THE EFFECT OF ANTIMICROBIALS USED FOR GENITAL DISCHARGE DISEASE ON *TRICHOMONAS VAGINALIS*

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DECLARATION

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

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CONFERENCE PRESENTATIONS

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ABSTRACT

Trichomoniasis is the most common sexually transmitted infection caused by the protozoan, *Trichomonas vaginalis*. *T. vaginalis* infection is often asymptomatic. This infection causes vaginal discharge in women and urethritis in men. It has been reported that trichomoniasis is associated with serious health complications and it increases the risk of HIV acquisition and transmission. The gold standard for trichomoniasis diagnosis is culture, however various point of care tests have been approved by the US FDA. Metronidazole is the standard treatment for trichomoniasis. Multiple cases of metronidazole-resistance have been reported since 1962. Syndromic management of STIs is used to treat multiple infections simultaneously based on the signs and symptom with which the patient presents. In South Africa, the vaginal discharge syndrome is managed with ceftriaxone, azithromycin and metronidazole.

Ten *T. vaginalis* isolate were tested. Each isolate was tested against six combinations of two antimicrobials by the checkerboard method, four combinations of three antimicrobials and two combinations of four antimicrobials. The results obtained from the checkerboard of two antimicrobials were used to design the experiments for three and four antimicrobials combinations.

The MICs for metronidazole ranged between 0.25 – 1 µg/ml and for doxycycline, they ranged between 64 – 128 µg/ml. Ceftriaxone and fluconazole showed no antitrichomonal activity. All combinations tested has an indifferent effect.

Combinations of metronidazole and antimicrobials used in syndromic management including fluconazole has no effect against *T. vaginalis*. However, in the combination of metronidazole and doxycycline a decrease in the MICs for these antibiotics was observed. Further studies are required to test this combination on a larger sample size.

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ABBREVIATIONS

^{o}C	degree Celsius
µl	microliter
µg	microgram
ATCC	American Type Culture Collection
ATP	adenosine 5’-triphosphate
BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards Institute
CO_2	carbon dioxide
CP	cysteine proteinase
CTX	ceftriaxone
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
DOX	doxycycline
ECM	extracellular matrix
EUCAST	European Union Committee on Antimicrobial Susceptibility
FBS	foetal bovine serum
FDA	Food and Drug Administration
FIC	fractional inhibitory concentration
FLC	fluconazole
g	gram
GC	growth control
GUD	genital ulcer syndrome
HIV	human immunodeficiency virus
IM	intra-muscularly

LAP	lower abdominal pain
mg	milligram
MIC	minimal inhibitory concentration
MIC_{50}	MIC required to inhibit the growth of 50% of organisms
MIC_{90}	MIC required to inhibit the growth of 90% of organisms
ml	millilitre
MLC	minimal lethal concentration
MTZ	metronidazole
MUS	male urethral syndrome
PFOR	pyruvate-ferredoxin oxidoreductase
PID	pelvic inflammatory disease
STI	sexually transmitted infection
TvFd	*Trichomonas vaginalis* ferredoxin
TVLG	*Trichomonas vaginalis* lipoglycan
TYM	trypticase–yeast–maltose
VDS	vaginal discharge syndrome
WHO	World Health Organisation

CHAPTER ONE

1.1 INTRODUCTION

Trichomonas vaginalis is an anaerobic parasitic protozoan that causes the sexually transmitted infection (STI) trichomoniasis. Trichomoniasis is the most common STI in man, with a reported prevalence of 276 million infections annually worldwide [1, 2]. In South African women, the prevalence of trichomoniasis ranges from 5% to 49% [3]. *T. vaginalis* infection is often asymptomatic, however when symptoms arise, women experience vaginal discharge, pruritus and/or dysuria, vaginitis and cervicitis[2, 4, 5]. In men, it is characterised by urethritis (dysuria with or without urethral discharge) which may lead to serious complications such as epididymitis, prostatitis and infertility [4, 6-8]. Trichomoniasis has been found to be associated with various health complications including pelvic inflammatory disease (PID), significant pregnancy complications (premature rupture of membranes, early delivery of the baby, and low baby weight), cervical cancer and high risk of acquisition and transmission of HIV [4, 7, 9, 10]. Previous studies have shown that infection with *T. vaginalis* may widen the portal of entry for HIV in an HIV-seronegative person whereas in a person infected with both *T. vaginalis* and HIV, it may increase shedding of HIV with increased transmission to others [11].

It is difficult to diagnose *T. vaginalis* infection clinically because of the non-specific nature of the disease and the problems associated with the diagnostic tests such as sensitivity. The diagnosis of trichomoniasis is in most settings by means of wet-mount microscopy of vaginal specimens or urethral secretions. The sensitivity of wet mount is between 35% and 80% when compared to culture. Wet mount examination requires a concentration of 10^4 organisms/ml. Lower concentrations of organisms will not be detected [12, 13]. Wet-mount microscopy is the most simple and inexpensive diagnostic test, however it is associated with under-diagnosis of the disease because of the lack of sensitivity [12]. The disadvantage of wet mount is that since

T. vaginalis is unstable in conditions outside the body, the specimen should be examined immediately after collection when the microbes are still highly motile. It becomes difficult to discriminate from cells with similar morphology if the typical motility is decreased or lacking [13].

For the past 40 years, *T. vaginalis* has been diagnosed using broth culture technique. The standard broth is Diamond's medium and it only requires an inoculum size of 10^2 organisms/ml. However, culture techniques are not available or used at the primary healthcare level because of the expense and the expertise needed[12]. Over the past decade, PCR has been used as a diagnostic test. This technique has a sensitivity of about 90% and a possibility of false positives because of DNA from dead organisms after treatment [12].

In 1995, syndromic management of STIs was introduced in KwaZulu-Natal [14]. Syndromic management is used to treat all possible causes of an STI syndrome simultaneously based on the signs and symptoms with which the patient presents. Most health care facilities in developing countries lack trained personnel and laboratory equipment required for diagnosis of STIs. The WHO has recommended syndromic treatment of STIs in resource poor countries since laboratory approach is not practical at the primary healthcare level [15]. In South Africa, Vaginal Discharge Syndrome (VDS) is currently managed with metronidazole 2g single dose orally, ceftriaxone 250mg single dose intra-muscularly (IM) and azithromycin 1g single dose orally [16]. Metronidazole is one of the group of 5-nitroimidazoles [17]. Metronidazole has been the standard treatment for trichomoniasis since 1959 [18] and in some countries it is the only antibiotic that is approved for trichomoniasis treatment [10]. It is usually prescribed as a single dose (2g) or multiple dose regimens of 250 mg, 3 times a day or 500mg, twice a day for 7 days. Some studies report that metronidazole resistance has been observed in clinical isolates derived from treatment failures [18]. Approximately 100 *T. vaginalis* strains that are metronidazole-resistant have been reported in the United States. Some reports have also been published from Russia, Africa and Europe. Patients have been treated with higher doses of metronidazole as means of overcoming the problem of metronidazole resistance [17]. The aim of this study was to investigate the combined effect of antimicrobial agents used in the management of vaginal discharge syndrome on *T. vaginalis* isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Trichomonas vaginalis

2.1.1 History

Trichomonas vaginalis is a parasitic protozoan which colonizes the urogenital tract of humans and causes the sexually transmitted infection (STI) trichomoniasis[5, 6, 19]. The organism was first described by Alfred F. Donné in 1836 [18]. He observed the motile parasites in the frothy, purulent mucous secretions of females that were presenting with vaginitis and vaginal discharge [20]. In 1916, Hohne first described *T. vaginalis* as an etiological or clinical agent of the disease [20]. Most research on *T. vaginalis* focused on microscopic examination and biochemical tests to study behavioural and growth characteristics. Studies on immunology and pathogenesis of the organism only began in the 1980s upon availability of molecular biology and immunological techniques [20].

2.1.2 Morphology

T. vaginalis is unicellular and its cells vary in size. The average length is 10µm and the width is 7µm [6, 8]. This parasite exists in several cellular forms or shapes. The best described forms are the pear-shaped (free-swimming) and the amoeboid form [18, 21]. The free-swimming, pear-shaped and flagellated cell is usually observed in axenic broth cultures (Figure 1 (A)). The amoeboid form is characterised by an increase in surface contact and it is observed upon attachment of the protozoan to the vaginal or urethral epithelial cells and extracellular matrix (ECM) proteins (Figure 1 B and C) [8, 21]. *T. vaginalis* has five flagella. The first four are located at the anterior end while the fifth flagellum is integrated within the undulating membrane [1, 6, 8, 18] . The undulating membrane and the flagella give *T. vaginalis* the characteristic rapid and jerky movement [6, 8]. *T. vaginalis* can internalize the flagella under adverse growth conditions [6, 8]. The cytoskeleton is made up of actin and tubulin fibers [8].

The nucleus in *T. vaginalis* is enclosed in a porous nuclear membrane and is situated in its anterior portion. The axostyle (hyaline, rod-like structure) begins at the nucleus and it runs through the parasite longitudinally thus protruding through the posterior end of the organism [6, 8]. The paraxostylar granules are a distinguishing characteristic of *T. vaginalis*. These granules are arranged in three parallel rows along the axostyle. The protozoan also has glycogen granules and phagosomes [6, 8]. The hydrogenosome (a mitochondrion-like organelle) is enclosed in a double membrane, has a diameter of 0.5 to 1 µm [8]. It lacks DNA and cristae [8].

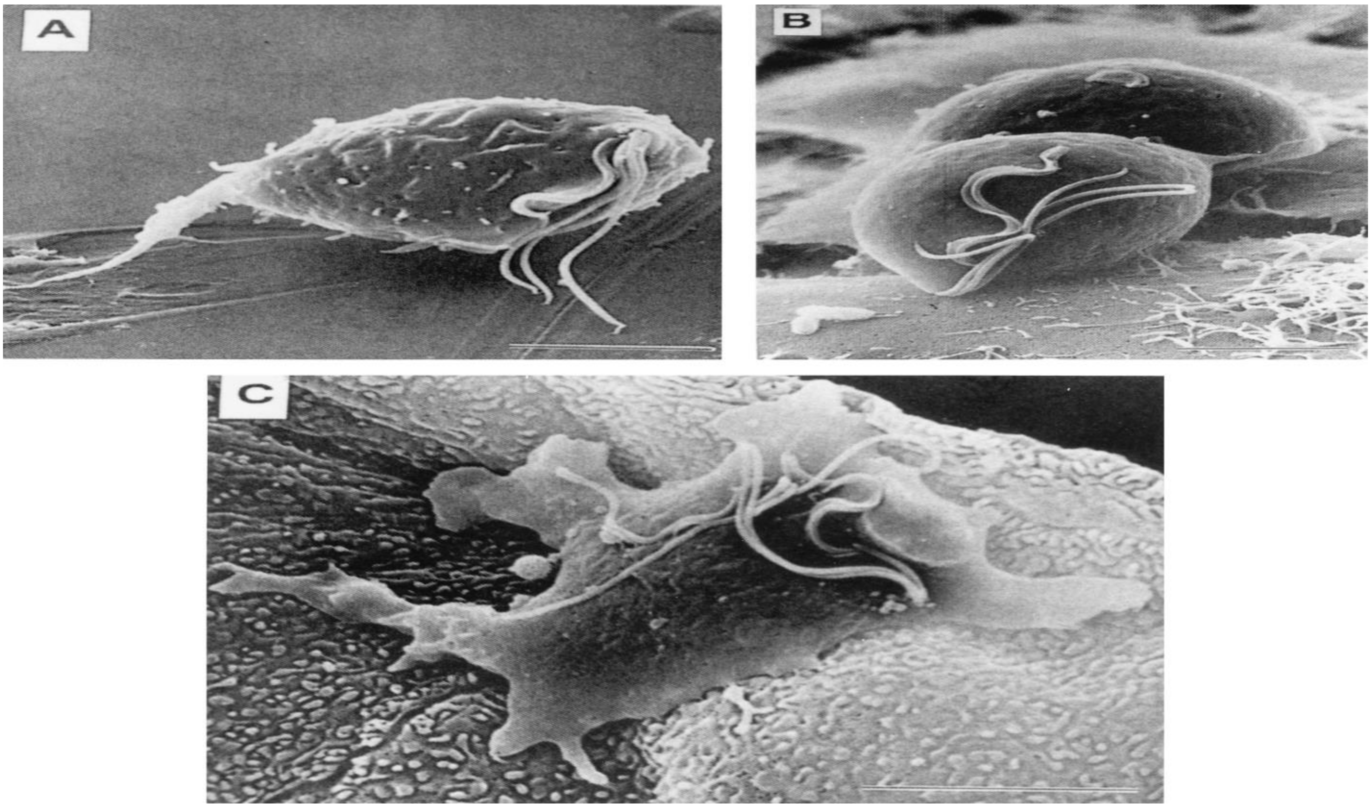


Figure 2.1.2: (A) *T. vaginalis* parasite as seen in broth culture. (B) *T. vaginalis* on the surface of a vaginal epithelial cell prior to amoeboid transformation. (C) Amoeboid

morphology of *T. vaginalis* as seen when grown on the surface of a cell culture (Arroyo *et al*., 1993 cited by Petrin *et al*., 1998) [8].

2.1.3 Growth requirements, Nutrition and Metabolism

T. vaginalis lives in anaerobic environments. However slightly increased levels of oxygen do not impair its replication rate as long as the carbon dioxide partial pressure is sufficient [1] . *T. vaginalis* is unable to produce many macromolecules such as lipids, purines and pyrimidines. Hence it is an obligate parasite. The protozoan acquires these nutrients from the vaginal secretions or by phagocytosing bacterial and host cells [8, 20]. Thus, the culture media for trichomonads needs to have all the vital minerals, vitamins and macromolecules. This is provided in the form of serum. Serum provides fatty acids, lipids, trace metals and amino acids. Therefore it is essential for the growth of *T. vaginalis* [8, 20].

Iron is essential for the maintenance of the activity of ferredoxin and pyruvate-ferredoxin oxidoreductase (PFOR) [8]. These enzymes are important for the metabolism of the organism. *Trichomonas vaginalis* ferredoxin (TvFd) is located in the hydrogenosome. Its main function is to transfer electrons from PFOR to the hydrogenase. The hydrogenase then transfers these electrons to protons to produce hydrogen (H_2) [22]. PFOR is located on the membrane of the hydrogenosome [23, 24]. This enzyme catalyses the oxidative decarboxylation of pyruvate. This reaction is CoA-dependent and it yields CO_2 and acetyl-CoA [23-25].

In *T. vaginalis* carbohydrate metabolism is fermentative, both under aerobic and anaerobic conditions. This carbohydrate metabolism occurs in the hydrogenosome and the cytoplasm. The functions of the hydrogenosome include the production of ATP and hydrogen. In addition, it provides the site for fermentative oxidation of pyruvate and processing of the carbohydrates [8]. The preferred substrate for energy production in *T. vaginalis* are carbohydrates however survival and growth of the trophozoite is sustained by amino acids in environments where carbohydrate levels are insufficient [8]. The carbohydrates provided in culture media are glucose or maltose. However, in vaginal secretions glycogen is the main carbohydrate. Recently, it was shown that replacing glucose and maltose in the culture media with glycogen has the same growth stimulating effect [26]

Since *T. vaginalis* lacks the ability to produce purines and pyrimidines, it has to adopt other pathways to produce nucleotides [8]. For growth, the trichomonad needs guanine and adenine or their nucleosides, uracil and /or uridine, thymidine and cytidine. *T. vaginalis* lacks most of the enzymes that are essential for production of complex phospholipids. Therefore it relies on external sources of lipids [8].

2.1.4 Life cycle and Reproduction

Reproduction in *T. vaginalis* has been extensively studied. However, it is still not completely understood [8]. It has been reported that *T. vaginalis* only exists as a trophozoite and it lacks the cystic stage [6, 8]. The trophozoites reproduce by longitudinal binary fission every 8-12 hours [27]. The nuclear envelop does not disappear during reproduction in this parasite [6, 8, 18, 19]. Pseudocyst formation has been observed in trophozoites that are experiencing environmental stress [18].

2.2 Epidemiology

Trichomoniasis is the most common STI, with a reported prevalence in 2008 of approximately 276 million new infections annually worldwide [1, 2]. About 90% of trichomoniasis cases occur in resource-limited countries [2]. In sub-Saharan Africa, nearly 30 million new cases are reported each year [28] and this is likely under-reported. Trichomoniasis has an estimated global prevalence of 1% and 8.1% in men and women, respectively [2]. Trichomoniasis has a world-wide distribution and it has been observed in all climate conditions [6, 20], racial groups and socio-economic strata [20]. The incidence rate is influenced by various factors such as sexual behaviour, age, other STIs, the number of sexual partners [1]. Clinical examination methods and laboratory diagnostic techniques also influence the measured incidence [6]. Several risk factors associated with acquisition of trichomoniasis and other STIs have been identified and these include high risk sexual behaviours, poverty, low educational level, socio-economic status, multiple sexual partners, HBV and/or HIV infection [29], the presence of bacterial vaginosis (BV) [2, 5], drug use, prostitution and older age [2, 4, 5]. Older age is thought to be a risk factor in trichomoniasis because the male population is usually not treated since the disease is mostly asymptomatic. Although trichomoniasis has the highest prevalence compared to other STIs, data describing prevalence and incidence of this infection in the total population is insufficient [20, 29].

2.3 Transmission and Pathogenesis

T. vaginalis can only thrive in humans as their host. The trichomonad is primarily transmitted through sexual intercourse [2, 5, 6, 8, 19]. However, it can also be transmitted during artificial insemination with infected semen, from mother to infant during delivery and through orogenital sexual activities. Orogenital sexual activities cause *T. vaginalis* infections in the lower respiratory tract and pharynx [27]. *T. vaginalis* invades or colonises the squamous epithelium in the genitourinary tract of both males and females [1, 2, 5]. *T.vaginalis* infection of the genitourinary tract causes inflammation of the vagina or urethra [30]. Adhesion of the protozoa to the epithelial cells is the first essential step in pathogenesis of *T. vaginalis* infection [6, 8, 30, 31]. Adherence to epithelial cells depends on the pH, exposure time and temperature [8]. Several adhesion molecules have been identified in *T. vaginalis* including AP23, AP33, AP51 and AP65 [8, 20, 30]. Binding of the trichomonad to the epithelial cells is prominently facilitated by AP65 and studies have shown that laminin may be the target protein. Gelactin-1, found on cervical epithelial cells, is the only host cell receptor known to bind to *T. vaginalis* lipoglycan (TVLG) [21, 30]. Contributing to the acquisition of nutrients such as iron (Fe) and lipids is the capacity of *T. vaginalis* to lyse and phagocytose host erythrocytes [6, 8, 20, 31]. Trichomonal cysteine proteinases (CPs) have been shown to also mediate adherence of the trichomonad to epithelial cells as well as haemolysis of red blood cells [6, 8].

T. vaginalis has the ability to escape the human immune response. CPs are capable of degrading IgG as well as IgA antibodies [1, 6, 8]. It has been documented that *T. vaginalis* can also degrade the C3 portion of complement thus escaping the complement-mediated immune response. Escaping the complement pathway is iron dependent, as high levels of iron upregulate CP expression [6]. *T. vaginalis* also uses the host's plasma proteins to coat itself thus avoiding recognition by the immune system as foreign. *T. vaginalis* can avoid complement mediated kill by activating the alternative pathway. It has also been shown that *T. vaginalis* secretes highly immunogenic soluble antigens which have the ability to neutralize cytotoxic T-lymphocytes and antibodies [6, 8].

2.4 Clinical manifestations and complications

T. vaginalis infection is often asymptomatic. The incubation period is usually 4 to 28 days. In men, symptomatic infections usually persists for less than 10 days, however in females it may persist for months or years [2, 5]. About 50% of women and most men with trichomoniasis show no clinical manifestations [8, 28]. When symptoms arise, women experience vaginal discharge, pruritus and/or dysuria, vaginitis, cervicitis and colpitis macularis (strawberry cervix) [2, 4, 5, 19, 20]. The vaginal discharge in *T. vaginalis* infection is often yellow-green, frothy, foul-smelling and mucopurulent [2, 5, 8]. The vaginal pH of infected females increases to above 5 compared to the normal pH of 4.5 [2, 5]. In chronic trichomoniasis, the symptoms are mild and associated with dyspareunia, pruritus and minimal vaginal discharge mixed with mucus [6, 8]. In men, trichomoniasis is characterised by urethritis, dysuria, pruritus and urethral discharge (clear or mucopurulent). These symptoms are usually self-limiting and of short duration [4, 6-8].

Trichomoniasis has been found to be associated with serious health complications including pelvic inflammatory disease (PID), significant pregnancy complications (premature rupture of membranes, early delivery of the baby, and low baby weight), high risk of acquisition and transmission of HIV [4, 7, 9, 10, 19, 20, 28, 32], cervical cancer [10, 28], infertility and bacterial vaginosis [28]. Previous studies have shown that infection with *T. vaginalis* may widen the portal of entry for HIV in an HIV-seronegative person whereas in a person infected with both *T. vaginalis* and HIV, it may increase shedding of HIV [11, 20]. It has also been reported that trichomoniasis is associated with PID in HIV positive females [4, 32]. Trichomoniasis in men may lead to serious complications such as epididymitis, prostatitis, infertility [7, 8], urethral disease and balanoposthitis [8].

2.5 Diagnosis

2.5.1 Microscopic techniques

The diagnosis of trichomoniasis has for a long time been dependent on microscopic detection of the protozoan in vaginal and urethral secretions [12]. Wet mount microscopy is the first method that was used for diagnosis of trichomoniasis and it remains the most frequently used method [13, 31]. Wet mount examination requires a minimal protozoal concentration of 10^4 organisms/ml, organisms fewer than this will not be detected [12, 31]. The sensitivity of wet mount microscopy is between 35% and 80% when compared to culture [8, 12, 13, 31]. Sensitivity of this method is greatly influenced by the experience of the observer, the presence and the concentration of viable and motile trichomonads in the specimen and the period of transportation of the specimen to the laboratory [12, 31]. This technique is the simplest and inexpensive diagnostic test[5, 8, 12, 13]. However it is associated with under-diagnosis of the disease because of the low sensitivity[5, 12]. The disadvantage of wet mount microscopy is that *T. vaginalis* is unstable in conditions outside the body, therefore, specimens should be examined immediately after collection [4, 13]. Outside the human body, *T. vaginalis* retracts its flagella, loses motility and changes shape (becomes rounder). Therefore, it becomes difficult to discriminate between trichomonads and cells with similar morphology, in particular white blood cells [13].

Several staining methods such as acridine orange (OA), Giemsa, Fontana-Masson silver stain, Periodic Acid-Schiff (PAS), and Leishman stain have been employed for diagnosis of *T. vaginalis* [8, 12, 13, 20, 31]. However, these techniques are associated with loss of morphologic and motility characteristics during the fixation and staining steps [8, 12, 31]. Also, it is difficult to interpret the stained smears because *T. vaginalis* can resemble polymorphonuclear leukocytes [8, 31].

