STUDY OF ANTIMICROBIAL SENSITIVITY PATTERN OF NEISSERIA GONORRHOEAE

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CERTIFICATE

Certified that this dissertation entitled **"STUDY OF ANTIMICROBIAL SENSITIVTY PATTERN OF NEISSERIA GONORRHOEAE"** is a bonafide work done by **DR. V.N.S. AHAMED SHARIFF,** Post Graduate Student in M.D. Dermatology, Venereology and Leprosy, Madras Medical College, Chennai – 600 003, during the academic year 2004 – 2007. This work has not been formed previously the basis for the award of any degree.

Prof. Dr. V.S. DORAIRAJ, M.D., D.V.,
Director Incharge,
Institute of Venereology,
Madras Medical College,
Chennai – 600 003.

Prof. DR. B. PARVEEN, M.D., D.D.,
Professor and Head,
Department of Dermatology & Leprosy,
Madras Medical College,
Chennai – 600 003.

Prof. Dr. Kalavathi Ponniraivan, B.Sc., M.D.,
Dean
Madras Medical College,
Chennai.

DECLARATION

I, Dr. V.N.S. Ahamed Shariff, solemnly declare that dissertation titled, **"STUDY OF ANTIMICROBIAL SENSITIVITY PATTERN OF NEISSERIA GONORRHOEAE"** is a bonafide work done by me at Madras Medical College, during 2004-2007 under the guidance and supervision of Prof. Dr.V.S. Dorairaj, M.D., D.V., Director Incharge , Institute of Venereology Madras Medical College, Chennai-3. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of M.D Degree in Dermatology, Venereology and Leprosy (Branch-XII A).

Place : Chennai
Date :

(Dr. V.N.S. Ahamed shariff)

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CONTENTS

APPENDIX

Criteria for interpretation of antibiotic sensitivity tests of stains of N. gonorrhoeae

Drug	Concentration of disk	Zone size (Diameters in mm)		
		Resistant	Moderately sensitive	Sensitive
Penicillin	10 I.U.	≤ 26	27-46	≥ 47
Ciprofloxacin	5 mcg	≤ 27	28-40	≥ 41
Ceftriaxone	30 mcg	-	-	≥ 35
Cefixime	5 mcg	-	-	≥ 31
Spectinomycin	100 mcg	≤ 14	15-17	≥ 18
Azithromycin	15 mcg	≤ 13	14-17	≥ 18

INTRODUCTION

Sexually transmitted diseases are prevalent through out the world. About 340 million new cases reported every year.[1] Incidence of new STD cases per year in India is about 40 million or 5% of population.[2] Globally around 39.4 million person are living with HIV/AIDS, and India has 5.13million HIV infections.[1] Sexually transmitted diseases (STD) play a major role in the transmission of HIV infection. Thus prevention and control of STD is one of the major strategies for the control of HIV.

STD can be grouped as ulcerative and non-ulcerative. The risk of acquiring HIV infection in ulcerative STD is 5 to 7 times and in non ulcerative STD to 3 to 5 times more than that in persons without any sexually transmitted infections.[3] Since the prevalence of non-ulcerative STD is higher than ulcerative STD, it represents the population at attributable risk in the transmission of HIV.[4]

One of the main non- ulcerative STD is Gonorrhoea. The relative incidence of gonococcal infections is about 10 to 13% of total sexually transmitted infections in STD clinics.[5,6] Common symptoms are urethritis in male and cervicitis is female. HIV positive men with urethritis had HIV concentrations in the semen more than those in seropositive men without urethritis. After treatment of urethritis the concentrations of HIV in semen decreased significantly.[7] Causative organism for gonorrhoea is Neisseria gonorrhoeae. Man being the only natural host. N. gonorrhoeae usually produces purulent exudates, but signs and symptoms of the

disease may be absent especially in women or indistinguishable from those of Chlamydial infection. Therefore, laboratory procedures are needed for the diagnosis.[8]

In recent years, there has been an alarming increase in ciprofloxacin and penicillin-resistant N. gonorrhoeae in India.[9] Establishing appropriate treatment strategies is further complicated by the rapidly changing resistance patterns. Hence monitoring of drug resistance patterns at regular intervals, is essential.

Before the emergence of penicillinase producing N. gonorrhoea in 1976, increased doses of penicillin remained the drug of choice for treating uncomplicated urogenital infections through out the world.[10] But now high doses of penicillin is ineffective in PPNG strains. Because of renewed interest in developing alternative gonococcal therapies, a number of compounds with proven efficacy against both PPNG and non PPNG have been described. Isolates from developing countries have shown decreased susceptibility to many of these, presumably as a result of intense antibiotic pressure favoring the selection of resistance strains.

In the management of gonorrhoea it is not practicable to delay treatment until the results of susceptibility tests are known. Hence alternative approach is to know the periodic patterns of gonococcal sensitivity for antibiotics. Culture is essential for surveillance for antimicrobial susceptibility which provides guidance for appropriate treatment depending on the strains prevalent in the region.

Other sexually transmitted infections like HIV, Syphilis, Trichomoniasis are common with gonorrhoea.[11] Presence of gonococcal infection aids in acquiring HIV infection. So proper diagnosis and treatment with sensitive antibiotics is necessary to prevent the complications of gonorrhoea and the spread of HIV.

