors (Table 4). Antibiotics susceptibility in subjects without previous treatment was 81.25% for ceftriaxone and 68.75% for cefixime; while in subjects who had received prior treatment, the percentage of susceptibility was 80% for ceftriaxone and 100% for cefixime. Antibiotics susceptibility in subjects who had never been infected with gonorrhoea was 76.47%

for ceftriaxone and 76.47% for cefixime; while in subjects who had previously been infected with gonorrhoea, the percentage of susceptibility was 88.89% for ceftriaxone and 88.89% for cefixime. Among HIV-reactive subjects, susceptibility to ceftriaxone and cefixime were each 66.67%, meanwhile, among non-reactive HIV subjects, susceptibility to ceftriaxone and cefixime were each 80.95%.

Table 3. Prior history of treatments at outpatient clinic of Dermatology and Venereology Department at RSSA Malang during July 2018 – October 2018

History of Treatment	Number (%)
Prior History of Treatment	
Treated	10 (38.46 %)
Untreated	16 (61.54%)
Prior Routes of Drug Administration	
Oral	10 (100%)
Intramuscular	0 %
Origin of Prior Treatment	
Buying drugs without prescription in pharmacy or drugstore	10 (100%)
Prescribed	0 %
Type of Drugs Used	
Antibiotics drugs	9 (90%)
Herbal drugs	1 (10%)



Graphic 1. Cefixime and ceftriaxone susceptibility of *Neisseria gonorrhoeae* isolates at outpatient clinic of Dermatology and Venereology Department at RSSA Malang during July 2018 – October 2018.

The most common risk factor for gonorrhea infection was heterosexuality. One of the high-risk groups mentioned in the Integrated Biological Behavior Survey of 2015 was male

homosexuality. The estimated risk for a homosexual male to be infected with gonorrhea in the survey was 12.72% lower than the results of this study.¹²

Table 4. Cefixime and ceftriaxone susceptibility of *Neisseria gonorrhoeae* isolates based on clinical manifestations and risk factors at outpatient clinic of Dermatology and Venereology Department at RSSA Malang during July 2018 – October 2018

Clinical manifestations and risk factors	Susceptibility	
	Ceftriaxone	Cefixime
Duration of symptoms		
≤ 1 week (n=21)	17 (80.95%)	18 (85.71%)
> 1 week (n=5)	4 (80.00%)	3 (60.00%)
History of prior treatment		
Untreated (n=16)	13 (81.25%)	11 (68.75%)
Treated (n=10)	8 (80%)	10 (100%)
History of previous gonorrhea infection		
No (n= 17)	13 (76.47%)	13 (76.47%)
Yes (n=9)	8 (88.89%)	8 (88.89%)
Risk factor		
Heterosexual (n=15)	12 (80%)	12 (80%)
Homosexual male (n=8)	6 (75%)	6 (75%)
Bisexual (n=3)	3 (100%)	3 (100%)
HIV status		
Non-reactive HIV (n=21)	17 (80.95%)	17(80.95%)
Reactive HIV (n=3)	2 (66.67%)	2 (66.67%)
Unchecked (n=2)	2 (100%)	2(100%)

HIV = human immunodeficiency virus

DISCUSSION

The increase in resistance of *Neisseria gonorrhoeae* to certain antibiotics has been reported in several studies in Indonesia and other countries. Guidelines from the Centers for Disease Control and Prevention in 2015 did not recommend cefixime as the primary therapy for gonorrhea due to

numerous reports of resistance in several countries.⁴ The study was conducted to determine the cefixime and ceftriaxone susceptibility pattern of *Neisseria gonorrhoeae* isolates in RSSA Malang.

Gonorrhea is currently still a common infection in the community. In the Outpatient Clinic of Dermatology and Venereology Department at RSSA

Malang, gonorrhea ranked the 5th of all the STI cases. This study also determined other STI risk factors which coincided with gonorrhea infection, one of which was HIV infection (9.4%) and male homosexuality (18.8%) in study subjects.