Papanicolaou (Pap) smear has a potential as a diagnostic procedure for trichomoniasis [8, 12]. However, for this purpose, Pap smears have a sensitivity as low as approximately 60% [4, 19] but a specificity of 95% [19]. Moreover, this technique is associated with false positive and false negative results [8, 12].

2.5.2 Culture techniques

For the past 40 years, *T. vaginalis* infection has been diagnosed using broth culture technique [12] and this has been considered as the gold standard [8, 12, 31, 33]. There are various culture media that have been shown to support *T. vaginalis* growth. These include: Kupferberg Trichosel medium, Lash serum medium, Diamond medium, Kupferberg STS medium and Difco Kupferberg medium [34].

The standard broth is Diamond’s medium [12] and it only requires an inoculum size of 300-500 organisms/ml [8, 13, 20]. However, culture techniques are not readily available at the primary healthcare level mainly because of the need for skilled staff [4, 5, 12]. The advantages of broth culture technique include easy interpretation [8, 12, 31], detection of relatively few organisms [31] resulting in a higher sensitivity compared to wet mount [5]. The major disadvantages of this technique are: it is expensive [4, 5, 12], insensitive in men and time consuming [5]. This method generally requires two to seven days of incubation [4, 8, 12] and the culture needs to be examined by wet mount microscopy every day [13]. Furthermore, the culture technique is prone to bacterial contamination which interferes with detection [12].

A practical improvement of the culture technique is the InPouch™ system (BioMed Diagnostics, USA). This system allows for easy inoculation, immediate observation, storage and transport of the specimen as well as microscopic observation inside the device [12, 20, 35]. The InPouch™ medium contains maltose and other sugars, salts, amino acids, peptones and antibiotics in a phosphate buffered saline (PBS) base. As shown in figure 2, the InPouch™ device is an oxygen resistant plastic pouch which has two chambers. These chambers are joined together by a thin passage [35].

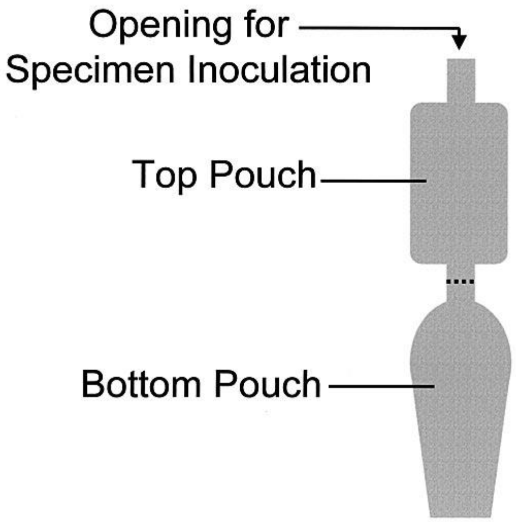


Figure 2.5.2: Schematic diagram of the InPouch™ system (Barenfanger *et al.,* 2002) [36].

The top chamber replaces the slide used in wet mount microscopy and is placed under the microscope and viewed without being opened [20, 35, 36]. If the microscopic examination is negative, the fluid in the top chamber is squeezed into the bottom chamber and further incubated [20, 36]. This system has sensitivity between 81-94% and specificity of 100% [35]. The cell culture technique can be used to recover or isolate *T. vaginalis* from specimens. This techniques uses different cell lines [8, 20] and it requires the specimen to be treated with antimicrobials prior to passage onto the cell culture [12]. Cell culture has high sensitivity and it only requires an inoculum of 3 trichomonads/ml [8, 12]. However, this technique is more susceptible to bacterial contamination [12], expensive and not suitable for routine and rapid diagnosis [8, 12].

2.5.3 Molecular techniques

Over the past decade, recombinant DNA technology has been used in diagnostic tests for trichomoniasis [12]. The Affirm VP III (Becton, Dickinson & Co.; USA) has been approved for *T. vaginalis* diagnosis in women by the US FDA[2, 5, 33]. The Affirm VP III is a nucleic acid probe test used for simultaneous detection of significant levels of *Gardnerella vaginalis,* *T. vaginalis* [5, 8, 37] and *Candida albicans* from vaginal specimens. The results are available in approximately 45 minutes[2, 5, 33, 37]. It has been reported that this test might be associated with false positives due to the presence of DNA from dead trichomonads after treatment [12]. The Affirm VP III test has sensitivity of about 83% and specificity of approximately 97% [2, 5, 33]. The APTIMA Analyte Specific Reagents test (ASR, manufactured by Gen-Probe, Inc.) is also available for the diagnosis of trichomoniasis. This diagnostic test was approved by the US FDA in 2011 [2]. Studies have reported sensitivity of about 74 to 98% and specificity of about 87 to 98% [2, 5, 33]. Amplicor (Roche Diagnostic Corp.) used for chlamydial and gonorrhoea infections has been modified for trichomoniasis diagnosis. This PCR assay can detect *T. vaginalis* in urine, vaginal and endocervical specimen of both men and women [5, 33].

Dot-blot hybridization uses a radioactive probe [8, 12, 31]. The disadvantage of this technique is that the probe is unstable as a radioactive probe, must be handled with care and requires proper disposal. This can be overcome by using a fluorescent-labelled probe [8, 12].

2.5.4 Antibody based techniques

Trichomonal antibodies can be detected using several techniques including ELISA, complement fixation, immune-fluorescence (IFT), hemagglutination and gel diffusion. However, these techniques cannot distinguish recent and past infections [8, 12, 31]. Assays such as the Fluorescent Direct Immunoassay and Trichomonas Direct Enzyme Immunoassay (California Integrated Diagnostics, Benica, Calif.) have been developed for detection of trichomonal antigens. These assays employ fluorochrome-labelled and peroxidase-labelled monoclonal antibodies to various antigens of *T. vaginalis* [8]. These tests are convenient because the results are available in 1 hour, thus diagnosis and treatment of the patient are possible in a single visit [8]. The OSOM test is based on immunochromatographic capillary flow dipstick technology [2, 5, 20, 33, 38] and the results are available in approximately 10 minutes[2, 5, 33]. This point of care test has sensitivity of approximately 83% and specificity of approximately 97% [2, 5, 20, 33]. The major disadvantage of this test is that it cannot be used for men [38].

2.6 Treatment

2.6.1 Trichomoniasis treatment

Before the introduction of 5-nitroimidazoles, treatment of trichomoniasis has been dependent on topical vaginal preparations [8]. These were not effective in curing trichomoniasis but they relieved symptoms [8]. The 5-nitroimidazole drug family include metronidazole, tinidazole, secnidazole, ornidazole, carnidazole, flunidazole and nimorazole [4, 8, 19]. Metronidazole has been the standard and approved treatment for trichomoniasis since 1959 [8, 18] and in some countries it is the only antibiotic that is approved for treatment of trichomoniasis [10]. The CDC treatment guidelines for this infection are metronidazole in a 2g single dose, a multiple dose regimen of 500mg, twice a day for 7 days [5, 18, 39] or 250 mg, 3 times a day for 7 days [9]. If treatment of the patient is unsuccessful with these regimens, the patient should be treated with 2g of metronidazole or tinidazole orally for 5 days [20]. In South Africa, trichomoniasis is currently treated with metronidazole 2g single dose, administered orally. If the patient does not respond to treatment, then 400mg of metronidazole is orally administered 12 hourly for 7 days [16]. Metronidazole can also be administered intravenously with a dosage of 500mg to 2g [9]. The mechanisms of action of metronidazole include impairment of molecular hydrogen production leading to elevated intracellular levels of hydrogen peroxide in the trichomonad [18] and production of toxic nitro-radicals upon activation of the drug [8, 9, 17, 18, 20]. The cytotoxic nitro-radicals are thought to bind to DNA, causing strand breakage and thus cell death [8, 9, 20]. The approved metronidazole regimens have up to 95% cure rates [5, 9, 20, 39].

Topical metronidazole in intravaginal gels are ineffective for treatment of trichomoniasis [4, 8, 9, 19]. In 2004, tinidazole was approved by the FDA for treatment of trichomoniasis [33]. It is administered as a 2g single dose orally [5, 39]. Tinidazole has cure rates ranging from 86-100% [20, 39]. Furazolidone and paromomycin have been shown to have anti-trichomonal activity [4, 17] and are used in patients with severe adverse effects from the nitro-imidazoles.

2.6.2 Resistance to metronidazole

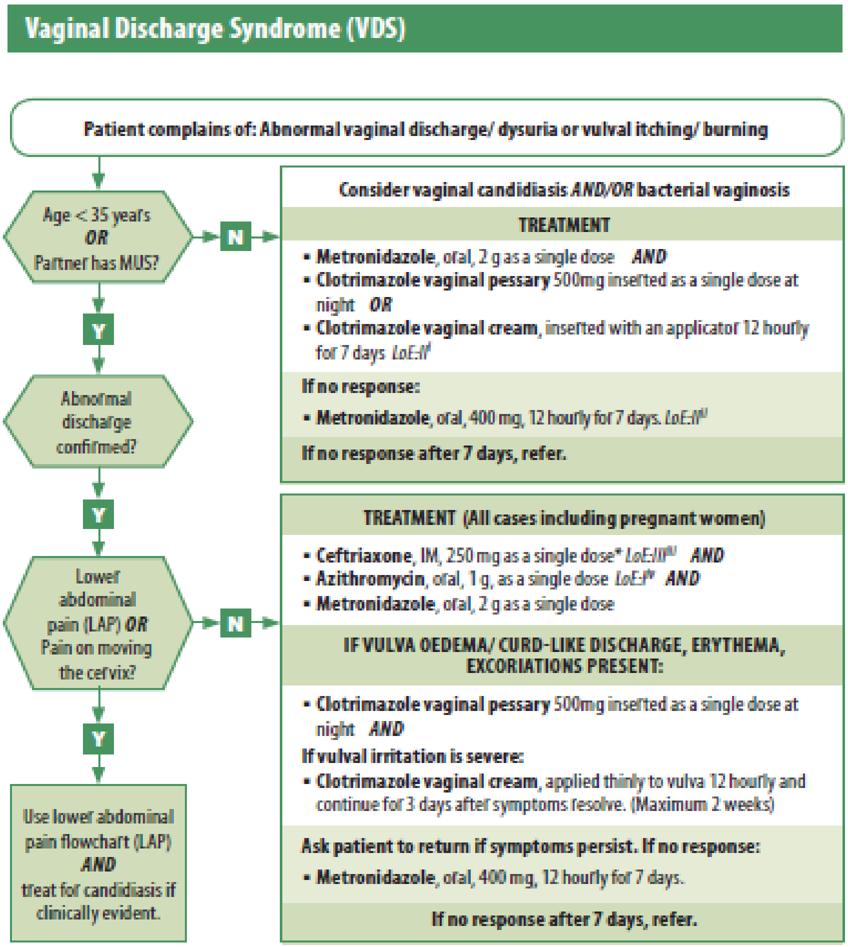
Several cases of metronidazole-resistance have been reported in clinical isolates since its introduction [18]. Mechanisms of metronidazole-resistance that have been suggested include: change in structure of the hydrogenosome, decreased levels of intracellular ferrodoxin, decreased activity of the pyruvate-ferredoxin oxidoreductase (PFOR) and ferrodoxin with abnormally high redox potential [17]. These mechanisms cause pyruvate fermentation to occur in the cytosol instead of in the hydrogenosome, thus preventing the action of the nitroimidazoles [17].

In aerobic resistance, high levels of oxygen in the cell lead to impaired activation and reduction of metronidazole. High levels of oxygen also lead to futile recycling thus limited cell damage rather than cell death [17, 18]. It is also thought that in aerobic resistance, ferredoxin gene transcription is downregulated resulting in decreased activation of the drug by the cell [8, 20]. Anaerobic resistance is characterised by high minimal lethal concentrations (MLCs) during susceptibility testing under anaerobic conditions [40, 41]. In this type of resistance, the activity of PFOR is reduced or absent, thus no reductive activation of metronidazole [8, 20, 40, 41]. The trichomonads are able to replicate in the presence of high levels of metronidazole (100µg/mL) in anaerobic resistance [40]. If treatment fails in patients that have been treated with high dose regimens (2g for 5 days) of metronidazole and tinidazole, susceptibility testing to metronidazole and tinidazole of *T. vaginalis* should be done [20].

2.6.3 Syndromic management

Many countries including South Africa, Tanzania, Ethiopia and India have implemented syndromic management of STIs [42]. In 1995, it was introduced in KwaZulu-Natal [43]. The primary objective of syndromic management is to treat STIs based on the signs and symptoms that the patient present with instead of being based on a laboratory diagnosis [15, 42-45]. Such treatment regimens should cover all causes of the clinical syndrome the patient presents with. With this approach, the patient is treated on the day of presentation thus reducing the spread of STIs, including HIV [44]. Most health care facilities in developing countries lack trained personnel and laboratory equipment required for diagnosis of STIs [43, 44]. The World Health Organisation (WHO) has therefore recommended syndromic treatment of STIs in resource poor countries since a laboratory approach to diagnosis is not practical at the primary healthcare level [44]. Syndromic management is based on the assumption that a group of symptoms and clinical signs are associated with a specific syndrome [46] and indicate infection with one or more of a group of specific pathogens [42, 46]. Several algorithms have been approved for clinical diagnosis of STI syndromes [46]. The major STI syndromes include Male Urethral Syndrome (MUS), Vaginal Discharge Syndrome (VDS), Genital Ulcer Disease (GUD), and Lower Abdominal Pain (LAP) [45-47].

The most common STI syndrome in women is VDS [15]. Most of the VDS cases are caused by *T. vaginalis*, bacterial vaginosis (BV), vulvovaginal candidiasis (CA) or any combination of these [15]. Less frequent causes are cervicitis with *Neisseria gonorrhoeae*, *Chlamydia trachomatis* or *Mycoplasma genitalium*. In South Africa, VDS is currently treated with metronidazole 2g single dose orally, ceftriaxone 250mg single dose intra-muscularly (IM) and azithromycin 1g single dose orally (Figure 3) [16]. The advantages of syndromic management are that the patients are treated at their first visit to the clinic [3, 42, 45], includes counselling, education and treatment to the patient and their sex partner(s), has a high sensitivity and it requires no laboratory diagnosis [42]. The major drawbacks of this approach include low specificity because of overtreatment, the VDS algorithm has low sensitivity and specificity and asymptomatic infections are undetected since it solely relies on symptoms and signs [42, 44]. Effective syndromic management requires regular aetiology and resistance surveillance for the different syndromes [45].



Consider vaginal candidiasis *AND/OR* bacterial vaginosis

TREATMENT

- **Metronidazole**, oral, 2 g as a single dose *AND*
- **Clotrimazole vaginal pessary** 500mg inserted as a single dose at night *OR*
- **Clotrimazole vaginal cream**, inserted with an applicator 12 hourly for 7 days *LoE:II*^{I}

If no response:

- **Metronidazole**, oral, 400 mg, 12 hourly for 7 days. *LoE:II*^{IV}

If no response after 7 days, refer.

TREATMENT (All cases including pregnant women)

- **Ceftriaxone**, IM, 250 mg as a single dose* *LoE:III*^{IV} *AND*
- **Azithromycin**, oral, 1 g, as a single dose *LoE:I*^{IV} *AND*
- **Metronidazole**, oral, 2 g as a single dose

IF VULVA OEDEMA/ CURD-LIKE DISCHARGE, ERYTHEMA, EXCORIATIONS PRESENT:

- **Clotrimazole vaginal pessary** 500mg inserted as a single dose at night *AND*

If vulval irritation is severe:

- **Clotrimazole vaginal cream**, applied thinly to vulva 12 hourly and continue for 3 days after symptoms resolve. (Maximum 2 weeks)

Ask patient to return if symptoms persist. If no response:

- **Metronidazole**, oral, 400 mg, 12 hourly for 7 days.

If no response after 7 days, refer.

Figure 2.6.3: Treatment guideline for Vaginal Discharge Syndrome in South Africa, 2015 [16]

2.7 Antimicrobial susceptibility testing and response to treatment

For susceptibility testing of bacteria standardised methods have been formulated by organisations such as European Committee on Antimicrobial Susceptibility Testing (EUCAST), Clinical and Laboratory Standards Institute (CLSI), and the British Society for Antimicrobial Chemotherapy (BSAC)[48]. Although no consensus exists regarding the methodology with which to test susceptibility of *T. vaginalis*, the techniques used are based on broth dilution. The test is incubated aerobically or anaerobically which results in different outcomes. In addition, reading can be done by wet-prep microscopy or directly in tissue culture plates using an inverted microscope. Different criteria are used for interpretation leading to differences in MIC values obtained by the different methods.

The checkerboard assay is used to test antibiotic combinations *in vitro* [49]. This assay is used to establish synergism or antagonism of combinations of drugs. The test is labor extensive and time consuming and therefore it is not applied routinely [50]. Synergism of antimicrobial agents can also be assessed using the E-test method [51]. However, the E-test cannot be applied for *T. vaginalis* since it needs organisms to grow on agar plates.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Culturing and maintenance of isolates

3.1.1 Trichomonas vaginalis isolates

Ten randomly chosen clinical isolates of *T. vaginalis* were obtained from the culture collection of the Medical Microbiology laboratory, University of KwaZulu-Natal. The initial plan was to use five isolates that are resistant and five susceptible isolates as previously tested in the department. However, this could not be achieved since most of the isolates could not be revived.

3.1.2 Culture Medium

Diamond's TYM medium was used for culturing and maintaining *T. vaginalis* isolates. The pH was adjusted to pH 6.0 with hydrochloric acid (HCl) before the medium was autoclaved at 121^{o}C for 15 minutes. The autoclaved medium was cooled to 50^{o}C following which it was supplemented with 10 % heat inactivated horse serum (Celtic Molecular Diagnostics, South Africa). Vancomycin (5 µg/mL), amphotericin B (5 µg/mL), amikacin (4 µg/mL), chloramphenicol (1 µg/mL) and ciprofloxacin (2 µg/mL) was then added. Five milliliter volumes of the complete medium were aliquoted in 15 ml polystyrene conical screw-cap tubes (NEST) with a diameter of 15 mm and stored at 4^{o}C.

3.1.3 Revival of T. vaginalis isolates from storage

Vials of stored axenic cultures were removed from -70^{o}C storage and placed for 2-4 minutes in a water bath set at 37^{o}C until completely thawed. The entire contents of each vial were then immediately inoculated into Diamond's media which was pre-warmed to 37^{o}C. The inoculated media were placed in a 37^{o}C incubator with 5% CO_2. The screw caps of the culture tubes were loosely screwed. After 24 hours, the cultures were examined by wet mount microscopy at x400 magnification. If sufficient growth was observed, 500µl was transferred aseptically into 5ml of Diamond's medium. If insufficient numbers of viable trichomonads were present, the culture was topped-up to 10 mL with fresh Diamond's media. This was done to dilute excess DMSO. Microscopic examination was thereafter performed after 48 and 72 hours of incubation to monitor growth. If growth was observed, a subculture was made into fresh antibiotic-free Diamond's medium.

3.1.4 Storage

Once axenic cultures were confirmed, the isolates were grown in drug free Diamond's media following which 10% dimethyl sulphoxide (DMSO) and 15 % heat inactivated foetal bovine serum (FBS) was added. The tubes were then inverted 3-4 times to obtain even distribution of the organisms in the broth. The cultures were then dispensed into cryovials in 2ml aliquots. These were then placed at -20^{o}C for approximately 1 hour, then subsequently transferred to -70^{o}C for long term storage.

3.2 Antimicrobial Susceptibility Testing

3.2.1 Preparation of antibiotic stock solutions

Commercially obtained powders of metronidazole, ceftriaxone, doxycycline and fluconazole (Sigma Aldrich, USA) were used to prepare stock solutions. The formula below was used to calculate the amount of powder required for each antibiotic:

Required concentration (µg/mL) x volume (ml) x $\frac{100}{\text{purity}}$ = weight (µg)

Stock solutions were prepared by dissolving the appropriate amount of powder in its respective solvent (Table 1). These stock solutions were then aliquoted and stored at -20^{o}C until required.

Table 3.2.1: Antibiotics used, their solvents, diluents and stock concentration.

Antibiotic	Solvent	Diluent	Stock solution concentration (µg/mL)
Metronidazole	Acetic Acid	Distilled water	6 400
Doxycycline	Distilled water	Distilled water	10 240
Ceftriaxone	Distilled water	Distilled water	6 400
Fluconazole	DMSO	Distilled water	51 200

3.2.2 Inoculum preparation and standardization

Each of the ten *T. vaginalis* isolates was grown in 5ml drug-free Diamond's media and incubated at 37°C in a 5% CO_2 incubator for 24 to 48 hours. For the inoculum preparation, 500 µl of *T. vaginalis* culture was added to 5 ml of fresh pre-warmed Diamonds medium and incubated as above. After 24 hours, the number of viable trophozoites was counted using a Neubauer haemocytometer. The haemocytometer was first disinfected with 70% ethanol and then rinsed with sterile distilled water. A glass coverslip was placed over the counting chambers of the haemocytometer. A drop of *T. vaginalis* culture was then placed on the side of the coverslip to fill the area under the coverslip by capillary action. Viability of the trophozoites was assessed by motility, flipping flagella and/or undulating membrane using a light microscope at x400 magnification. The number of viable trophozoites per millilitre was then calculated using the following formula:

$$\text{Concentration} = \frac{\text{number of trophozoites}}{\text{squares counted}} \text{ x}10^4$$

The desired inoculum of 1.5 x 10^3 trichomonads per well was obtained by dilution with antibiotic free pre-warmed Diamond's medium.

3.2.3 Checkerboard Assay

Six antibiotic combinations were tested in this experiment (Table 3.2.3a).

Table 3.2.3a: Antibiotic combinations tested in the checkerboard assay

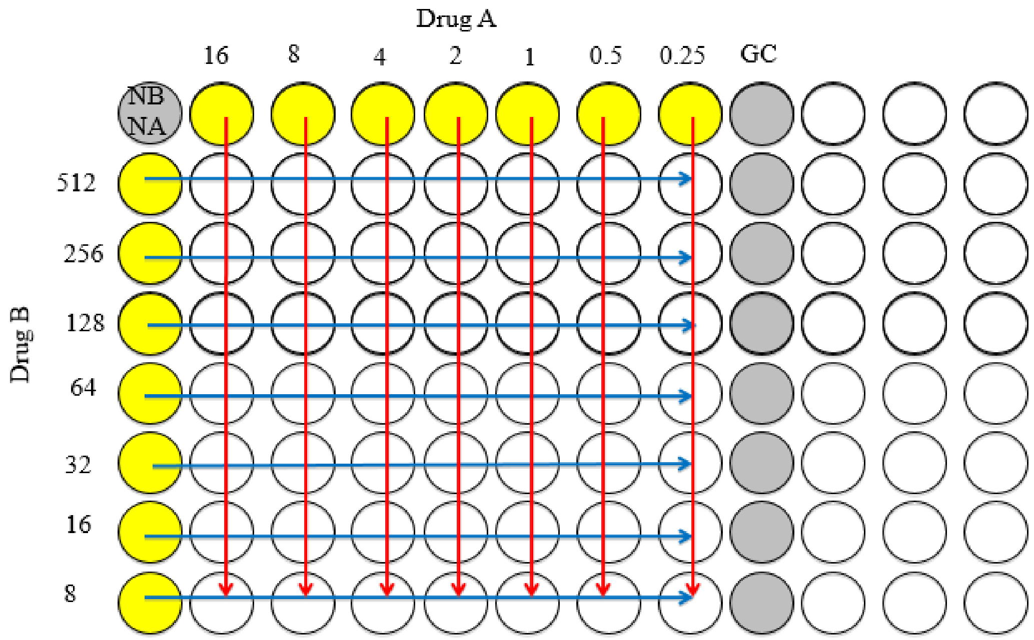
No.	**Antibiotics in combination**
1.	Metronidazole + Doxycycline
2.	Metronidazole + Ceftriaxone
3.	Metronidazole + Fluconazole
4.	Doxycycline + Ceftriaxone
5.	Doxycycline + Fluconazole
6.	Ceftriaxone + Fluconazole

The Minimal Inhibitory Concentrations (MICs) of metronidazole, doxycycline, ceftriaxone and fluconazole (each drug alone and in combination (Table 2)) were determined for each isolate. All experiments were performed in triplicate in flat bottomed micro-titer plates. Dilutions of all antibiotic stock solutions were prepared to four times the required concentration. Macro-dilution was performed for each antibiotic in 15 ml polypropylene tubes using drug free Diamond's medium. Two-fold serial dilutions were performed using pre-diluted antibiotic stock solutions for each antibiotic. The highest MIC concentration used in this study for metronidazole was chosen per previously published data. For doxycycline we used concentrations that were previously tested by Naidoo and Sturm, (2015) [52]. For ceftriaxone, we used a concentration range that is normally used for *N. gonorrhoeae*. Concentrations for fluconazole were chosen based on Naidoo and Sturm, (2015) work [52]. The resulting concentrations are shown in Table 3.2.3b.

Table 3.2.3b: Concentrations of antimicrobial drugs after broth macro-dilution and final concentrations in the checkerboard assay.

Antibiotic	Macro-broth dilutions (4x) (µg/mL)	Final concentration in the assay (µg/mL)
Metronidazole	16 - 0.25	4 - 0.0625
Doxycycline	2048 - 32	512 - 8
Ceftriaxone	64 - 1	16 - 0.25
Fluconazole	2048 - 32	512 - 8

Figure 3.2.3 shows the set-up of the checkerboard. Fifty (50) µl of antibiotic-free Diamond's media was added in wells A1- A8 and B1- H1 and 100 µl was added in the Growth Control (GC) wells (Column 9). Fifty µl volumes of each dilution were then transferred to the flat bottom 96-well plate (NEST). 50 µl of antibiotic A was added along the *x*-axis (column 2 to column 8) and 50 µl of antibiotic B was added along the *y*-axis (row B to row H). Each microtiter well was then inoculated with 100 µl of *T. vaginalis* standardized inoculum ($1.5x10^3$ trichomonads per well). The same volume of a suspension with + $1x10^4$ cfu/mL of the bacterial or yeast control strains (2.3.5) was added to the remaining columns. The plates were then incubated in air-tight 2.5L anaerobic jar containing Anaerogen Gas Packs (Thermo Scientific) at 37^oC, 5% CO_2 incubator. Anaerobic indicator strips (Thermo Scientific) were used to monitor anaerobiosis. The plates were read after 48 and 72 hours of incubation using an inverted microscope at x40. The gas pack and indicator strip were changed after the first reading (48 hours).



NB- No Bacteria or trichomonads

NA- No Antimicrobial

GC- Growth Control

Figure 3.2.3: Schematic presentation of the checkerboard assay. The yellow-shaded wells only had one antibiotic.

3.2.4 Combinations of three antibiotics

Four antibiotic combinations were tested in this assay (Table 3.2.4).

Table 3.2.4: Antibiotic combinations tested in the combination of three antimicrobials experiment

No.	**Antibiotics in combination**
1.	Metronidazole (A) + Doxycycline (B) + Ceftriaxone (C)
2.	Metronidazole (A)+ Doxycycline (B) + Fluconazole (C)
3.	Metronidazole (A) + Ceftriaxone (B) + Fluconazole (C)
4.	Doxycycline (A) + Ceftriaxone (B) + Fluconazole (C)

This assay was designed using results obtained from the checkerboard assay of combinations of two antibiotics (Fig 3.2.4). For each isolate, the concentration two times lower than the MIC obtained was used for metronidazole and doxycycline. For ceftriaxone and fluconazole, the highest concentrations were used (16 and 512 µg/mL, respectively). The working stock solutions of antibiotic A and antibiotic B were prepared at concentrations four and eight times higher than the required concentration. Antibiotic C working stock solution was prepared to four times higher the required concentration. Fifty µl of antibiotic A and B (8x) (each) was added in row A and 50 µl of antibiotic A and B (4x) in row B-G. One hundred µl of antibiotic C (4x) was added in row A. Two-fold serial dilutions were performed by using 100 µl from row A to row G and 100 µl was discarded at row G. The concentration of antibiotic A and B remained the same (fixed) in all wells after serial dilutions. Hundred µl of antibiotic-free Diamond’s media was added in row H followed by 100 µl of standardized *T. vaginalis* inoculum (1.5x10^3 trichomonads /well) into each well. Plates were then incubated as previously described. Scoring or reading was done after 48 and 72 hours. The assay was performed in triplicate for each isolate.

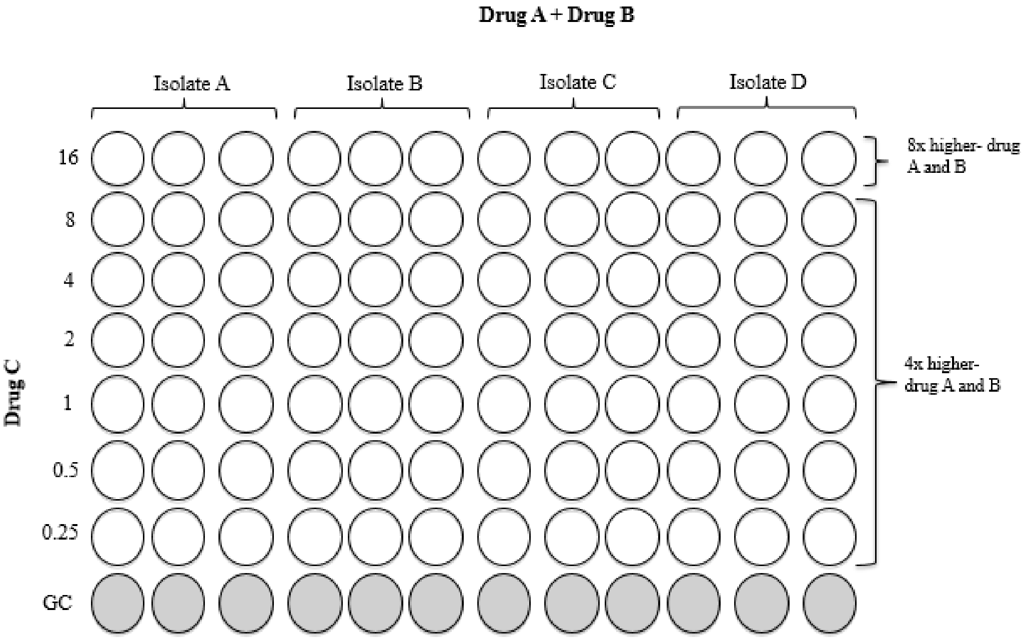


Figure 3.2.4: Schematic presentation of the assay for combinations of 3 antimicrobials

3.2.5 Combinations of four antibiotics

The combination of metronidazole + doxycycline + ceftriaxone + fluconazole was tested in two ways:

1. [Metronidazole + doxycycline + ceftriaxone] (fixed concentration) + fluconazole.
2. [Metronidazole + doxycycline + fluconazole] (fixed concentration) + ceftriaxone.

In the first combination, the concentration of metronidazole, doxycycline and ceftriaxone were fixed, only fluconazole was serially diluted. In the second combination, the concentration of metronidazole, doxycycline and fluconazole were fixed, only ceftriaxone was serially diluted.

This assay was designed using results obtained from the checkerboard assay of combinations of two and three antibiotics. For each isolate, the concentration two times lower than the MIC obtained from the checkerboard assay was used for metronidazole and doxycycline. For ceftriaxone and fluconazole, the highest concentrations were used (16 and 512 µg/mL, respectively). The working stock solutions of antibiotic A (MTZ) and antibiotic B (DOX) were prepared to sixteen and eight times higher the required concentration. Antibiotic C (CTX or FLC, depending on the combination) working stock solution was prepared to four times higher the required concentration. Working stock solution for antibiotic D (CTX or FLC) was prepared to eight times higher the required concentration. Twenty-five µl of antibiotic A (MTZ) (16x) and 25 µl of antibiotic B (DOX) (16x) were added in row A. Twenty-five µl of A(8x) and 25 µl of B (8x) were added in in row B-G as well as 50µl of antibiotic D (8x) in row A. Two-fold serial dilutions were performed from row A to row G using 50 µl and 50 µl was discarded at row G. Fifty µl of antibiotic C (4x) was added in all wells. The concentration of the first three antibiotics (A, B, C) remained fixed in all wells. One hundred µl of antibiotic-free Diamond's media was added in row H. Finally, one hundred µl of standardized *T. vaginalis* inoculum (1.5x10^3 trichomonads /well) was added into each well. Plates were then incubated as previously described. Scoring or reading was done after 48 and 72 hours. The assay was performed in triplicate for each isolate.

3.2.6 Controls

Three bacterial control strains with known MICs were used in this study: *Bacteroides fragilis* ATCC 25285 to test metronidazole concentrations, *Klebsiella pneumoniae* ATCC 700603 to test doxycycline and ceftriaxone concentrations and *Candida albicans* ATCC 14053 to test fluconazole concentrations.

3.2.7 Scoring of Trichomonas vaginalis

The plates were read using an inverted microscope (x400), after 48 and 72 hours of incubation. *T. vaginalis* growth and motility was scored according to the scoring criteria in Table 3.2.7. The MIC was the lowest antimicrobial concentration with a score of +1.

Table 3.2.7: Scoring of *Trichomonas vaginalis* growth (Upcroft and Upcroft, 2001)

Score	Interpretation
1+	0-10 motile parasites; not more than 20% coverage of well surface
2+	> 10 motile parasites; 20% to 50% coverage of the well surface
3+	> 50 % coverage of the well surface = almost confluent growth with much motility
4+	confluent growth with full motility

3.2.8 Interpretation

The following formulas were used to calculate the fractional inhibitory concentration (FIC) index:

$$\text{The FIC of drug A} = \frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}}$$

$$\text{The FIC of drug B} = \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}}$$

The FIC index = $FIC_A + FIC_B$

Synergy was defined as an FIC index of ≤ 0.5. Indifference was defined as an FIC of > 0.5 but of < 4. Antagonism was defined as an FIC index of ≥ 4 [53].

3.3 ETHICAL APPROVAL

The study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal. Ethics approval number: BE287/15.

CHAPTER FOUR

RESULTS

4.1 MICs of single antimicrobial drugs

The distribution of the MICs obtained with metronidazole and doxycycline for the ten isolates are shown in table 4.1 a and b. For metronidazole, the values ranged from 0.25 to 1 µg/mL. For doxycycline, the MICs were between 64 and 128 µg/mL. Ceftriaxone and fluconazole had no antimicrobial activity against *T. vaginalis* isolates tested (MICs >16µg/mL and >512µg/mL, respectively). A summary of the results is given in table 4.1.c.

Table 4.1a: Distribution of MICs obtained with metronidazole (n=10)

Antibiotic	**No of isolates with MIC (µg/mL):**						
	0.0625	0.125	0.25	0.5	1	2	4
Metronidazole	-	-	1	4	5	-	-

Table 4.1b: Distribution of MICs obtained with doxycycline (n=10)

Antibiotic	**No of isolates with MIC (µg/mL):**						
	8	16	32	64	128	256	512
Doxycycline	-	-	-	5	5	-	-

Table 4.1c: MIC range, MIC_{50} and MIC_{90} of the antimicrobial drugs tested

Antibiotic	MIC range (µg/mL)	MIC_{50} (µg/mL)	MIC $_{90}$ (µg/mL)
MTZ	0.25-1	0.5	1
DOX	64-128	64	128
CTX	>16	>16	>16
FLC	>512	>512	>512

MTZ- Metronidazole, DOX- Doxycycline, CTX- Ceftriaxone, FLC-Fluconazole

4.2 Combinations of two antimicrobial drugs

Tables 4.2 a-f show the results for the combinations of two drugs. The MIC of metronidazole was decrease in combination with doxycycline for five isolates The MIC of all other isolates for all the other antimicrobial agents remained the same, even in combination.

The FIC Indices for the combination of metronidazole and doxycycline ranged between 0.75 to 1.125. The interaction between metronidazole and doxycycline was therefore indifferent for all isolates.

The FIC Index for the remaining five drug combinations was 2 for all ten isolates and therefore also indifferent.

Table 4.2a: Results obtained with metronidazole and doxycycline alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	MTZ	DOX	MTZ	DOX	MTZ	DOX	
63	0.5	128	0.25	64	0.5	0.5	1
94	1	64	0.0625	64	0.0625	1	1.0625
95	1	64	0.0625	64	0.0625	1	1.0625
264	1	128	0.25	64	0.25	0.5	0.75
304	0.5	128	0.25	64	0.5	0.5	1
652	1	128	0.5	32	0.5	0.25	0.75
696	0.25	64	0.0625	64	0.25	1	1.25
716	0.5	128	0.25	64	0.5	0.5	1
727	0.5	64	0.0625	64	0.125	1	1.125
777	1	64	0.0625	64	0.0625	1	1.0625

Table 4.2b: Results obtained with metronidazole and ceftriaxone alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	MTZ	CTX	MTZ	CTX	MTZ	CTX	
63	0.5	>16	0.5	>16	1	1	2
94	1	>16	1	>16	1	1	2
95	1	>16	1	>16	1	1	2
264	0.5	>16	0.5	>16	1	1	2
304	0.5	>16	0.5	>16	1	1	2
652	1	>16	1	>16	1	1	2
696	0.25	>16	0.25	>16	1	1	2
716	0.5	>16	0.5	>16	1	1	2
727	0.5	>16	0.5	>16	1	1	2
777	1	>16	1	>16	1	1	2

Table 4.2c: Results obtained with metronidazole and fluconazole alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	MTZ	FLC	MTZ	FLC	MTZ	FLC	
63	0.5	>512	0.5	>512	1	1	2
94	1	>512	1	>512	1	1	2
95	1	>512	1	>512	1	1	2
264	0.5	>512	0.5	>512	1	1	2
304	0.5	>512	0.5	>512	1	1	2
652	0.5	>512	0.5	>512	1	1	2
696	0.25	>512	0.25	>512	1	1	2
716	0.5	>512	0.5	>512	1	1	2
727	0.5	>512	0.5	>512	1	1	2
777	1	>512	1	>512	1	1	2

Table 4.2d: Results obtained with doxycycline and ceftriaxone alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	DOX	CTX	DOX	CTX	DOX	CTX	
63	128	>16	128	>16	1	1	2
94	64	>16	64	>16	1	1	2
95	64	>16	64	>16	1	1	2
264	128	>16	128	>16	1	1	2
304	128	>16	128	>16	1	1	2
652	128	>16	128	>16	1	1	2
696	64	>16	64	>16	1	1	2
716	128	>16	128	>16	1	1	2
727	64	>16	64	>16	1	1	2
777	64	>16	64	>16	1	1	2

Table 4.2e: Results obtained with doxycycline and fluconazole alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	DOX	FLC	DOX	FLC	DOX	FLC	
63	128	>512	128	>512	1	1	2
94	64	>512	64	>512	1	1	2
95	64	>512	64	>512	1	1	2
264	128	>512	128	>512	1	1	2
304	128	>512	128	>512	1	1	2
652	128	>512	128	>512	1	1	2
696	64	>512	64	>512	1	1	2
716	128	>512	128	>512	1	1	2
727	64	>512	64	>512	1	1	2
777	64	>512	64	>512	1	1	2

Table 4.2f: Results obtained with ceftriaxone and fluconazole alone and in combination

ISOLATE	MIC alone (µg/mL)		MIC in comb (µg/mL)		FIC		FIC INDEX
	CTX	FLC	CTX	FLC	CTX	FLC	
63	>16	>512	>16	>512	1	1	2
94	>16	>512	>16	>512	1	1	2
95	>16	>512	>16	>512	1	1	2
264	>16	>512	>16	>512	1	1	2
304	>16	>512	>16	>512	1	1	2
652	>16	>512	>16	>512	1	1	2
696	>16	>512	>16	>512	1	1	2
716	>16	>512	>16	>512	1	1	2
727	>16	>512	>16	>512	1	1	2
777	>16	>512	>16	>512	1	1	2

4.3 Combinations of three antimicrobial drugs

In this assay, a fixed concentration of metronidazole and doxycycline was used. The concentrations used were obtained from the checkerboard assay (combinations of two antimicrobial drugs). A concentration 0.5 x MIC was used for each drug.

For all four combinations tested, the effect was indifferent for all isolates (FIC Indices = 3) (Table 4.3a, b and c).

Table 4.3a: Results with the MTZ + DOX + CTX and MTZ + DOX + FLC combinations

Isolate	Concentration used (µg/mL)		3rd antimicrobial drug MIC (µg/mL)		FIC Index	
	MTZ	DOX	CTX	FLC	MTZ+DOX+CTX	MTZ+DOX+FLC
63	0.125	32	>16	>512	3	3
94	0.03125	32	>16	>512	3	3
95	0.03125	32	>16	>512	3	3
264	0.125	32	>16	>512	3	3
304	0.125	32	>16	>512	3	3
652	0.125	32	>16	>512	3	3
696	0.03125	32	>16	>512	3	3
716	0.125	32	>16	>512	3	3
727	0.03125	32	>16	>512	3	3
777	0.03125	32	>16	>512	3	3

Table 4.3b: Results obtained with the MTZ + CTX + FLC combination

Isolate	Concentration used (µg/mL)		3rd antimicrobial drug MIC (µg/mL)	FIC Index
	MTZ	CTX	FLC	MTZ+CTX+FLC
63	0.25	16	>512	3
94	0.5	16	>512	3
95	0.5	16	>512	3
264	0.25	16	>512	3
304	0.25	16	>512	3
652	0.5	16	>512	3
696	0.125	16	>512	3
716	0.25	16	>512	3
727	0.25	16	>512	3
777	0.5	16	>512	3

Table 4.3c: Results obtained with the DOX + CTX + FLC combination

Isolate	Concentration used (µg/mL)		3rd antimicrobial drug MIC (µg/mL)	FIC Index
	DOX	CTX	FLC	DOX+CTX+FLC
63	64	16	>512	3
94	32	16	>512	3
95	32	16	>512	3
264	64	16	>512	3
304	64	16	>512	3
652	64	16	>512	3
696	32	16	>512	3
716	64	16	>512	3
727	32	16	>512	3
777	32	16	>512	3

4.4 Combinations of four antibiotics

All the combinations in this assay had an indifferent effect on all the isolates tested (FIC Indices =4). This is shown in table 4.4a and 4.4b.

Table 4.4a: Results obtained with the MTX + DOX + CTX + FLC combination

Isolate	**Concentration used (µg/mL)**			**4rd antimicrobial drug MIC (µg/mL)**	**FIC Index**
	MTZ	DOX	CTX	FLC	MTZ+DOX+CTX+FLC
63	0.125	64	16	>512	4
94	0.03125	32	16	>512	4
95	0.03125	32	16	>512	4
264	0.125	64	16	>512	4
304	0.125	64	16	>512	4
652	0.125	64	16	>512	4
696	0.03125	32	16	>512	4
716	0.125	64	16	>512	4
727	0.03125	32	16	>512	4
777	0.03125	32	16	>512	4

Table 4.4b: Results obtained with the MTX + DOX + FLC + CTX combination

Isolate	Concentration used (µg/mL)			4^{rd} antimicrobial drug MIC (µg/mL)	FIC Index
	MTZ	DOX	FLC	CTX	MTZ+DOX+FLC+CTX
63	0.125	64	512	>16	4
94	0.03125	32	512	>16	4
95	0.03125	32	512	>16	4
264	0.125	64	512	>16	4
304	0.125	64	512	>16	4
652	0.125	64	512	>16	4
696	0.03125	32	512	>16	4
716	0.125	64	512	>16	4
727	0.03125	32	512	>16	4
777	0.03125	32	512	>16	4

All the combinations tested in this study had an indifferent effect on the isolates. Table 4.5a, b and c show the summary of the results obtained with all the combinations.

Table 4.5a: FIC indices and effect of the combinations of two antimicrobial drugs

Isolate	MTZ+ DOX		MTZ+ CTX		MTZ + FLC	
	∑ FIC	Activity	∑ FIC	Activity	∑ FIC	Activity
63	1	I	2	I	2	I
94	1.0625	I	2	I	2	I
95	1.0625	I	2	I	2	I
264	0.75	I	2	I	2	I
304	1	I	2	I	2	I
652	0.75	I	2	I	2	I
696	1.25	I	2	I	2	I
716	1	I	2	I	2	I
727	1.125	I	2	I	2	I
777	1.0625	I	2	I	2	I

*I – indifferent effect

Isolate	DOX + CTX		DOX + FLC		CTX + FLC	
	∑ FIC	Activity	∑ FIC	Activity	∑ FIC	Activity
63	2	I	2	I	2	I
94	2	I	2	I	2	I
95	2	I	2	I	2	I
264	2	I	2	I	2	I
304	2	I	2	I	2	I
652	2	I	2	I	2	I
696	2	I	2	I	2	I
716	2	I	2	I	2	I
727	2	I	2	I	2	I
777	2	I	2	I	2	I

Table 4.5b: FIC indices and effect of the combinations of three antimicrobial drugs

Isolate	MTZ+DOX+CTX		MTZ+DOX+FLC		MTZ+CTX+FLUC		DOX+CTX+FLC	
	∑ FIC	Activity	∑ FIC	Activity	∑ FIC	Activity	∑ FIC	Activity
63	3	I	3	I	3	I	3	I
94	3	I	3	I	3	I	3	I
95	3	I	3	I	3	I	3	I
264	3	I	3	I	3	I	3	I
304	3	I	3	I	3	I	3	I
652	3	I	3	I	3	I	3	I
696	3	I	3	I	3	I	3	I
716	3	I	3	I	3	I	3	I
727	3	I	3	I	3	I	3	I
777	3	I	3	I	3	I	3	I

Table 4.5c: FIC indices and effect of the combinations of four antimicrobial drugs

Isolate	MTZ+DOX+CTX+FLC		MTZ+DOX+FLC+CTX	
	∑ FIC	Activity	∑ FIC	Activity
63	4	I	4	I
94	4	I	4	I
95	4	I	4	I
264	4	I	4	I
304	4	I	4	I
652	4	I	4	I
696	4	I	4	I
716	4	I	4	I
727	4	I	4	I
777	4	I	4	I

CHAPTER FIVE

5.1 DISCUSSION

Metronidazole resistance as well as treatment failure has been reported as early as 1962, after its introduction [18, 33, 54]. The nitroimidazoles metronidazole and tinidazole are the only antimicrobials approved by the FDA for treating trichomoniasis [55] while in South Africa only metronidazole is registered by the Medicine Council for this indication. It has also been reported that *T. vaginalis* infection is associated with increased risk of HIV acquisition [7, 9, 10, 19, 20] transmission and cervical cancer [10, 28]. Therefore, there is a great need for alternative therapeutic agents that can be used to effectively treat trichomoniasis.