Gonorrhea infection or other STIs can serve as risk factors which influence HIV transmission. Weire *et al.* found that female sex workers infected with HIV who participated in his study had also previously been infected with gonorrhea.¹³ The risk of HIV infection and gonorrhea in homosexual male risk group is caused by insufficient lubrication in the rectal region, thus rendering higher risk for microlesion. Furthermore, the absorption rate in the area is greater, thereby increasing the risk of transmitting HIV or other STIs. *Neisseria gonorrhoeae* infection may also induce the production of cytokines and chemokines in monocytes and modulates T cell activation. *In vivo* studies showed an increase in the number of CD4+ T-Cells in the endocervical region of gonorrhea-infected subjects (CD4+ T-cell is a target cell for HIV infection).¹⁴ Bernstein *et al.* conducted a study on male homosexuals who suffered from gonorrhea and chlamydia in the rectal region and suggested that those who previously had two histories of rectal infections will have an 8-fold higher risk for HIV infection.¹⁵

Suspected intercourse in the study subjects mostly occurred in less than one week preceding the symptoms. This is in accordance with several works of literature which state that the suspected intercourse in gonorrhea patients occurred 2 – 5 days preceding the symptoms,^{16,17} whereas, in female patients, symptoms often occur approximately 10 days after infection.¹⁸ History of recurrent gonorrhea infection was found in 30.76% of subjects. Brooks *et al.* revealed that 29.4% of cases of gonorrhea were reported to occur more than once, especially in the younger age group, which is associated with higher rates of sexual activity and a higher risk of having multiple sexual partners. In addition, the risk of recurrence is also found especially in the male homosexual group.¹⁸ Study in Thailand found that 40.3% of their male homosexual subjects had a history of recurrent gonorrhea infection that 68.7% of them had recurrent infections in the urethra, 6.8% of them had recurrent infections in the rectum, and 25% of them had recurrent infections in the rectum and urethra.¹⁹

An increase in resistance of *Neisseria gonorrhoeae* is associated with improper selection of drug, drug dosage, and duration of drug administration. In this study, 34.61% of the study subjects had previously received treatment. Previously consumed antibiotics were also found from other

classes of antibiotic, which were not recommended for gonorrhea therapy as high levels of resistance and inappropriate duration of drug administration have been reported.²⁰

Susceptibility test results showed that 80.77% of isolates were still susceptible to cefixime and ceftriaxone, whereas 19.23% had decreased susceptibility to cefixime and ceftriaxone. A study conducted in Surabaya by Setyaningrum *et al.* found that 33.3% of their isolates were resistant to cefixime, and 66.7% were susceptible to cefixime.²² Another study by Pidari in Denpasar showed that out of 45 obtained isolates, 51.2% were susceptible to cefixime and 48.8% had decreased susceptibility.⁷ The results of this study were in contrast with the study of Hananta *et al.* conducted in three major cities in Indonesia (Jakarta, Yogyakarta and Denpasar), in which out of 79 urogenital culture isolates, all isolates were found to be 100% susceptible to ceftriaxone and cefixime. On the contrary, the isolates were found to be mostly resistant to doxycycline (98.7%) and ciprofloxacin (97.4%).⁶ Saroh's study in Semarang showed that 77% of samples were resistant and only 23% were susceptible to ceftriaxone.⁸ The results of this study indicate that the susceptibility of *Neisseria gonorrhoeae* towards cefixime and ceftriaxone in RSSA is still higher compared to studies in other cities in Indonesia.

Factors which caused cefixime and ceftriaxone resistance found in this study cannot be ascertained because it may be influenced by many factors, such as changes in penB, mtrR, and penC genes as well as mutations in penA genes in PBP2. The disrupted penA gene may result from mutations or genetic recombination.^{22,23} Changes in PBP2 encoded by the penA gene are the cause of decreased binding to penicillin through a single insertion of amino acids. This change was also found in cephalosporin-resistant isolates.²⁴ Further evaluation is needed to determine the significance of the possible causes of decreased susceptibility to cefixime and ceftriaxone in this study. Limitation of this study was associated with the chosen antibiotic susceptibility testing method, which is the Kirby Bauer method, as it is not the current gold standard method for assessing the susceptibility of antibiotics. The gold standard for antibiotic susceptibility testing is by agar dilution method to quantitatively determine the Minimum Inhibitory Concentration (MIC) (pg/ml).²²

The results of antibiotic susceptibility test for cefixime and ceftriaxone against *Neisseria gonorrhoeae* showed that 80.77% of isolates were susceptible to cefixime, and 80.77% of isolates were susceptible to ceftriaxone. Meanwhile, the remaining

19.23% of isolates were resistant to cefixime, and 19.23% were resistant to ceftriaxone. As conclusion, cefixime and ceftriaxone are still effective as gonorrhea therapy in RSSA Malang.

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