There is no clear association between in-vitro resistance and treatment failure. Also, in-vitro susceptibility does not equate response to treatment. In a study by Naidoo and Sturm [52], 5 of 6 patients infected with a metronidazole-resistant *T. vaginalis* responded to treatment with eradication of the organism while 4 of 28 patients whose organism was susceptible remained symptomatic and infected. It was postulated that response to treatment of patients with a resistant organism could be the result of concentrations of metronidazole in the vaginal secretions above the breakpoint used to interpret the susceptibility tests. Alternatively, drugs administered in combination with metronidazole in the syndromic management (SM) regimen could work synergistically.

In this study, we investigated the latter hypothesis by testing the effect of antibiotics used in syndromic management of VDS in South Africa in combination on *T. vaginalis* isolates. These are metronidazole, doxycycline and ceftriaxone. In the new guidelines doxycycline has been replaced with azithromycin [16] but this drug was not included in this study. Fluconazole was included since this drug is often added to the SM combination.

The findings of this study show that ceftriaxone and fluconazole had no antitrichomonal activity. These findings correlate with those of Naidoo and Sturm [52]. There was no additional effect of ceftriaxone or fluconazole in combination with metronidazole. Doxycycline had an effect against *T. vaginalis* even though the effect was seen at higher concentrations that are considered resistant for bacterial isolates.

Using the Upcroft and Upcroft breakpoints [10], Naidoo and Sturm found 57% of 160 isolates susceptible, 32% intermediate and 11% resistant to metronidazole [52]. The ten isolates used for our synergy test project were stored susceptible isolates that were part of a study done in 2015 by Mtshali et al. [56]. This included 94 isolates and, using the same breakpoints, the results were with 55 % susceptible, 27 % intermediate and 12 % resistant, similar to those of Naidoo and Sturm, 10 years earlier.

Reports on the association between in-vitro susceptibility test results and response to treatment are conflicting. Naidoo and Sturm [52] and Schwebke and Barrientes [57] found that there was no correlation between *in vitro* resistance and treatment outcome. In the study by Naidoo and Sturm, out of six patients that were infected with resistant isolates, only one patient had treatment failure. The other five patients responded to treatment [52]. Lossick et al. reported that *in vitro* metronidazole susceptibility values provide a relative estimate of the antibiotic-dosage required for treatment and the level of clinical resistance [58]. In their study, 87% (n=53) of patients infected with clinically resistant *T. vaginalis* were cured with doses of metronidazole as high 3g of metronidazole per day for approximately 13 days. They also found that aerobic Minimal Lethal Concentrations (MLCs) were best suitable or accurate in estimating *in vivo* resistance of *T. vaginalis* isolates compared to anaerobic MLCs [58]. However, it is difficult to test *T. vaginalis* under aerobic conditions. In this study, we attempted incubating *T. vaginalis* aerobically in a 37°C; 5% CO_2 incubator. There was no trichomonal growth after 48 and 72 hours of incubation. The findings of a study conducted by Muller et al. also demonstrate that clinical resistance does not correlate with *in vitro* susceptibility [59].

Doxycycline is a broad-spectrum antimicrobial that is derived from tetracycline. It has been shown to have anti-protozoal activity and it is used in malaria prophylaxis since 1994 [60]. In this study, doxycycline had some antitrichomonal activity with MICs of 64 and 128 µg/mL. In a study conducted by Naidoo and Sturm [52], 33% of isolates tested had an MIC of ≤ 8 µg/mL. The concentration of 8 µg/ml has been shown to be achievable in plasma or serum with a dose of 500mg, orally [52]. A study by Mahdi et al. showed that doxycycline has no antitrichomonal activity *in vivo*. In this study, only three patients were treated with 200mg of doxycycline. Interestingly, they found that the tetracycline derivative, oxytetracycline was effective against *T. vaginalis* in vitro [61]. The effect of this antimicrobial needs to be further investigated with a larger sample size.

Antibiotic combination therapy is getting more attention with the increase in infections that are caused by multi-drug resistant organisms. Therefore, there is a greater need for development of a standardised and simple protocol for testing antibiotic combinations to different organisms [49]. The checkerboard assay is the commonly used technique for assessing synergism of antimicrobials. This technique has not been previously used for assessing the effect combination therapy on *T. vaginalis*. The major disadvantage of this techniques is that it is labour intensive [50]. The E-test method has also been used to assess synergism of antimicrobials. This method has been reported to be less time consuming [51] and it can be performed routinely in laboratories [51, 62]. However, this method cannot be used for *T. vaginalis* because it is performed on solid agar.

In this study, we designed the checkerboard assay using the method described by Orhan [51]. This method results in each well containing two antimicrobials with different concentrations. In a recent study by El-Azizi, they describe a method for testing synergism that they report as simple and rapid for testing double and triple antimicrobial combinations [49]. Briefly, they used one 96-well plate to test susceptibility to each antimicrobial alone and in combination of two and three antimicrobials. After adding 50µl of growth medium to each well, they added 50µl of antimicrobials at 4x the highest concentration (alone, double and triple combination). These were then serially diluted and then 50µl of the inoculum was added. However, this technique does not result in a checkerboard and few concentration combinations may be tested. Researchers use different FIC Index values to interpret synergism, antagonism and / or indifference. Orhan describes synergism as an antibiotic combination with an FIC index of 0.5, indifference when the FIC index is 0.5-2, and antagonism when the FIC index is ≥2 [51]. El-Azizi; Mackay et al. and White et al. defined synergism as combination with an FIC index of 0.5 or less, indifference when the FIC index is greater than 0.5 but less than 4, and antagonism when the FIC index is greater than 4 [49, 50, 62]. In this study, we used the latter interpretation. The results obtained in this study suggest that all the combinations of two antimicrobials tested had an indifferent effect on the *T. vaginalis* isolates. However, with metronidazole and doxycycline combination there was a decrease in the MICs of both antibiotics in combination for some isolates. For metronidazole, there was a decrease in MICs of all isolates. Five isolates had MICs that were one dilution below the MIC obtained with metronidazole alone and five had MICs of ≤ 0.0625µg/mL in combination with doxycycline. Although based on the FIC index the effect of this combination is indifferent, this might suggest that these two antibiotics have some synergistic effect. This needs to be further investigated with a larger sample size. All other combinations (three and four antimicrobials) also had an indifferent effect on *T. vaginalis*. Azithromycin has also been shown to have some inhibitory effect on *T. vaginalis* [52]. Possible synergistic effect with this drug and metronidazole needs to be investigated.

Since there are limited treatment options for trichomoniasis, it is essential that other antimicrobial combinations are investigated.

Several antimicrobials have been shown to have antitrichomonal activity. These include other 5-nitroimidazoles (tinidazole, ornidazole) [17], furazolidone [4, 17, 54, 63], anisomycin, and mebendazole [63]. These antimicrobials may be useful alternatives for treating *T. vaginalis* infections resistant to metronidazole and for patients with allergic reactions to metronidazole. These may further be investigated for combination therapy with metronidazole.

The limitation in this study was that out of the 94 *T. vaginalis* isolates that were available in the Medical Microbiology laboratory, only few isolates could be revived. We were planning to use five isolates that are resistant and five susceptible isolates as previously tested in the department. However, this could not be achieved since most of the isolates could not be revived. The other limitation was that since the checkerboard assay is labour extensive, only a few isolates could be tested. In this study, we aimed to perform the experiments aerobically and anaerobically. However, this was not achievable since the revived *T. vaginalis* isolates did not grow aerobically, even after incubation in permeable plastic bags. We used bacterial controls in this study to confirm that all calculations and dilutions were accurate. For the other antimicrobials, bacterial controls were used because *Trichomonas vaginalis* strains with known MIC values were not available.

5.2 CONCLUSION

Our findings indicate that the combinations of antimicrobials used in syndromic management of the Vaginal Discharge Syndrome have an indifferent effect on *T. vaginalis* isolates. The decrease in MICs of metronidazole and doxycycline in their combination suggests that these two antimicrobials have some degree of synergism although it was interpreted as indifferent by the FIC Indices. Therefore, it is essential to further investigate this combination with a larger sample size. Given that treatment choices are limited for trichomoniasis, more combination studies of metronidazole and the other antimicrobials that have been shown to have antitrichomonal effect need to be conducted.

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APPENDICES

APPENDIX A

Diamond’s TYM Medium preparation (1 Litre)

20g	BBL™ trypticase peptone
10g	Yeast extract
5g	Maltose
1g	L-cysteine hydrochloride
0.2g	L-ascorbic acid
0.5g	Agar
900ml	Distilled water

All the powder components were dissolved in 900ml distilled water and adjusted to a pH of 6.0. The medium was then autoclaved at 121^{o}C for 15 minutes and cooled to 50^{o}C in the water bath. 100ml of heat inactivated horse serum was then added. The medium was then aliquoted into 5ml in polystyrene 15ml tubes and stored at 4^{o}C.

Antimicrobials were not added in the medium that was to be used for susceptibility testing. If contamination was observed in cultures then vancomycin (5 µg/ml), amphotericin B (5 µg/ml), amikacin (4 µg/ml), chloramphenicol (1 µg/ml) and ciprofloxacin (2 µg/ml) was added in the medium during preparation.

APPENDIX B

Antimicrobial Agents

1. Antimicrobial Agents stock preparation

Antimicrobial stocks for media supplementation were prepared as shown in table 1. The powder for each antimicrobial was weighed and dissolved in the appropriate solvent. The stocks were then stored at -20^{o}C.

Table 1: Preparation of antimicrobials for media supplementation

Antimicrobial	Solvent	Purity	Stock concentration (µg/ml)	Concentration in Diamonds medium (µg/ml)
Amikacin	Water	100	400	4
Amphotericin B	DMSO	80	500	5
Ciprofloxacin	Acetic acid	98	200	2
Chloramphenicol	Ethanol	98	100	1
Vancomycin	Water	100	500	5

APPENDIX C

Table1 was used to score *T. vaginalis* growth after 48 and 72 hours of incubation. The MIC was the lowest antimicrobial concentration with a score of +1.

Table 1: Scoring of *Trichomonas vaginalis* growth (Upcroft and Upcroft, 2001)

Score	Interpretation
1+	0-10 motile parasites; not more than 20% coverage of well surface
2+	> 10 motile parasites; 20% to 50% coverage of the well surface
3+	> 50 % coverage of the well surface = almost confluent growth with much motility
4+	confluent growth with full motility

1. Combinations of two antimicrobials

The yellow-shaded wells only had one antimicrobial. The bold highlighted 1+ score after 72 hours represent overgrowth or dead organisms due to overgrowth.

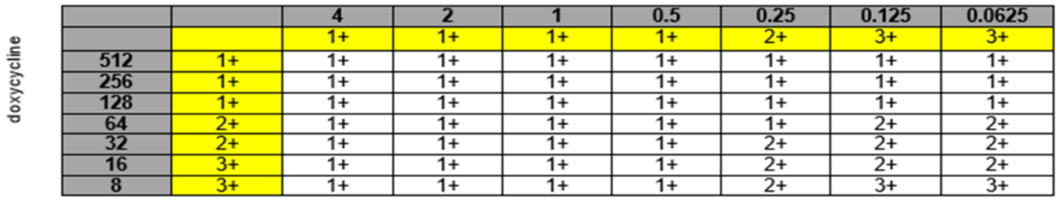
1.1 Metronidazole and doxycycline combination.

Date: 19 Oct 2016 **Isolate: 63**

Reading after 48 hours

metronidazole

doxycycline



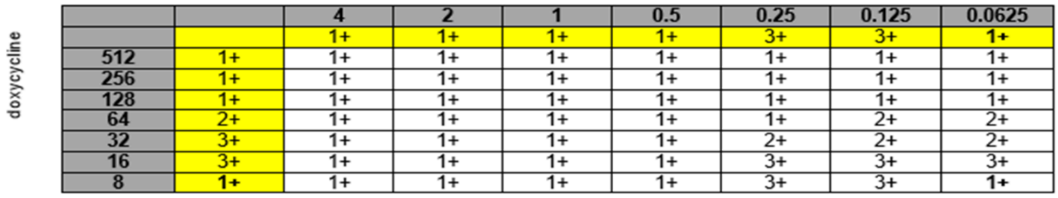
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	2+	2+	2+
8	3+	1+	1+	1+	1+	2+	3+	3+

Date: 20 Oct 2016

Reading after 72 hours

metronidazole

doxycycline



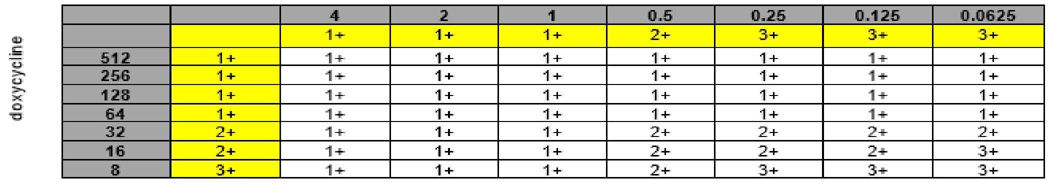
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	3+	3+	**1+**
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	3+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	3+	3+	3+
8	**1+**	1+	1+	1+	1+	3+	3+	**1+**

Date: 01 Oct 2016 **Isolate: 94**

Reading after 48 hours

metronidazole

doxycycline



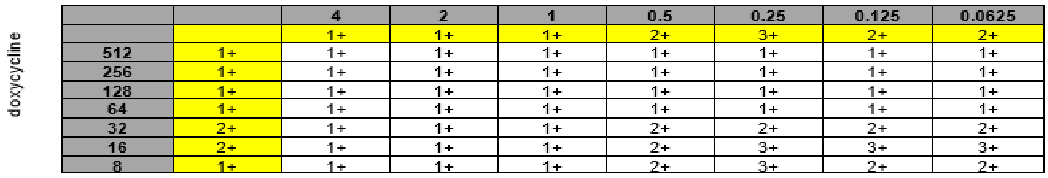
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	2+	2+	2+	2+
16	2+	1+	1+	1+	2+	2+	2+	3+
8	3+	1+	1+	1+	2+	3+	3+	3+

Date: 02 Oct 2016

Reading after 72 hours

metronidazole

doxycycline



		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	2+	2+	2+	2+
16	2+	1+	1+	1+	2+	3+	3+	3+
8	1+	1+	1+	1+	2+	3+	2+	2+

Date: **03 Oct 2016** **Isolate:** **95**

Reading after 48 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	2+	1+	1+	1+	2+	2+	2+	2+
8	2+	1+	1+	1+	2+	3+	3+	3+

Date: **04 Oct 2016**

Reading after 72 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	1+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	2+	2+	2+	2+
16	2+	1+	1+	1+	2+	3+	3+	3+
8	1+	1+	1+	1+	2+	3+	1+	1+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	1+	2+	2+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

Date: 06 Oct 2016

Reading after 72 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	3+	1+	1+	1+	1+	2+	3+	3+
16	2+	1+	1+	1+	1+	2+	3+	3+
8	2+	1+	1+	1+	1+	2+	3+	3+

Date: 04 Oct 2016 **Isolate:** 304

Reading after 48 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	1+	2+	2+
16	2+	1+	1+	1+	1+	2+	2+	2+
8	3+	1+	1+	1+	1+	2+	2+	2+

Date: 05 Oct 2016

Reading after 72 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	3+	1+	1+	1+	1+	2+	2+	2+
16	2+	1+	1+	1+	1+	2+	3+	2+
8	1+	1+	1+	1+	1+	2+	3+	1+

Date: 09 Oct 2016 **Isolate:** 652

Reading after 48 hours

metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	2+	2+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

Date: 10 Oct 2016

Reading after 72 hours

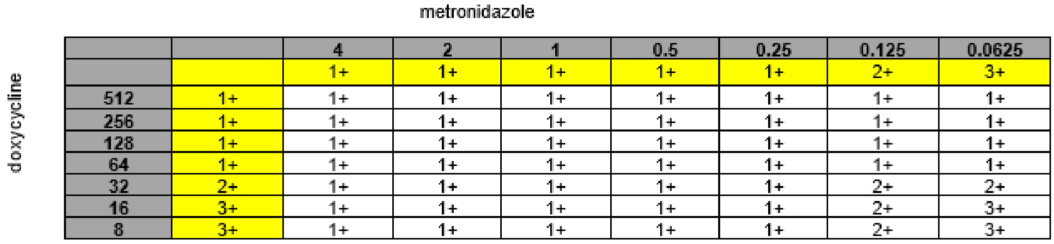
metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	2+	1+	1+	1+	1+	2+	3+	2+
16	2+	1+	1+	1+	2+	3+	2+	2+
8	1+	1+	1+	1+	2+	2+	1+	1+

Date: 30 Oct 2016 **Isolate:** 696

Reading after 48 hours



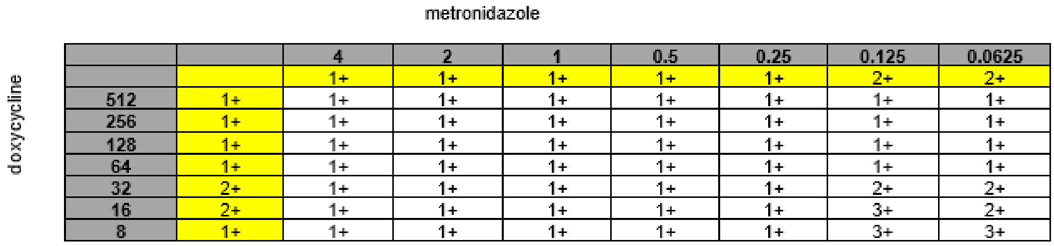
metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	2+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	1+	2+	2+
16	3+	1+	1+	1+	1+	1+	2+	3+
8	3+	1+	1+	1+	1+	1+	2+	3+

Date: 01 Nov 2016

Reading after 72 hours



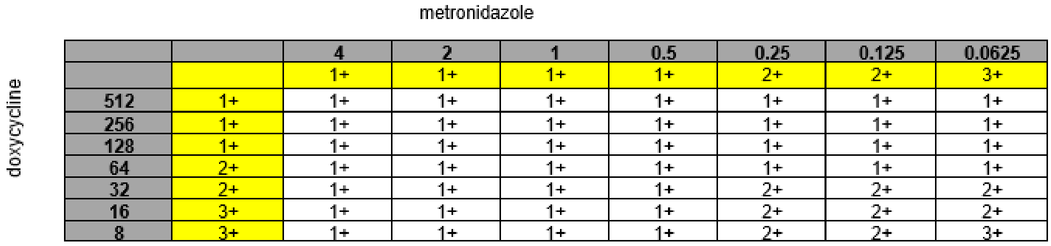
metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	1+	2+	2+
16	2+	1+	1+	1+	1+	1+	3+	2+
8	1+	1+	1+	1+	1+	1+	3+	3+

Date: 12 Oct 2016 **Isolate:** 716

Reading after 48 hours



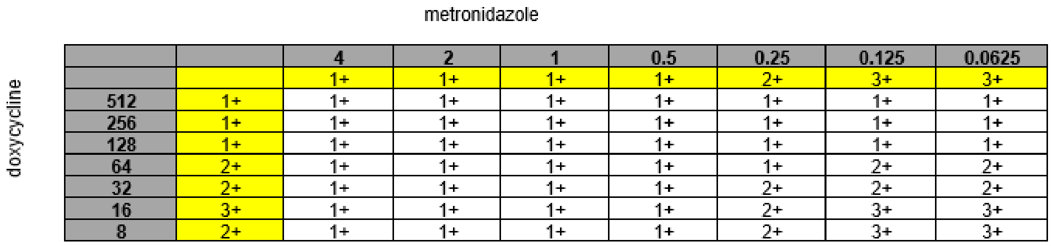
metronidazole

doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	2+	2+	2+
8	3+	1+	1+	1+	1+	2+	2+	3+

Date: 13 Oct 2016

Reading after 72 hours



metronidazole

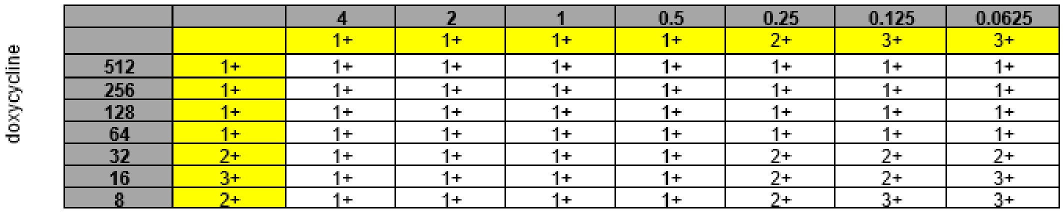
doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	1+	1+	1+	1+	1+	2+	2+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	2+	1+	1+	1+	1+	2+	3+	3+

Date: 20 Oct 2016 **Isolate:** 727

Reading after 48 hours

metronidazole



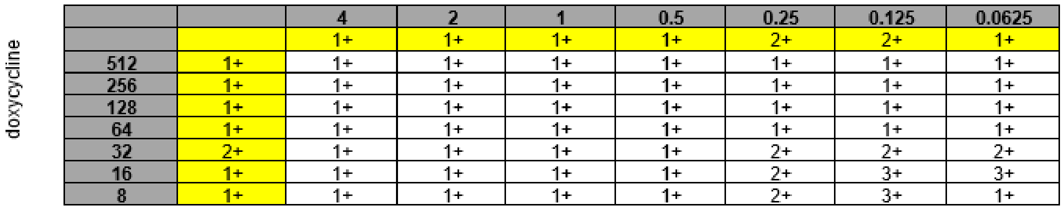
doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	3+	1+	1+	1+	1+	2+	2+	3+
8	2+	1+	1+	1+	1+	2+	3+	3+

Date: 21 Oct 2016

Reading after 72 hours

metronidazole



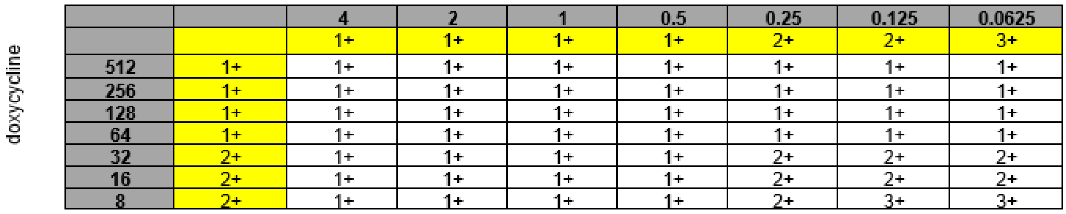
doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	1+	1+	1+	1+	1+	2+	3+	3+
8	1+	1+	1+	1+	1+	2+	3+	1+

Date: 11 Oct 2016 **Isolate:** 777

Reading after 48 hours

metronidazole



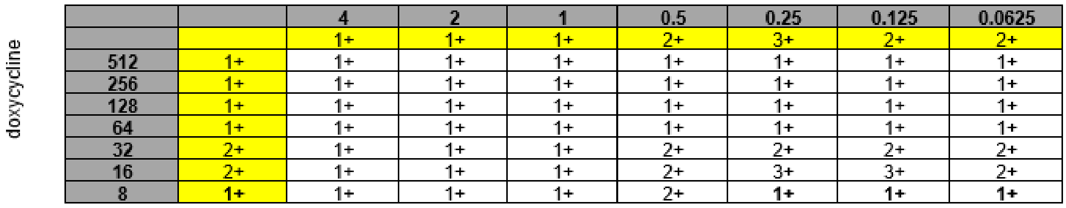
doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	1+	2+	2+	2+
16	2+	1+	1+	1+	1+	2+	2+	2+
8	2+	1+	1+	1+	1+	2+	3+	3+

Date: 12 Oct 2016

Reading after 72 hours

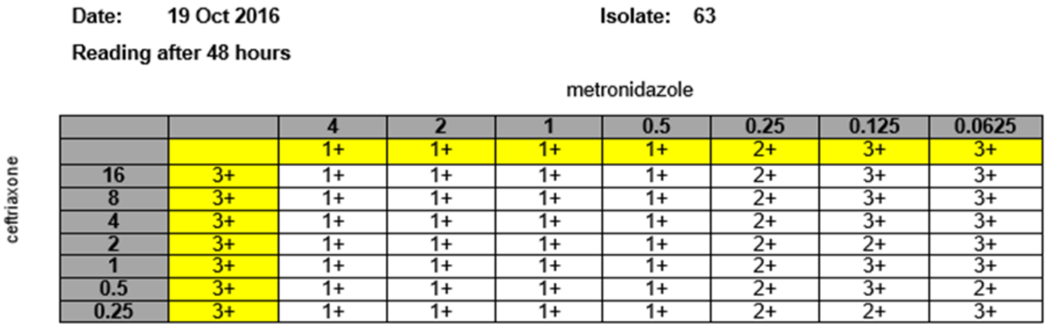
metronidazole



doxycycline

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	1+	1+	1+	2+	2+	2+	2+
16	2+	1+	1+	1+	2+	3+	3+	2+
8	1+	1+	1+	1+	2+	1+	1+	1+

1.2 Metronidazole and ceftriaxone combination

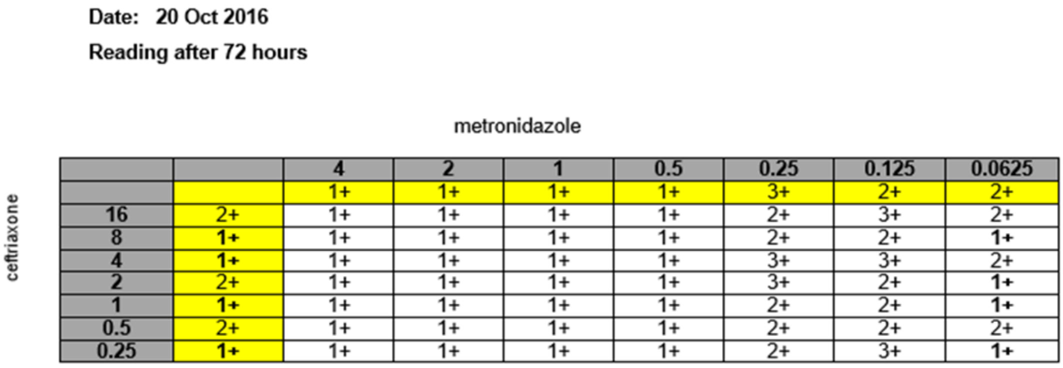

Date: 19 Oct 2016 **Isolate:** 63

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+
4	3+	1+	1+	1+	1+	2+	3+	3+
2	3+	1+	1+	1+	1+	2+	2+	3+
1	3+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	2+
0.25	3+	1+	1+	1+	1+	2+	2+	3+

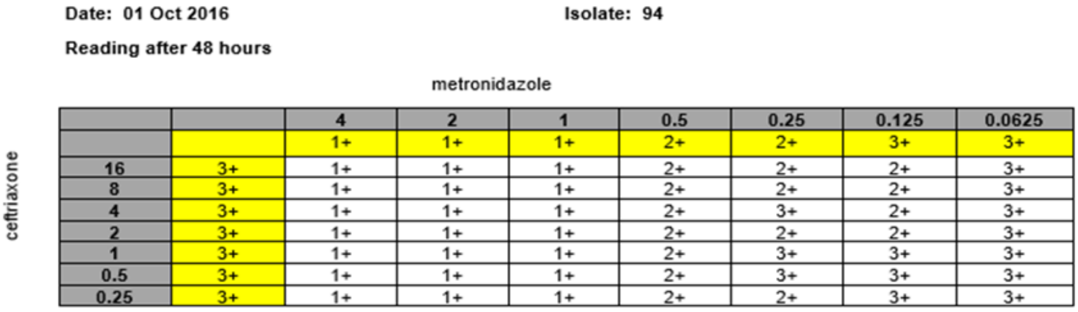

Date: 20 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	3+	2+	2+
16	2+	1+	1+	1+	1+	2+	3+	2+
8	1+	1+	1+	1+	1+	2+	2+	1+
4	1+	1+	1+	1+	1+	3+	3+	2+
2	2+	1+	1+	1+	1+	3+	2+	1+
1	1+	1+	1+	1+	1+	2+	2+	1+
0.5	2+	1+	1+	1+	1+	2+	2+	2+
0.25	1+	1+	1+	1+	1+	2+	3+	1+

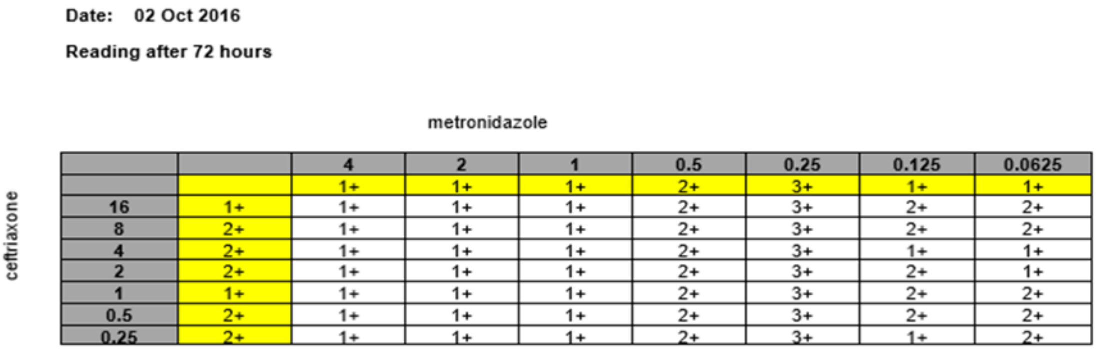

Date: 01 Oct 2016 **Isolate:** 94

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	2+	3+	3+
16	3+	1+	1+	1+	2+	2+	2+	3+
8	3+	1+	1+	1+	2+	2+	2+	3+
4	3+	1+	1+	1+	2+	3+	2+	3+
2	3+	1+	1+	1+	2+	2+	2+	3+
1	3+	1+	1+	1+	2+	3+	3+	3+
0.5	3+	1+	1+	1+	2+	3+	3+	3+
0.25	3+	1+	1+	1+	2+	2+	3+	3+


Date: 02 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	1+	1+
16	1+	1+	1+	1+	2+	3+	2+	2+
8	2+	1+	1+	1+	2+	3+	2+	2+
4	2+	1+	1+	1+	2+	3+	1+	1+
2	2+	1+	1+	1+	2+	3+	2+	1+
1	1+	1+	1+	1+	2+	3+	2+	2+
0.5	2+	1+	1+	1+	2+	3+	2+	2+
0.25	2+	1+	1+	1+	2+	3+	1+	2+

Date: 03 Oct 2016 **Isolate:** 95

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	3+	3+
16	3+	1+	1+	1+	2+	3+	3+	3+
8	3+	1+	1+	1+	2+	3+	3+	3+
4	3+	1+	1+	1+	2+	3+	2+	3+
2	3+	1+	1+	1+	2+	3+	3+	3+
1	3+	1+	1+	1+	2+	3+	2+	2+
0.5	3+	1+	1+	1+	2+	3+	2+	3+
0.25	3+	1+	1+	1+	2+	3+	3+	3+

Date: 04 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	1+	1+
16	1+	1+	1+	1+	2+	3+	2+	2+
8	2+	1+	1+	1+	2+	4+	2+	2+
4	2+	1+	1+	1+	2+	3+	2+	1+
2	1+	1+	1+	1+	2+	3+	1+	2+
1	2+	1+	1+	1+	2+	3+	1+	2+
0.5	2+	1+	1+	1+	2+	3+	2+	1+
0.25	2+	1+	1+	1+	3+	4+	2+	2+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	2+	3+
4	3+	1+	1+	1+	1+	2+	3+	3+
2	3+	1+	1+	1+	1+	2+	2+	3+
1	3+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	3+
0.25	3+	1+	1+	1+	1+	2+	3+	3+

Date: 06 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	2+	1+	1+	1+	1+	2+	3+	3+
8	1+	1+	1+	1+	1+	3+	3+	3+
4	2+	1+	1+	1+	1+	2+	3+	3+
2	1+	1+	1+	1+	1+	2+	3+	2+
1	2+	1+	1+	1+	1+	3+	3+	3+
0.5	2+	1+	1+	1+	1+	2+	3+	2+
0.25	1+	1+	1+	1+	1+	2+	3+	2+

Date: 04 Oct 2016 **Isolate:** 304

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+
4	3+	1+	1+	1+	1+	2+	2+	3+
2	3+	1+	1+	1+	1+	2+	3+	3+
1	3+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	3+
0.25	3+	1+	1+	1+	1+	2+	2+	3+

Date: 05 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
16	2+	1+	1+	1+	1+	2+	3+	3+
8	1+	1+	1+	1+	1+	2+	3+	2+
4	2+	1+	1+	1+	1+	2+	3+	3+
2	2+	1+	1+	1+	1+	2+	3+	2+
1	1+	1+	1+	1+	1+	2+	3+	2+
0.5	2+	1+	1+	1+	1+	2+	3+	2+
0.25	1+	1+	1+	1+	1+	2+	3+	3+

Date: 09 Oct 2016 **Isolate:** 652

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+
4	3+	1+	1+	1+	1+	2+	3+	3+
2	3+	1+	1+	1+	1+	2+	3+	3+
1	3+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	3+
0.25	3+	1+	1+	1+	1+	2+	3+	3+

Date: 10 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	2+	1+	1+
16	2+	1+	1+	1+	2+	2+	2+	2+
8	2+	1+	1+	1+	2+	3+	2+	2+
4	1+	1+	1+	1+	2+	2+	3+	1+
2	2+	1+	1+	1+	2+	3+	2+	1+
1	2+	1+	1+	1+	2+	3+	3+	2+
0.5	1+	1+	1+	1+	2+	3+	1+	1+
0.25	2+	1+	1+	1+	2+	3+	2+	2+

Date: 30 Oct 2016 **Isolate:** 696

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	2+	3+
16	3+	1+	1+	1+	1+	1+	2+	3+
8	3+	1+	1+	1+	1+	1+	2+	3+
4	3+	1+	1+	1+	1+	1+	2+	3+
2	3+	1+	1+	1+	1+	1+	2+	3+
1	3+	1+	1+	1+	1+	1+	2+	2+
0.5	3+	1+	1+	1+	1+	1+	2+	3+
0.25	3+	1+	1+	1+	1+	1+	2+	3+

Date: 01 Nov 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	3+	2+
16	2+	1+	1+	1+	1+	1+	2+	2+
8	1+	1+	1+	1+	1+	1+	3+	2+
4	2+	1+	1+	1+	1+	1+	2+	3+
2	1+	1+	1+	1+	1+	1+	3+	3+
1	1+	1+	1+	1+	1+	1+	3+	3+
0.5	2+	1+	1+	1+	1+	1+	3+	2+
0.25	1+	1+	1+	1+	1+	1+	3+	3+

Date: 12 Oct 2016 **Isolate:** 716

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	2+	3+
8	3+	1+	1+	1+	1+	2+	2+	3+
4	3+	1+	1+	1+	1+	2+	2+	3+
2	3+	1+	1+	1+	1+	2+	2+	3+
1	3+	1+	1+	1+	1+	2+	2+	3+
0.5	3+	1+	1+	1+	1+	2+	2+	3+
0.25	3+	1+	1+	1+	1+	2+	2+	3+

Date: 13 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	2+
8	3+	1+	1+	1+	1+	2+	2+	3+
4	3+	1+	1+	1+	1+	2+	3+	2+
2	3+	1+	1+	1+	1+	2+	3+	2+
1	2+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	2+
0.25	3+	1+	1+	1+	1+	2+	2+	3+

Date: 20 Oct 2016 **Isolate: 727**

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	2+	3+
8	3+	1+	1+	1+	1+	2+	3+	2+
4	3+	1+	1+	1+	1+	2+	2+	3+
2	3+	1+	1+	1+	1+	2+	2+	3+
1	3+	1+	1+	1+	1+	2+	3+	3+
0.5	3+	1+	1+	1+	1+	2+	3+	3+
0.25	3+	1+	1+	1+	1+	2+	2+	3+

Date: 21 Oct 2016

Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	2+
16	1+	1+	1+	1+	1+	2+	3+	1+
8	2+	1+	1+	1+	1+	2+	2+	2+
4	1+	1+	1+	1+	1+	2+	2+	1+
2	1+	1+	1+	1+	1+	2+	3+	1+
1	2+	1+	1+	1+	1+	2+	3+	2+
0.5	2+	1+	1+	1+	1+	2+	2+	1+
0.25	1+	1+	1+	1+	1+	2+	3+	1+

Date: 11 Oct 2016 **Isolate: 777**

Reading after 48 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+
4	2+	1+	1+	1+	1+	2+	2+	3+
2	3+	1+	1+	1+	1+	2+	3+	3+
1	2+	1+	1+	1+	1+	2+	3+	3+
0.5	2+	1+	1+	1+	1+	2+	3+	3+
0.25	2+	1+	1+	1+	1+	2+	3+	3+

Date: 12 Oct 2016

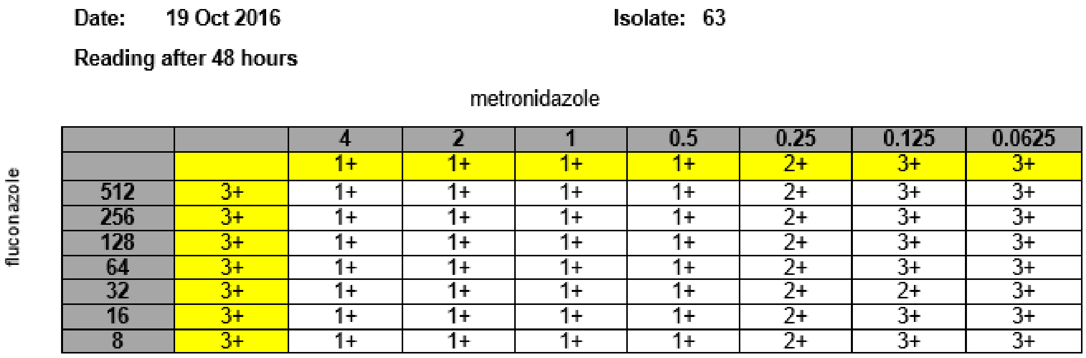
Reading after 72 hours

metronidazole

ceftriaxone

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	2+	1+
16	1+	1+	1+	1+	2+	3+	1+	1+
8	2+	1+	1+	1+	2+	3+	2+	2+
4	1+	1+	1+	1+	2+	3+	1+	2+
2	1+	1+	1+	1+	2+	3+	2+	1+
1	2+	1+	1+	1+	2+	3+	2+	1+
0.5	2+	1+	1+	1+	2+	3+	2+	2+
0.25	1+	1+	1+	1+	2+	2+	1+	1+

1.3 Metronidazole and fluconazole combination



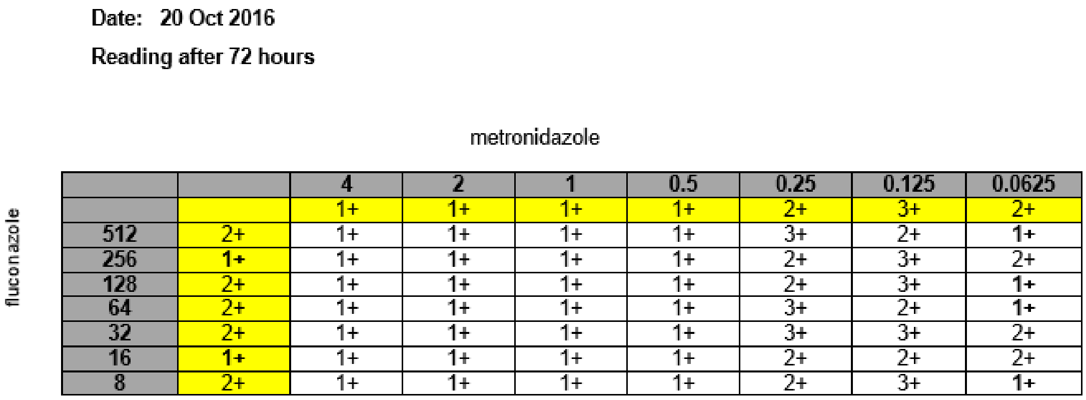
Date: 19 Oct 2016 **Isolate:** 63

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	3+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	3+	3+
32	3+	1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+



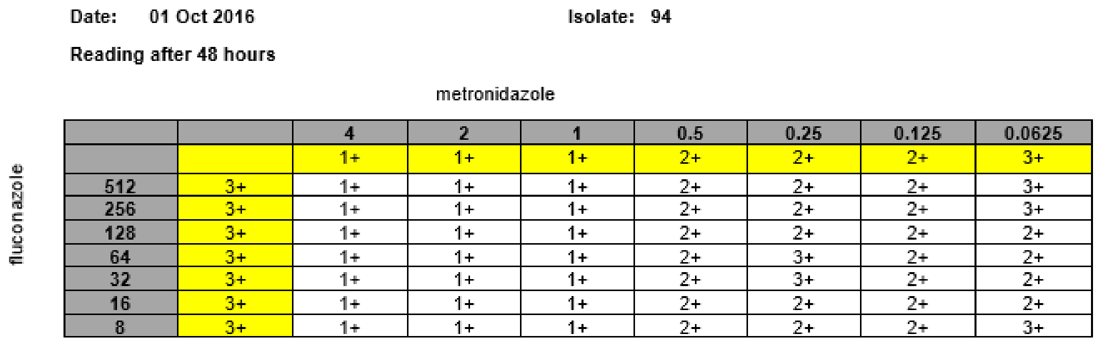
Date: 20 Oct 2016

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	2+	1+	1+	1+	1+	3+	2+	1+
256	1+	1+	1+	1+	1+	2+	3+	2+
128	2+	1+	1+	1+	1+	2+	3+	1+
64	2+	1+	1+	1+	1+	3+	2+	1+
32	2+	1+	1+	1+	1+	3+	3+	2+
16	1+	1+	1+	1+	1+	2+	2+	2+
8	2+	1+	1+	1+	1+	2+	3+	1+



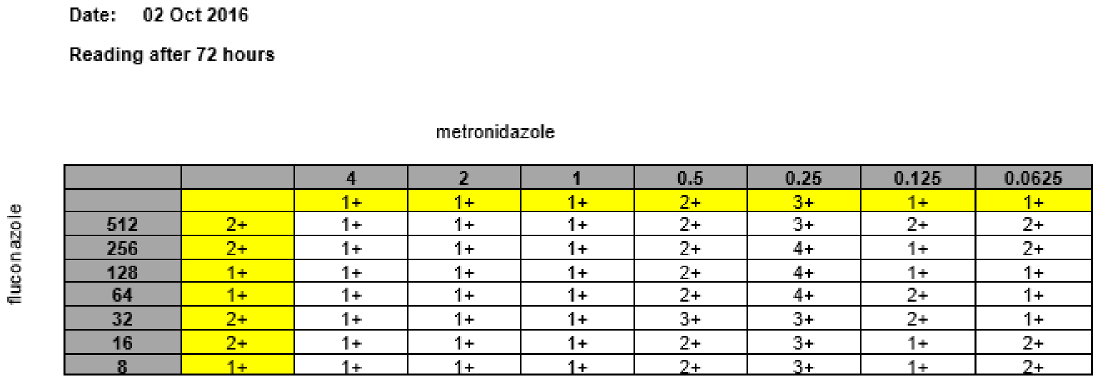
Date: 01 Oct 2016 **Isolate:** 94

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	2+	2+	3+
512	3+	1+	1+	1+	2+	2+	2+	3+
256	3+	1+	1+	1+	2+	2+	2+	3+
128	3+	1+	1+	1+	2+	2+	2+	2+
64	3+	1+	1+	1+	2+	3+	2+	2+
32	3+	1+	1+	1+	2+	3+	2+	2+
16	3+	1+	1+	1+	2+	2+	2+	2+
8	3+	1+	1+	1+	2+	2+	2+	3+



Date: 02 Oct 2016

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	1+	1+
512	2+	1+	1+	1+	2+	3+	2+	2+
256	2+	1+	1+	1+	2+	4+	1+	2+
128	1+	1+	1+	1+	2+	4+	1+	1+
64	1+	1+	1+	1+	2+	4+	2+	1+
32	2+	1+	1+	1+	3+	3+	2+	1+
16	2+	1+	1+	1+	2+	3+	1+	2+
8	1+	1+	1+	1+	2+	3+	1+	2+

Date: 03 Oct 2016 **Isolate:** 95

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	3+	3+
512	3+	1+	1+	1+	2+	2+	3+	2+
256	3+	1+	1+	1+	2+	3+	3+	3+
128	3+	1+	1+	1+	2+	3+	3+	3+
64	3+	1+	1+	1+	2+	3+	3+	3+
32	3+	1+	1+	1+	2+	3+	3+	2+
16	3+	1+	1+	1+	2+	3+	3+	3+
8	3+	1+	1+	1+	2+	3+	3+	3+

Date: 04 Oct 2016

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	2+	1+
512	1+	1+	1+	1+	2+	3+	2+	1+
256	2+	1+	1+	1+	2+	3+	1+	2+
128	1+	1+	1+	1+	2+	3+	2+	1+
64	2+	1+	1+	1+	2+	3+	2+	1+
32	2+	1+	1+	1+	2+	4+	2+	2+
16	2+	1+	1+	1+	2+	3+	1+	2+
8	1+	1+	1+	1+	2+	3+	2+	1+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	2+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	3+	3+
32	3+	1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

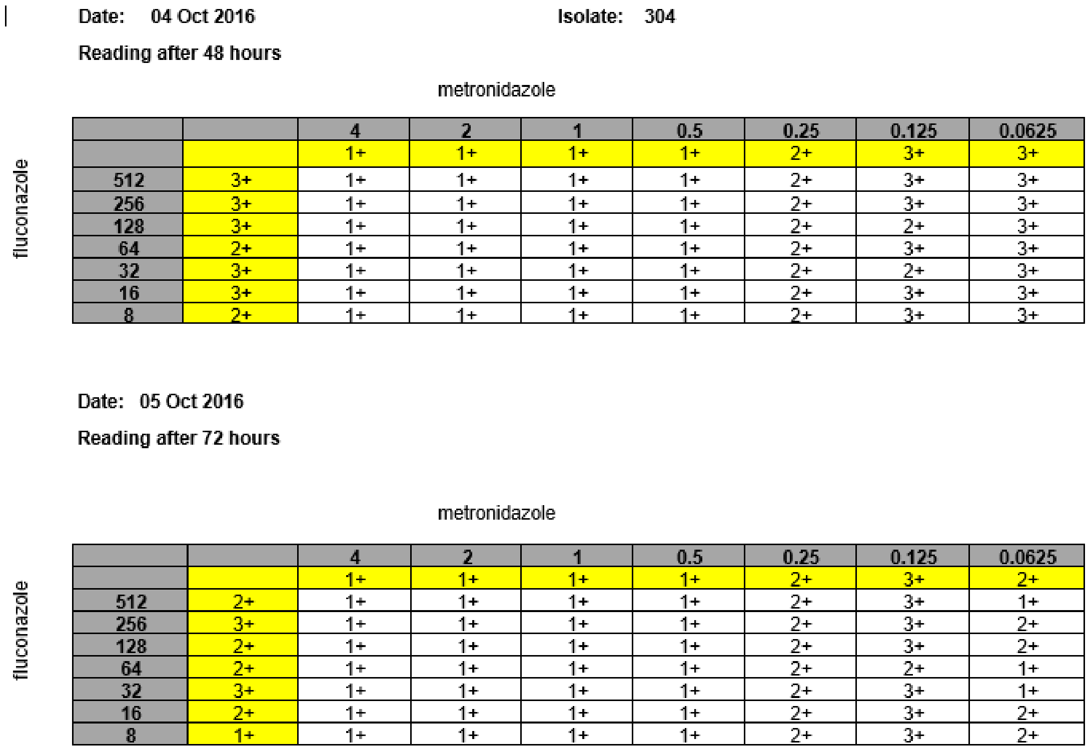
Date: 06 Oct 2016

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	2+	1+	1+	1+	1+	2+	3+	2+
256	2+	1+	1+	1+	1+	2+	2+	3+
128	1+	1+	1+	1+	1+	2+	3+	2+
64	2+	1+	1+	1+	1+	2+	2+	2+
32	2+	1+	1+	1+	1+	2+	3+	2+
16	2+	1+	1+	1+	1+	2+	3+	3+
8	1+	1+	1+	1+	1+	2+	2+	2+



Date: 04 Oct 2016 Isolate: 304

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	3+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	2+	3+
64	2+	1+	1+	1+	1+	2+	3+	3+
32	3+	1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	2+	1+	1+	1+	1+	2+	3+	3+

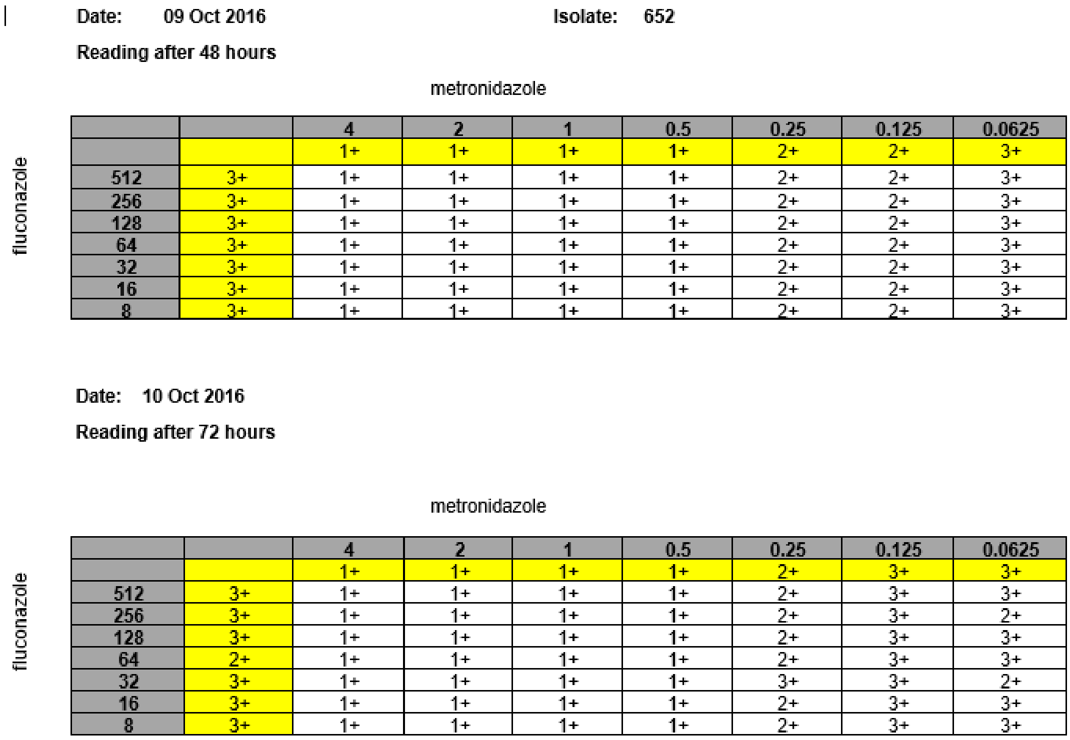
Date: 05 Oct 2016

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	2+	1+	1+	1+	1+	2+	3+	1+
256	3+	1+	1+	1+	1+	2+	3+	2+
128	2+	1+	1+	1+	1+	2+	3+	2+
64	2+	1+	1+	1+	1+	2+	2+	1+
32	3+	1+	1+	1+	1+	2+	3+	1+
16	2+	1+	1+	1+	1+	2+	3+	2+
8	1+	1+	1+	1+	1+	2+	3+	2+



Date: 09 Oct 2016 Isolate: 652

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	2+	3+
512	3+	1+	1+	1+	1+	2+	2+	3+
256	3+	1+	1+	1+	1+	2+	2+	3+
128	3+	1+	1+	1+	1+	2+	2+	3+
64	3+	1+	1+	1+	1+	2+	2+	3+
32	3+	1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	2+	3+
8	3+	1+	1+	1+	1+	2+	2+	3+

Date: 10 Oct 2016

Reading after 72 hours

metronidazole

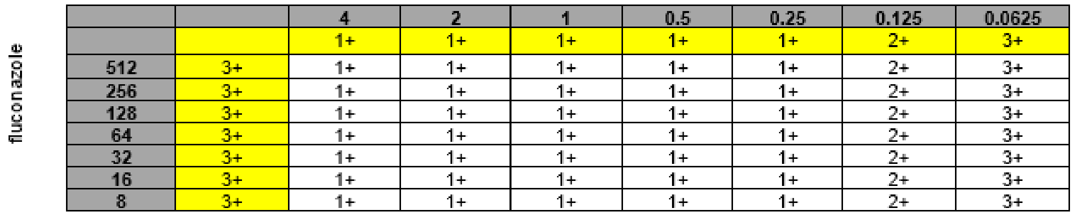
fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	3+	3+
256	3+	1+	1+	1+	1+	2+	3+	2+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	2+	1+	1+	1+	1+	2+	3+	3+
32	3+	1+	1+	1+	1+	3+	3+	2+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

Date: 30 Oct 2016 **Isolate:** 696

Reading after 48 hours

metronidazole



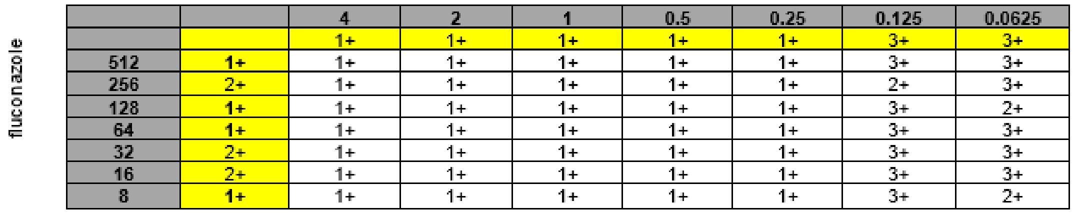
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	2+	3+
512	3+	1+	1+	1+	1+	1+	2+	3+
256	3+	1+	1+	1+	1+	1+	2+	3+
128	3+	1+	1+	1+	1+	1+	2+	3+
64	3+	1+	1+	1+	1+	1+	2+	3+
32	3+	1+	1+	1+	1+	1+	2+	3+
16	3+	1+	1+	1+	1+	1+	2+	3+
8	3+	1+	1+	1+	1+	1+	2+	3+

fluconazole

Date: 01 Nov 2016

Reading after 72 hours

metronidazole



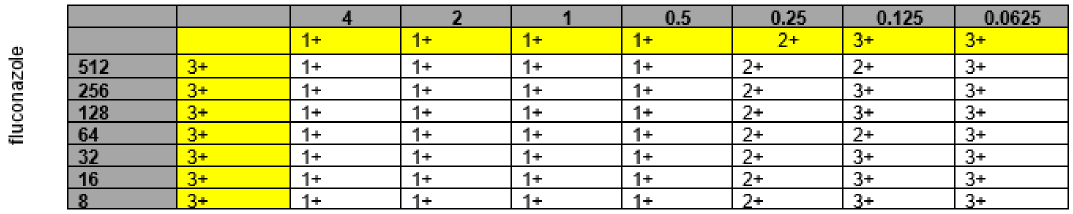
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	1+	3+	3+
512	1+	1+	1+	1+	1+	1+	3+	3+
256	2+	1+	1+	1+	1+	1+	2+	3+
128	1+	1+	1+	1+	1+	1+	3+	2+
64	1+	1+	1+	1+	1+	1+	3+	3+
32	2+	1+	1+	1+	1+	1+	3+	3+
16	2+	1+	1+	1+	1+	1+	3+	3+
8	1+	1+	1+	1+	1+	1+	3+	2+

fluconazole

Date: 12 Oct 2016 **Isolate:** 716

Reading after 48 hours

metronidazole



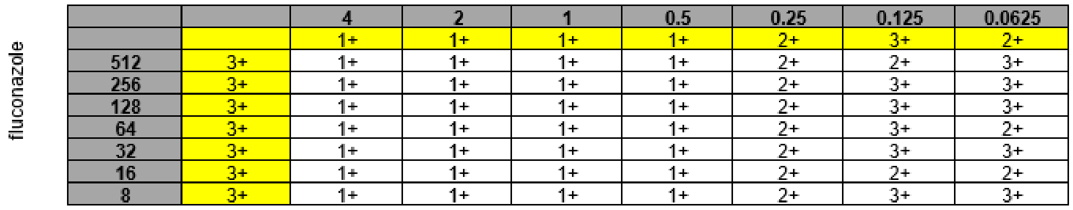
		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	2+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	2+	3+
32	3+	1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

fluconazole

Date: 13 Oct 2016

Reading after 72 hours

metronidazole



		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	2+
512	3+	1+	1+	1+	1+	2+	2+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	3+	2+
32	3+	1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	2+	2+
8	3+	1+	1+	1+	1+	2+	3+	3+

fluconazole

Date: **20 Oct 2016** **Isolate:** **727**

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	2+	3+
256	3+	1+	1+	1+	1+	2+	3+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	3+	3+
32	3+	1+	1+	1+	1+	2+	2+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	3+	3+

Date: **21 Oct 2016**

Reading after 72 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	1+
512	2+	1+	1+	1+	1+	2+	3+	1+
256	1+	1+	1+	1+	1+	2+	2+	2+
128	2+	1+	1+	1+	1+	2+	2+	2+
64	1+	1+	1+	1+	1+	2+	1+	1+
32	1+	1+	1+	1+	1+	2+	2+	1+
16	2+	1+	1+	1+	1+	2+	2+	2+
8	1+	1+	1+	1+	1+	2+	1+	1+

Date: **11 Oct 2016** **Isolate:** **777**

Reading after 48 hours

metronidazole

fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	1+	2+	3+	3+
512	3+	1+	1+	1+	1+	2+	3+	3+
256	3+	1+	1+	1+	1+	2+	2+	3+
128	3+	1+	1+	1+	1+	2+	3+	3+
64	3+	1+	1+	1+	1+	2+	2+	3+
32	3+	1+	1+	1+	1+	2+	3+	3+
16	3+	1+	1+	1+	1+	2+	3+	3+
8	3+	1+	1+	1+	1+	2+	2+	3+

Date: **12 Oct 2016**

Reading after 72 hours

metronidazole

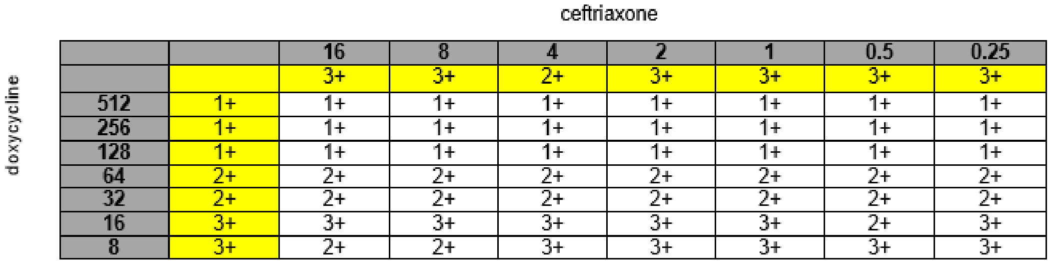
fluconazole

		4	2	1	0.5	0.25	0.125	0.0625
		1+	1+	1+	2+	3+	3+	2+
512	2+	1+	1+	1+	2+	2+	3+	1+
256	2+	1+	1+	1+	2+	3+	3+	1+
128	1+	1+	1+	1+	2+	2+	2+	2+
64	2+	1+	1+	1+	2+	2+	3+	1+
32	1+	1+	1+	1+	2+	3+	3+	2+
16	2+	1+	1+	1+	2+	3+	3+	1+
8	2+	1+	1+	1+	2+	3+	3+	2+

1.4 Doxycycline and ceftriaxone combination

Date: 19 Oct 2016 **Isolate:** 63

Reading after 48 hours



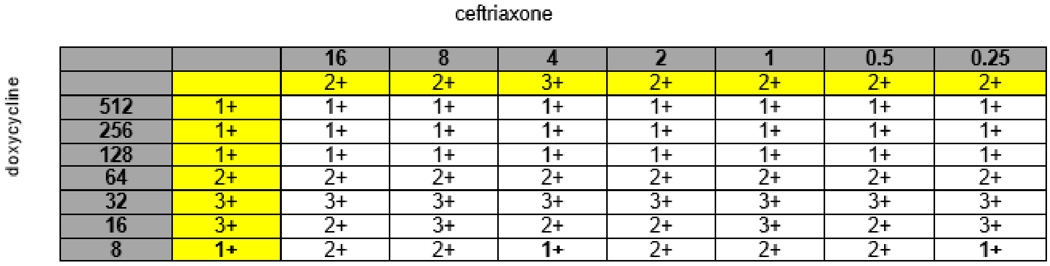
ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	2+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	3+	3+	3+	3+	3+	2+	3+
8	3+	2+	2+	3+	3+	3+	3+	3+

Date: 20 Oct 2016

Reading after 72 hours



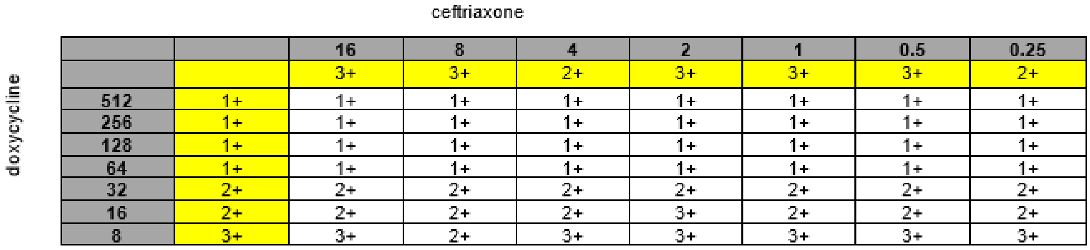
ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	2+	3+	2+	2+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	3+	2+	3+	2+	2+	3+	2+	3+
8	1+	2+	2+	1+	2+	2+	2+	1+

Date: 01 Oct 2016 **Isolate:** 94

Reading after 48 hours



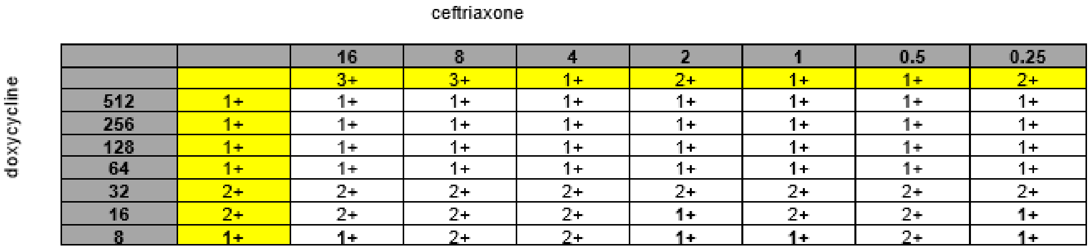
ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	2+	3+	3+	3+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	3+	2+	2+	2+
8	3+	3+	2+	3+	3+	3+	3+	3+

Date: 02 Oct 2016

Reading after 72 hours



ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	1+	2+	1+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	1+	2+	2+	1+
8	1+	1+	2+	2+	1+	1+	2+	1+

Date: 03 Oct 2016 **Isolate:** 95

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	2+	3+	3+	3+	3+	3+	3+	3+

Date: 04 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	1+	2+	2+	1+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	2+	3+	3+	2+	2+	3+
8	1+	1+	2+	2+	1+	2+	1+	2+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 06 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	2+	2+	2+	2+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	2+	3+	3+	2+	3+	3+	3+	3+
8	2+	2+	2+	2+	2+	2+	2+	2+

Date: 04 Oct 2016 **Isolate:** 304

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	3+	3+	3+	2+	3+	2+	3+	3+

Date: 05 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	2+	3+	2+	2+	2+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	2+	2+	2+	3+
16	1+	2+	3+	2+	3+	2+	3+	2+
8	1+	1+	2+	1+	2+	2+	2+	1+

Date: 09 Oct 2016 **Isolate:** 652

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	3+	3+	3+	2+	3+	3+	3+	3+

Date: 10 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	2+	1+	2+	2+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	2+	3+	3+
16	2+	2+	3+	2+	3+	3+	2+	3+
8	1+	1+	2+	2+	1+	2+	2+	1+

Date: 30 Oct 2016 **Isolate:** 696

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	2+	2+	2+	2+	2+	2+	2+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 01 Nov 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	2+	1+	2+	2+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	3+	2+	3+	2+	2+	2+	3+
8	1+	2+	1+	2+	2+	2+	1+	1+

Date: 12 Oct 2016 **Isolate:** 716

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	2+	3+	3+	3+	2+	3+	2+
16	3+	3+	2+	3+	3+	3+	2+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 13 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	2+	3+	3+	3+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	3+	3+	3+	2+	3+	3+	2+	3+
8	2+	3+	2+	3+	3+	2+	3+	3+

Date: 20 Oct 2016 **Isolate:** 727

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	3+	3+	3+	2+	3+	2+
8	2+	3+	3+	3+	2+	3+	3+	3+

Date: 21 Oct 2016

Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	1+	2+	2+	1+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	3+	3+	3+	2+	2+	3+	2+
8	2+	2+	3+	2+	2+	1+	2+	1+

Date: 11 Oct 2016 **Isolate:** 777

Reading after 48 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	3+	3+	3+	3+	3+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	2+	3+	3+	2+	2+	2+	2+	3+

Date: 12 Oct 2016

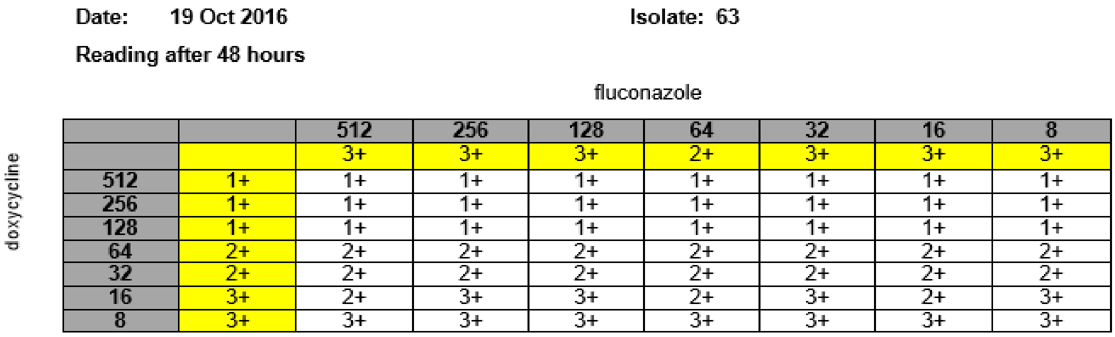
Reading after 72 hours

ceftriaxone

doxycycline

		16	8	4	2	1	0.5	0.25
		2+	1+	1+	2+	2+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	1+	2+	1+	2+	2+	2+	2+
8	1+	1+	2+	1+	2+	2+	2+	1+

1.5 Doxycycline and fluconazole combination



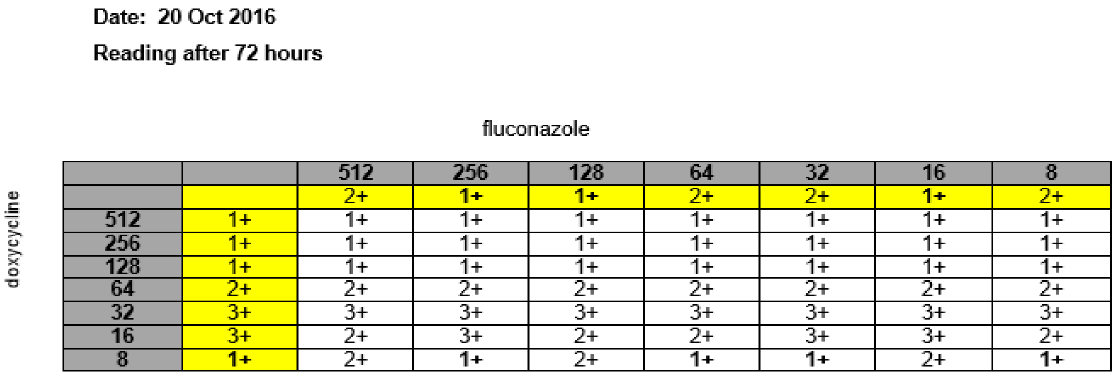
Date: 19 Oct 2016 **Isolate:** 63

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	2+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	3+	3+	2+	3+	2+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+



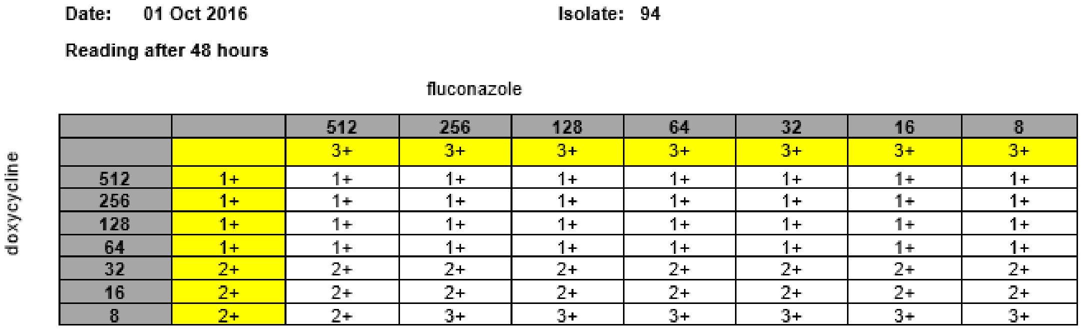
Date: 20 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	1+	1+	2+	2+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	3+	2+	3+	2+	2+	3+	3+	2+
8	1+	2+	1+	2+	1+	1+	2+	1+



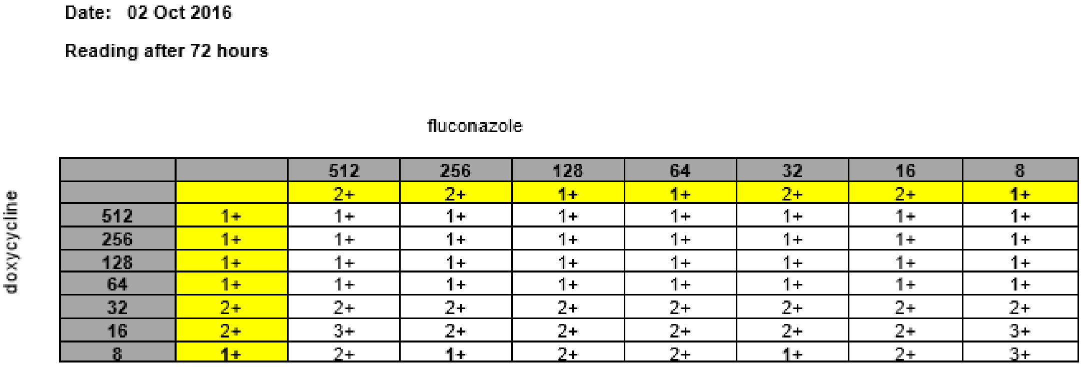
Date: 01 Oct 2016 **Isolate:** 94

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	2+	2+	3+	3+	3+	3+	3+	3+



Date: 02 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	2+	1+	1+	2+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	3+	2+	2+	2+	2+	2+	3+
8	1+	2+	1+	2+	2+	1+	2+	3+

Date: 03 Oct 2016 **Isolate:** 95

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	2+	2+	3+	2+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	2+	3+	3+	2+	3+	2+	2+	2+

Date: 04 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	1+	2+	2+	1+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	3+	2+	2+	2+	2+	3+	2+
8	1+	1+	2+	1+	2+	2+	2+	1+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	3+	2+	3+	2+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 05 Oct 2016

Reading after 72 hours

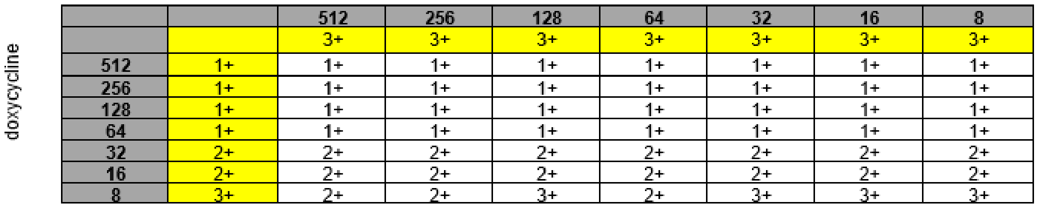
fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	2+	1+	2+	2+	2+	1+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	2+	1+	2+	1+	2+	2+	2+	1+

Date: 04 Oct 2016 **Isolate:** 304

Reading after 48 hours



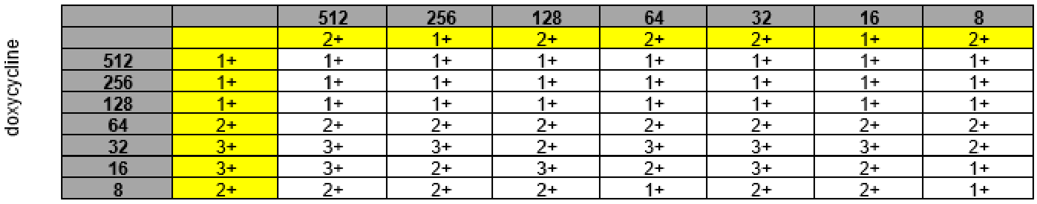
fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	3+	2+	2+	3+	2+	3+	3+	3+

Date: 05 Oct 2016

Reading after 72 hours



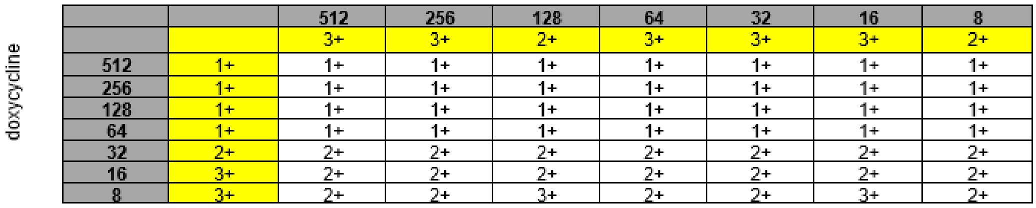
fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	1+	2+	2+	2+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	2+	3+	3+	3+	2+
16	3+	3+	2+	3+	2+	3+	2+	1+
8	2+	2+	2+	2+	1+	2+	2+	1+

Date: 09 Oct 2016 **Isolate:** 652

Reading after 48 hours



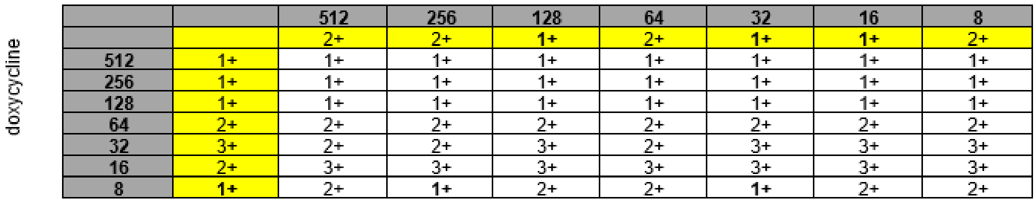
fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	2+	3+	3+	3+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	2+	2+	2+	2+	2+	2+
8	3+	2+	2+	3+	2+	2+	3+	2+

Date: 10 Oct 2016

Reading after 72 hours



fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	2+	1+	2+	1+	1+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	2+	2+	3+	2+	3+	3+	3+
16	2+	3+	3+	3+	3+	3+	3+	3+
8	1+	2+	1+	2+	2+	1+	2+	2+

Date: 30 Oct 2016 **Isolate:** 696

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	2+	2+	2+	2+	2+	2+	2+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 01 Nov 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	2+	2+	2+	2+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	3+	2+	3+	2+	2+	2+	3+
8	1+	2+	1+	1+	2+	2+	2+	1+

Date: 12 Oct 2016 **Isolate:** 716

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	2+	3+	3+	3+	2+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 13 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	2+	3+	2+	2+	2+	3+	3+	3+

Date: 20 Oct 2016 **Isolate: 727**

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	2+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 21 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	1+	2+	2+	1+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	3+	2+	2+	3+	2+	2+
8	1+	2+	1+	1+	1+	2+	1+	2+

Date: 11 Oct 2016 **Isolate: 777**

Reading after 48 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		3+	3+	3+	3+	3+	3+	3+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	3+	3+	3+	2+	3+	3+	3+	2+

Date: 12 Oct 2016

Reading after 72 hours

fluconazole

doxycycline

		512	256	128	64	32	16	8
		2+	2+	1+	2+	2+	2+	2+
512	1+	1+	1+	1+	1+	1+	1+	1+
256	1+	1+	1+	1+	1+	1+	1+	1+
128	1+	1+	1+	1+	1+	1+	1+	1+
64	1+	1+	1+	1+	1+	1+	1+	1+
32	2+	2+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	2+	2+	2+	2+	2+
8	1+	2+	1+	2+	2+	1+	2+	2+

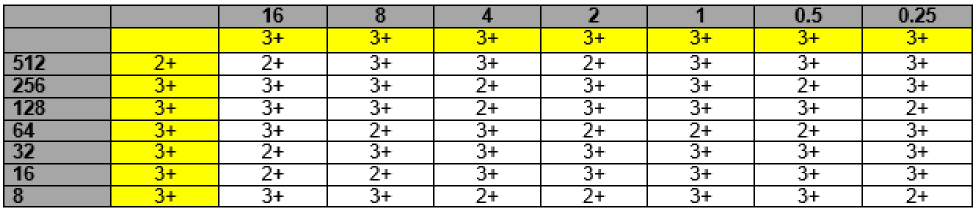
1.6 Ceftriaxone and fluconazole combination

Date: 10 Oct 2016 **Isolate:** 63

Reading after 48 hours

ceftriaxone

fluconazole



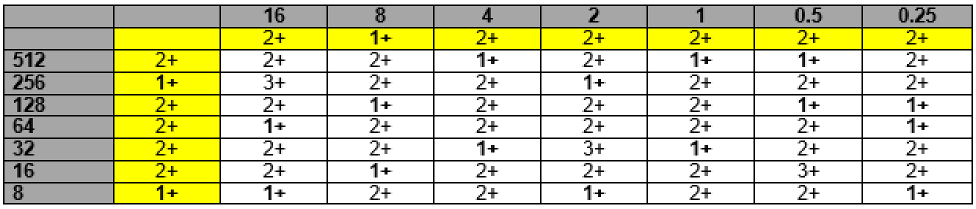
		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	2+	2+	3+	3+	2+	3+	3+	3+
256	3+	3+	3+	2+	3+	3+	2+	3+
128	3+	3+	3+	2+	3+	3+	3+	2+
64	3+	3+	2+	3+	2+	2+	2+	3+
32	3+	2+	3+	3+	3+	3+	3+	3+
16	3+	2+	2+	3+	3+	3+	3+	3+
8	3+	3+	3+	2+	2+	3+	3+	2+

Date: 20 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole



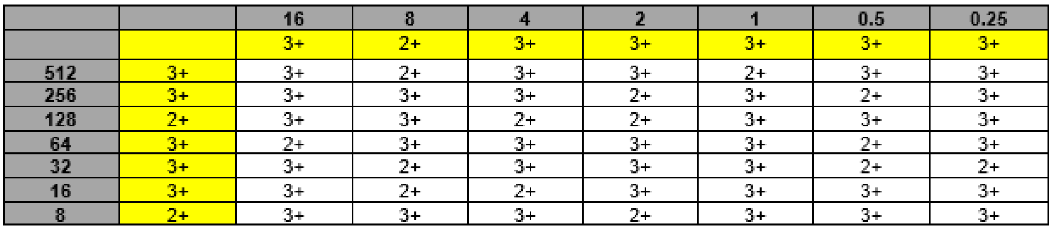
		16	8	4	2	1	0.5	0.25
		2+	1+	2+	2+	2+	2+	2+
512	2+	2+	2+	1+	2+	1+	1+	2+
256	1+	3+	2+	2+	1+	2+	2+	2+
128	2+	2+	1+	2+	2+	2+	1+	1+
64	2+	1+	2+	2+	2+	2+	2+	1+
32	2+	2+	2+	1+	3+	1+	2+	2+
16	2+	2+	1+	2+	2+	2+	3+	2+
8	1+	1+	2+	2+	1+	2+	2+	1+

Date: 01 Oct 2016 **Isolate:** 94

Reading after 48 hours

ceftriaxone

fluconazole



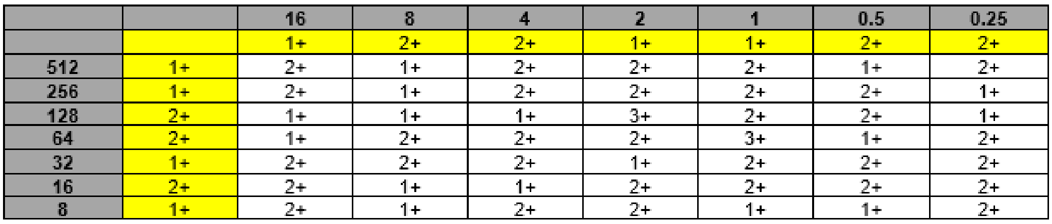
		16	8	4	2	1	0.5	0.25
		3+	2+	3+	3+	3+	3+	3+
512	3+	3+	2+	3+	3+	2+	3+	3+
256	3+	3+	3+	3+	2+	3+	2+	3+
128	2+	3+	3+	2+	2+	3+	3+	3+
64	3+	2+	3+	3+	3+	3+	2+	3+
32	3+	3+	2+	3+	3+	3+	2+	2+
16	3+	3+	2+	2+	3+	3+	3+	3+
8	2+	3+	3+	3+	2+	3+	3+	3+

Date: 02 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole



		16	8	4	2	1	0.5	0.25
		1+	2+	2+	1+	1+	2+	2+
512	1+	2+	1+	2+	2+	2+	1+	2+
256	1+	2+	1+	2+	2+	2+	2+	1+
128	2+	1+	1+	1+	3+	2+	2+	1+
64	2+	1+	2+	2+	2+	3+	1+	2+
32	1+	2+	2+	2+	1+	2+	2+	2+
16	2+	2+	1+	1+	2+	2+	2+	2+
8	1+	2+	1+	2+	2+	1+	1+	2+

Date: 03 Oct 2016 **Isolate:** 95

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	2+	3+	3+	3+	2+	3+
512	3+	2+	3+	2+	3+	3+	2+	3+
256	3+	3+	2+	3+	2+	2+	3+	2+
128	3+	3+	2+	3+	3+	2+	2+	2+
64	3+	2+	3+	2+	2+	3+	3+	2+
32	3+	3+	2+	3+	2+	2+	2+	3+
16	3+	2+	3+	2+	2+	3+	3+	3+
8	3+	2+	3+	2+	3+	3+	2+	2+

Date: 04 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		2+	2+	3+	1+	2+	2+	2+
512	2+	1+	2+	2+	2+	2+	2+	1+
256	1+	2+	1+	2+	1+	1+	1+	2+
128	2+	2+	2+	3+	2+	2+	2+	2+
64	1+	2+	1+	2+	2+	1+	2+	3+
32	2+	1+	1+	1+	2+	2+	1+	2+
16	2+	2+	2+	2+	3+	2+	2+	2+
8	1+	1+	2+	3+	2+	1+	1+	3+

Date: 05 Oct 2016 **Isolate:** 264

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	3+	3+	3+	3+	2+	3+	2+	2+
256	3+	2+	3+	3+	3+	3+	3+	3+
128	3+	3+	2+	2+	3+	2+	3+	3+
64	3+	3+	3+	3+	2+	3+	2+	2+
32	3+	3+	3+	3+	3+	2+	3+	3+
16	3+	3+	2+	2+	3+	3+	3+	3+
8	3+	2+	3+	3+	3+	3+	2+	3+

Date: 06 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		2+	2+	1+	2+	2+	1+	2+
512	2+	2+	2+	2+	2+	1+	2+	2+
256	2+	2+	1+	2+	2+	2+	2+	1+
128	1+	2+	2+	2+	1+	2+	1+	2+
64	2+	1+	2+	1+	2+	2+	2+	2+
32	2+	2+	1+	2+	2+	1+	2+	1+
16	2+	2+	1+	2+	1+	2+	1+	2+
8	1+	2+	2+	2+	2+	2+	1+	2+

Date: 04 Oct 2016 **Isolate:** 304

Reading after 48 hours

ceftriaxone

fluconazole

		16	**8**	**4**	**2**	**1**	**0.5**	**0.25**
		3+	3+	3+	3+	2+	3+	3+
512	3+	3+	3+	2+	2+	3+	3+	2+
256	3+	2+	2+	3+	2+	3+	3+	3+
128	3+	3+	3+	2+	3+	2+	2+	2+
64	3+	3+	2+	2+	2+	3+	2+	3+
32	3+	2+	3+	3+	2+	2+	3+	3+
16	3+	3+	3+	3+	3+	3+	2+	2+
8	3+	3+	2+	3+	2+	3+	3+	3+

Date: 05 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	**8**	**4**	**2**	**1**	**0.5**	**0.25**
		1+	2+	2+	1+	2+	2+	2+
512	2+	2+	2+	1+	2+	2+	1+	1+
256	1+	2+	3+	2+	2+	1+	2+	2+
128	2+	1+	2+	2+	3+	2+	3+	3+
64	2+	2+	3+	1+	2+	2+	2+	2+
32	1+	3+	2+	1+	2+	1+	2+	2+
16	2+	2+	1+	2+	1+	2+	1+	3+
8	1+	1+	2+	2+	2+	1+	2+	2+

Date: 09 Oct 2016 **Isolate:** 652

Reading after 48 hours

ceftriaxone

fluconazole

		16	**8**	**4**	**2**	**1**	**0.5**	**0.25**
		3+	3+	3+	3+	3+	3+	3+
512	3+	3+	3+	2+	3+	2+	3+	3+
256	3+	3+	2+	3+	2+	3+	3+	3+
128	3+	2+	3+	3+	3+	3+	3+	2+
64	3+	3+	2+	2+	3+	2+	3+	3+
32	3+	3+	2+	2+	3+	3+	2+	3+
16	3+	2+	3+	3+	2+	3+	3+	2+
8	3+	2+	3+	3+	3+	2+	3+	3+

Date: 10 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	**8**	**4**	**2**	**1**	**0.5**	**0.25**
		2+	2+	2+	2+	1+	2+	1+
512	2+	2+	2+	3+	2+	2+	2+	2+
256	2+	2+	1+	2+	3+	2+	3+	2+
128	1+	2+	2+	2+	2+	2+	2+	2+
64	2+	2+	2+	2+	3+	1+	2+	2+
32	2+	1+	2+	3+	2+	2+	2+	2+
16	1+	2+	2+	2+	1+	2+	2+	1+
8	2+	3+	2+	2+	2+	2+	1+	2+

Date: 30 Oct 2016 **Isolate: 696**

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	3+	3+	3+	3+	3+	2+	3+	3+
256	3+	3+	2+	3+	3+	3+	3+	3+
128	3+	2+	3+	3+	3+	3+	3+	3+
64	3+	3+	3+	3+	3+	3+	3+	3+
32	3+	3+	3+	3+	3+	3+	3+	3+
16	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+

Date: 01 Nov 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		2+	1+	2+	1+	2+	2+	1+
512	2+	1+	2+	2+	2+	1+	1+	2+
256	1+	2+	1+	1+	1+	2+	2+	1+
128	2+	2+	2+	1+	2+	1+	1+	1+
64	2+	1+	1+	2+	2+	1+	2+	1+
32	2+	2+	1+	2+	1+	2+	1+	2+
16	1+	2+	2+	2+	1+	2+	1+	1+
8	1+	2+	2+	1+	2+	1+	2+	2+

Date: 12 Oct 2016 **Isolate: 716**

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	2+	3+	3+	3+	3+	2+
512	3+	2+	3+	3+	3+	2+	3+	2+
256	3+	3+	3+	2+	2+	3+	2+	3+
128	3+	2+	3+	2+	3+	2+	3+	3+
64	3+	2+	2+	3+	3+	2+	3+	2+
32	3+	3+	3+	3+	2+	3+	2+	3+
16	3+	3+	2+	2+	3+	3+	3+	2+
8	3+	2+	3+	3+	2+	2+	2+	3+

Date: 13 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	3+	2+	3+	3+	2+	3+	3+	3+
256	2+	3+	2+	3+	3+	2+	2+	3+
128	3+	3+	3+	3+	3+	3+	3+	3+
64	2+	3+	3+	2+	3+	3+	3+	3+
32	2+	3+	3+	3+	3+	2+	3+	2+
16	3+	2+	3+	3+	2+	3+	3+	3+
8	2+	3+	2+	3+	3+	3+	3+	3+

Date: 20 Oct 2016 **Isolate:** 727

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	2+	3+	3+	3+	3+	2+
512	3+	2+	3+	2+	3+	3+	3+	3+
256	3+	3+	3+	3+	3+	2+	3+	3+
128	3+	3+	2+	3+	3+	3+	3+	2+
64	3+	2+	3+	2+	3+	3+	2+	3+
32	3+	2+	2+	3+	2+	3+	3+	3+
16	3+	3+	3+	2+	3+	3+	2+	2+
8	3+	3+	2+	3+	3+	2+	3+	3+

Date: 21 Oct 2016

Reading after 72 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		1+	2+	2+	2+	2+	2+	1+
512	2+	2+	1+	2+	2+	3+	1+	2+
256	1+	2+	2+	1+	1+	2+	2+	2+
128	2+	1+	2+	2+	2+	2+	2+	2+
64	1+	2+	1+	2+	3+	2+	1+	2+
32	1+	2+	1+	2+	2+	2+	2+	1+
16	2+	2+	2+	2+	2+	3+	2+	2+
8	2+	1+	2+	3+	2+	2+	2+	2+

Date: 11 Oct 2016 **Isolate:** 777

Reading after 48 hours

ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		3+	3+	3+	3+	3+	3+	3+
512	3+	2+	3+	3+	3+	3+	3+	3+
256	3+	3+	3+	3+	3+	3+	2+	2+
128	2+	3+	2+	3+	2+	2+	3+	3+
64	3+	3+	2+	3+	3+	2+	3+	3+
32	3+	3+	3+	2+	2+	3+	2+	3+
16	3+	2+	3+	3+	3+	2+	3+	2+
8	3+	3+	3+	2+	3+	2+	3+	2+

Date: 12 Oct 2016

Reading after 72 hours

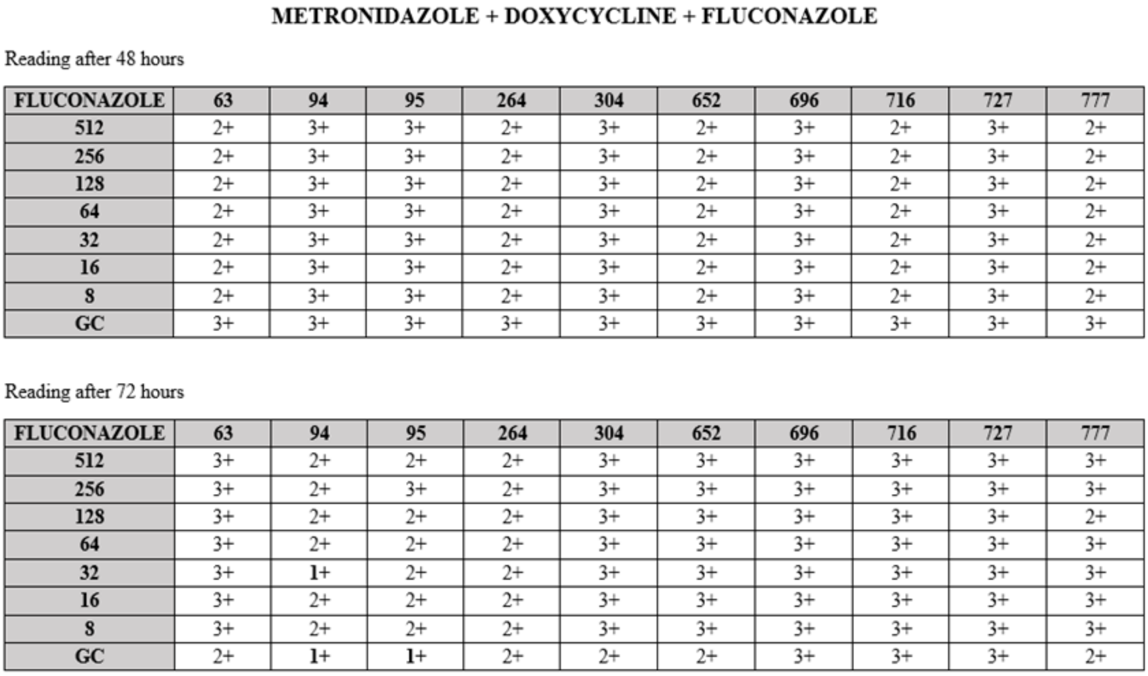
ceftriaxone

fluconazole

		16	8	4	2	1	0.5	0.25
		1+	2+	2+	2+	2+	1+	2+
512	2+	2+	2+	3+	2+	2+	2+	1+
256	1+	2+	2+	2+	2+	2+	2+	2+
128	2+	1+	1+	1+	2+	2+	2+	2+
64	2+	2+	2+	2+	1+	2+	1+	1+
32	2+	3+	1+	1+	2+	2+	2+	2+
16	1+	2+	2+	2+	2+	2+	2+	2+
8	2+	2+	2+	2+	2+	1+	1+	2+

2. Combinations of three antimicrobials

2.1 Metronidazole + Doxycycline + Fluconazole



METRONIDAZOLE + DOXYCYCLINE + FLUCONAZOLE

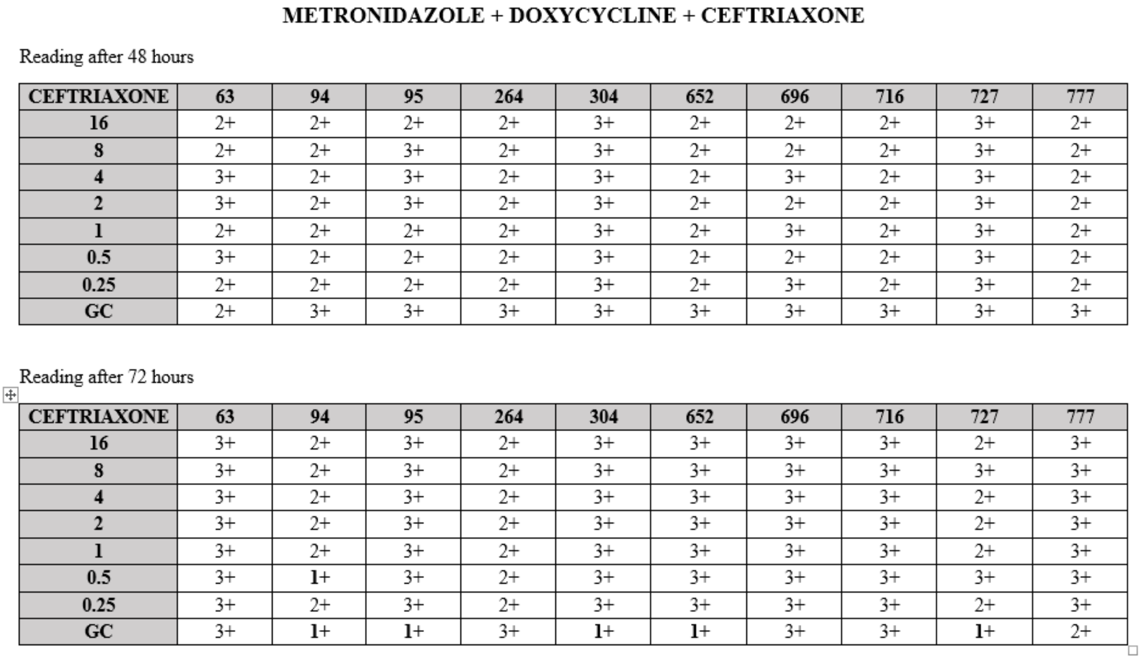
Reading after 48 hours

FLUCONAZOLE	63	94	95	264	304	652	696	716	727	777
512	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
256	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
128	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
64	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
32	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
16	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
8	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
GC	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Reading after 72 hours

FLUCONAZOLE	63	94	95	264	304	652	696	716	727	777
512	3+	2+	2+	2+	3+	3+	3+	3+	3+	3+
256	3+	2+	3+	2+	3+	3+	3+	3+	3+	3+
128	3+	2+	2+	2+	3+	3+	3+	3+	3+	2+
64	3+	2+	2+	2+	3+	3+	3+	3+	3+	3+
32	3+	**1+**	2+	2+	3+	3+	3+	3+	3+	3+
16	3+	2+	2+	2+	3+	3+	3+	3+	3+	3+
8	3+	2+	2+	2+	3+	3+	3+	3+	3+	3+
GC	2+	**1+**	**1+**	2+	2+	2+	3+	3+	3+	2+

2.2 Metronidazole + Doxycycline + Ceftriaxone



METRONIDAZOLE + DOXYCYCLINE + CEFTRIAXONE

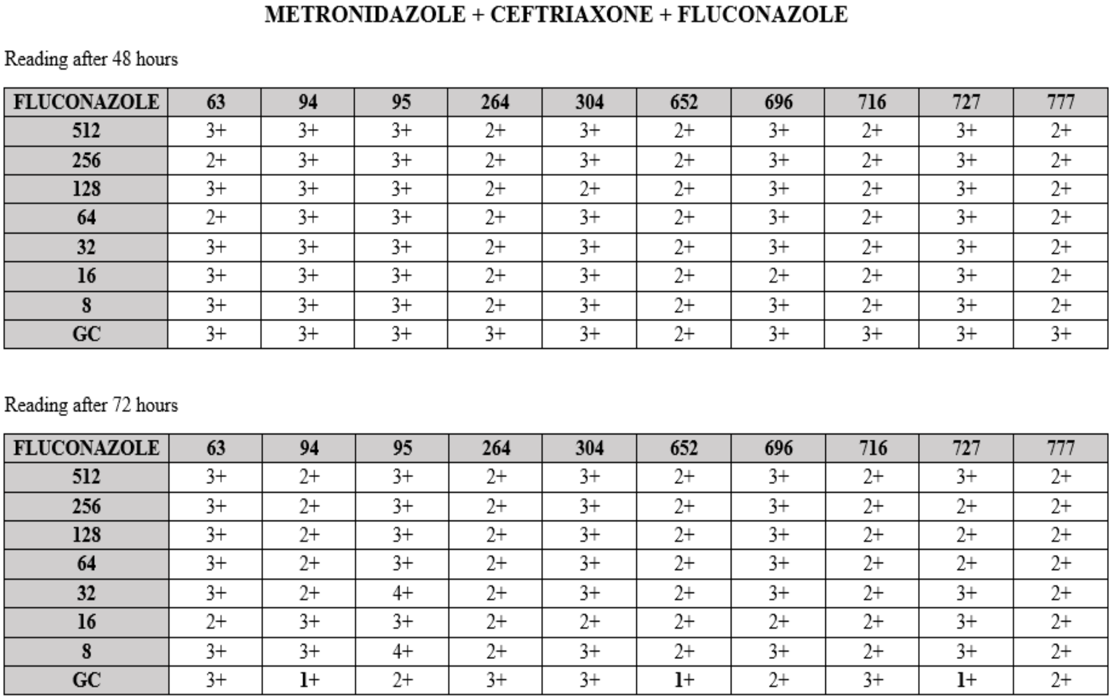
Reading after 48 hours

CEFTRIAXONE	63	94	95	264	304	652	696	716	727	777
16	2+	2+	2+	2+	3+	2+	2+	2+	3+	2+
8	2+	2+	3+	2+	3+	2+	2+	2+	3+	2+
4	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+
2	3+	2+	3+	2+	3+	2+	2+	2+	3+	2+
1	2+	2+	2+	2+	3+	2+	3+	2+	3+	2+
0.5	3+	2+	2+	2+	3+	2+	2+	2+	3+	2+
0.25	2+	2+	2+	2+	3+	2+	3+	2+	3+	2+
GC	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Reading after 72 hours

CEFTRIAXONE	63	94	95	264	304	652	696	716	727	777
16	3+	2+	3+	2+	3+	3+	3+	3+	2+	3+
8	3+	2+	3+	2+	3+	3+	3+	3+	3+	3+
4	3+	2+	3+	2+	3+	3+	3+	3+	2+	3+
2	3+	2+	3+	2+	3+	3+	3+	3+	2+	3+
1	3+	2+	3+	2+	3+	3+	3+	3+	2+	3+
0.5	3+	**1+**	3+	2+	3+	3+	3+	3+	3+	3+
0.25	3+	2+	3+	2+	3+	3+	3+	3+	2+	3+
GC	3+	**1+**	**1+**	3+	**1+**	**1+**	3+	3+	**1+**	2+

2.3 Metronidazole + Ceftriaxone + Fluconazole



METRONIDAZOLE + CEFTRIAXONE + FLUCONAZOLE

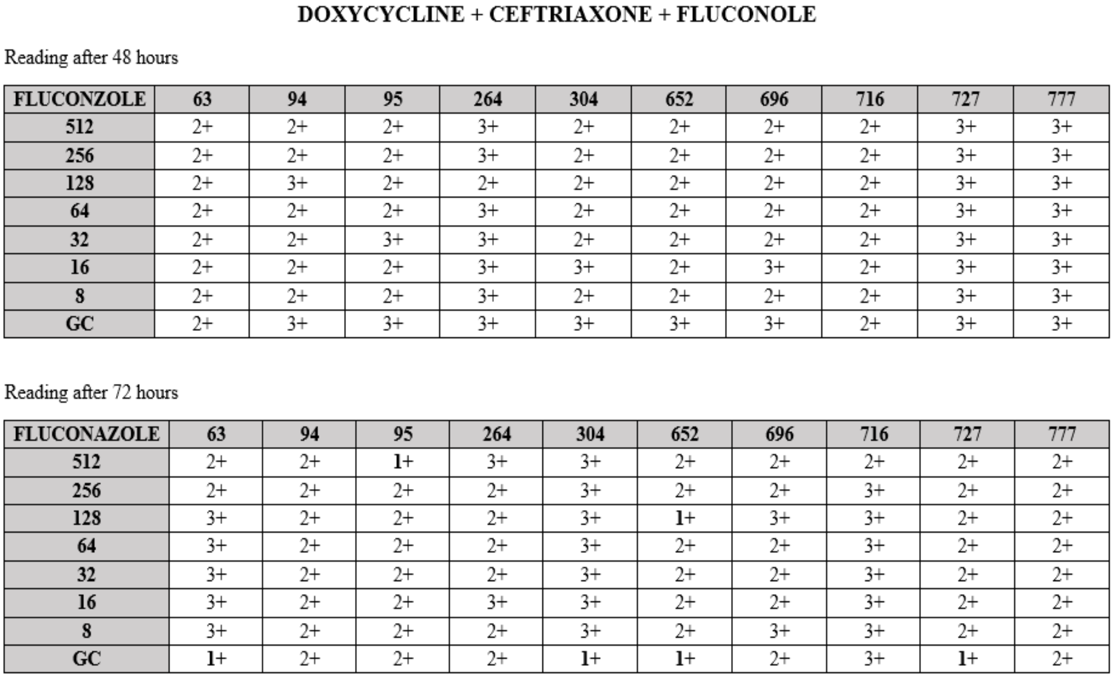
Reading after 48 hours

FLUCONAZOLE	**63**	**94**	**95**	**264**	**304**	**652**	**696**	**716**	**727**	**777**
512	3+	3+	3+	2+	3+	2+	3+	2+	3+	2+
256	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
128	3+	3+	3+	2+	2+	2+	3+	2+	3+	2+
64	2+	3+	3+	2+	3+	2+	3+	2+	3+	2+
32	3+	3+	3+	2+	3+	2+	3+	2+	3+	2+
16	3+	3+	3+	2+	3+	2+	2+	2+	3+	2+
8	3+	3+	3+	2+	3+	2+	3+	2+	3+	2+
GC	3+	3+	3+	3+	3+	2+	3+	3+	3+	3+

Reading after 72 hours

FLUCONAZOLE	**63**	**94**	**95**	**264**	**304**	**652**	**696**	**716**	**727**	**777**
512	3+	2+	3+	2+	3+	2+	3+	2+	3+	2+
256	3+	2+	3+	2+	3+	2+	3+	2+	2+	2+
128	3+	2+	3+	2+	3+	2+	3+	2+	2+	2+
64	3+	2+	3+	2+	3+	2+	3+	2+	2+	2+
32	3+	2+	4+	2+	3+	2+	3+	2+	3+	2+
16	2+	3+	3+	2+	2+	2+	2+	2+	3+	2+
8	3+	3+	4+	2+	3+	2+	3+	2+	3+	2+
GC	3+	**1+**	2+	3+	3+	**1+**	2+	3+	**1+**	2+

2.4 Doxycycline + Ceftriaxone + Fluconazole



DOXYCYCLINE + CEFTRIAXONE + FLUCONOLE

Reading after 48 hours

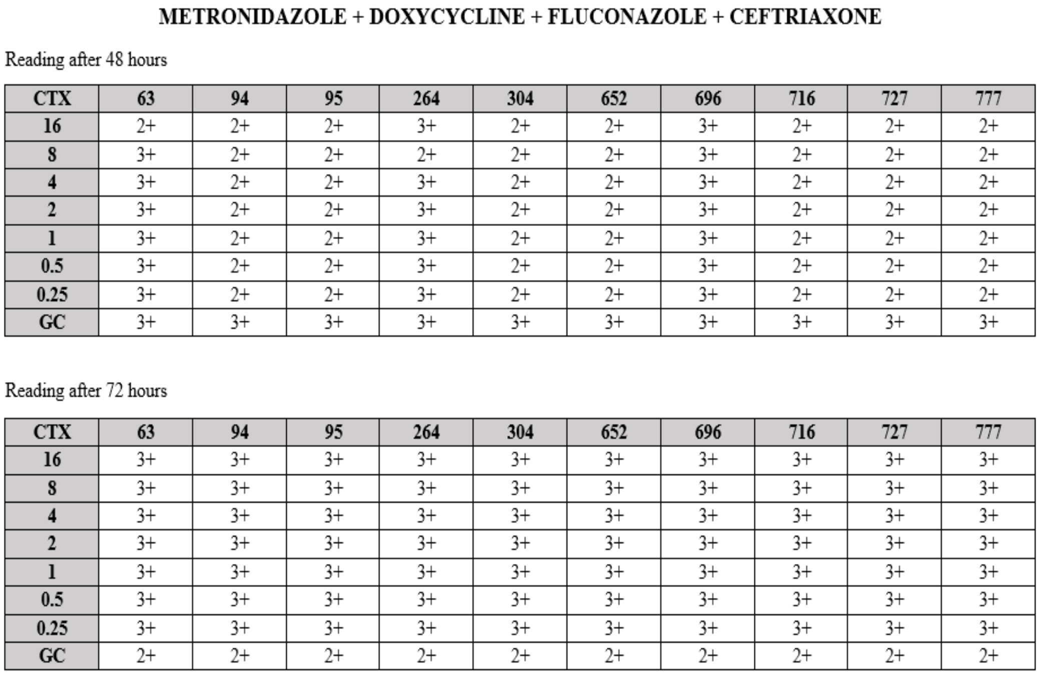
FLUCONZOLE	**63**	**94**	**95**	**264**	**304**	**652**	**696**	**716**	**727**	**777**
512	2+	2+	2+	3+	2+	2+	2+	2+	3+	3+
256	2+	2+	2+	3+	2+	2+	2+	2+	3+	3+
128	2+	3+	2+	2+	2+	2+	2+	2+	3+	3+
64	2+	2+	2+	3+	2+	2+	2+	2+	3+	3+
32	2+	2+	3+	3+	2+	2+	2+	2+	3+	3+
16	2+	2+	2+	3+	3+	2+	3+	2+	3+	3+
8	2+	2+	2+	3+	2+	2+	2+	2+	3+	3+
GC	2+	3+	3+	3+	3+	3+	3+	2+	3+	3+

Reading after 72 hours

FLUCONAZOLE	**63**	**94**	**95**	**264**	**304**	**652**	**696**	**716**	**727**	**777**
512	2+	2+	**1+**	3+	3+	2+	2+	2+	2+	2+
256	2+	2+	2+	2+	3+	2+	2+	3+	2+	2+
128	3+	2+	2+	2+	3+	**1+**	3+	3+	2+	2+
64	3+	2+	2+	2+	3+	2+	2+	3+	2+	2+
32	3+	2+	2+	2+	3+	2+	2+	3+	2+	2+
16	3+	2+	2+	3+	3+	2+	2+	3+	2+	2+
8	3+	2+	2+	2+	3+	2+	3+	3+	2+	2+
GC	**1+**	2+	2+	2+	**1+**	**1+**	2+	3+	**1+**	2+

3. Combinations of four antimicrobials

3.1 Metronidazole + Doxycycline + Fluconazole + Ceftriaxone



METRONIDAZOLE + DOXYCYCLINE + FLUCONAZOLE + CEFTRIAXONE

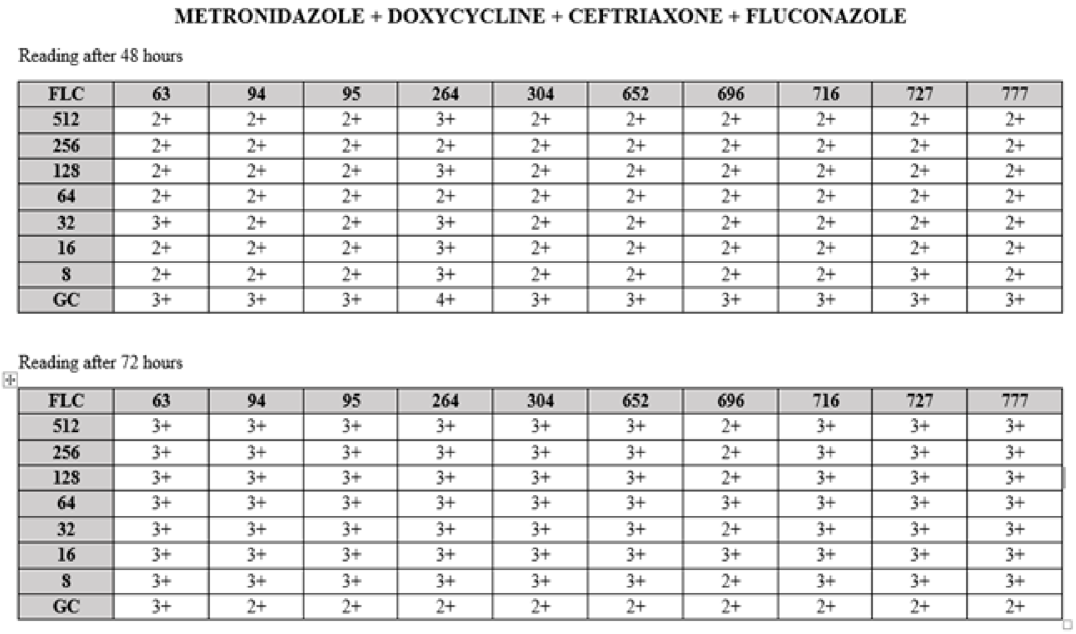
Reading after 48 hours

CTX	63	94	95	264	304	652	696	716	727	777
16	2+	2+	2+	3+	2+	2+	3+	2+	2+	2+
8	3+	2+	2+	2+	2+	2+	3+	2+	2+	2+
4	3+	2+	2+	3+	2+	2+	3+	2+	2+	2+
2	3+	2+	2+	3+	2+	2+	3+	2+	2+	2+
1	3+	2+	2+	3+	2+	2+	3+	2+	2+	2+
0.5	3+	2+	2+	3+	2+	2+	3+	2+	2+	2+
0.25	3+	2+	2+	3+	2+	2+	3+	2+	2+	2+
GC	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

Reading after 72 hours

CTX	63	94	95	264	304	652	696	716	727	777
16	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
2	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
0.5	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
0.25	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
GC	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

3.2 Metronidazole + Doxycycline + Ceftriaxone + Fluconazole



METRONIDAZOLE + DOXYCYCLINE + CEFTRIAXONE + FLUCONAZOLE

Reading after 48 hours

FLC	63	94	95	264	304	652	696	716	727	777
512	2+	2+	2+	3+	2+	2+	2+	2+	2+	2+
256	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
128	2+	2+	2+	3+	2+	2+	2+	2+	2+	2+
64	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
32	3+	2+	2+	3+	2+	2+	2+	2+	2+	2+
16	2+	2+	2+	3+	2+	2+	2+	2+	2+	2+
8	2+	2+	2+	3+	2+	2+	2+	2+	3+	2+
GC	3+	3+	3+	4+	3+	3+	3+	3+	3+	3+

Reading after 72 hours

FLC	63	94	95	264	304	652	696	716	727	777
512	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+
256	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+
128	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+
64	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
32	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+
16	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
8	3+	3+	3+	3+	3+	3+	2+	3+	3+	3+
GC	3+	2+	2+	2+	2+	2+	2+	2+	2+	2+