REVIEW OF LITERATURE

Historical Perspectives

Gonorrhoea is one of the oldest known diseases of humans. Thedisease was described in the old Testament in chapter Leviticus, 15:2. Hippocrates (460-350 BC) called acute Gonorrhoea as "Strangury" and said resulted from the "Pleasures of Venus".[12] Galen (130-200 AD) a Greek hysician coined the term "gonorrhoea" (**gono**=seed; **rrhoea**= flow).[13] In ancient medical treatise, the condition "dysuria" was dealt exhaustively under the chapter "Diseases of urinary passages" by Sushrutha (500 AD).[14]

Ambroise Pare (sixth century) and John Hunter (eighth century) considered Syphilis and Gonorrhoea to be different manifestations of a single disease. Distinction between the two was first clearly achieved by Phillippe Ricord in 1831.[15] The etiologic agent was identified in 1879 byAlbert Ludwig Neisser from Poland the stained smears of inflammatory urethral exudates.[16] Leistikow and Leoffler in 1882[17] and Bumm in 1885 grew the organism in culture media of blood serum, gelatin and coagulated human serum respectively. Hans Gram demonstrated the organism by grams staining in 1884.[18] Gonococcal complement fixation test and Oxidase test were introduced by Muller and Oppenheim in 1906, and Gordon and Mcleod in 1988 respectively.[19] Diagnostic procedure further augmented in 20th century with development

of fluorescent antibody technique, serological tests for gonococcal antibodies and DNA amplification by LCR.[20-21] Variance in virulence of Gonococcus with different colonial Morphology was demonstrated by Kelogg and co workers in 1963.[22]

MORPHOLOGY

Gonorrhoea is caused by Neisseria gonorrhoeae belonging to the Family, Neisseriaceae and genus Neisseria. Gonococci are gram negative, non-motile, non-sporulating cocci measuring 0.8 x 0.6 mm in size, typically arranged in pairs (diplococci) with sides concave, reniform or pear-shaped. They grow poorly on ordinary media requiring serum or blood. Catalase and Oxidase positive, indole negative and reduce nitrite but not nitrate.

In smears from urethral discharge gonococci are predominantly within the polymorphs but in cultures they appear as oval or spherical cocci without the typical diplococcal arrangement. The organism lacks a true outer polysaccharide capsule. Instead, it produces a surface polyphate or 'pseudocapsule' which is responsible for the hydrophilic negatively charged cell surface of the organism.

ULTRASTRUCTURE AND ANTIGENIC PROPERTIES

Inner to the cell wall is the outer cell membrane made up of Lipo-oligosaccharide (LOS). Six distinct entities of LOS varying in size from 3

to 7 kDa are recognized. LOS act as endotoxins. Exotoxins are not elaborated by gonococci. LOS is a target for bactericidal antibodies.[23-24]

PILI

These are protein polymers that project from the surface of bacterial cell and mediate attachment. They promote virulence by preventing neutrophilic phagocytosis and involved in the exchange of genetic material.[25]

PORIN

Extends through the gonococcal cell membrane through which some nutrients enter the cell. Each strain of gonococcus expresses only one type of Por but different strains are antigenically different. It is a target for bactericidal opsonic antibodies.[26-27]

Opa (protein II) functions in adhesion of gonococci within colonies and in attachment of gonococci to host cell. A strain can possess 1-3 types of Opa. Each strain has 10 or more genes for different opas. Opa is responsible for opaque colonies.

Rmp (Protein III) is a reduction modifiable protein. It associates with Por in the formation of pores in the cell surface.

Iron- or oxygen-repressible proteins are expressed by gonococcal membrane under limited growth situations, such as iron deficiency or anaerobic environment. They perform the function of iron transport.[28-29]

Ig A1 protease: Gonococci elaborate two genetically and biochemically distinct variants of protease called Ig A1 protease. It recognizes both serum and secretory IgA 1 antibody and cleaves it at the hinge region forming Fab and Fc fragments, thus inactivating the mucosal immune defenses.

Auxotypes

Strains of gonococci that need specific requirements for certain nutritional growth factors are known as auxotypes. They are important for epidemiological studies in order to differentiate one strain from other. A strain unable to grow without arginine is designated as Arg- or without leucine is Leu-. This is helpful in assessing the potential virulence, invasiveness, antimicrobial susceptibility and genetic constitution of various gonococcal strains.[30]

Arg-Hyx-Ura- (AHU) strains are recovered from patients with disseminated gonococcal infection, and are frequently resistant to normal bactericidal action of human serum. Further they are associated with asymptomatic urethral infection in men, are highly susceptible to penicillins, and form atypical colonies on culture requiring 72 hours or more of incubation time for growth.[31] Gonococci requiring none of the growth factors are termed prototrophic or the wild type.

GENETIC CHARACTERISTICS

Transformation

The pilated gonococci are capable of undergoing chromosomal DNA transformation by exogenous DNA at all stages of their life cycle. This property may be responsible for transfer of genes between different strains in chromosomally mediated antibiotic resistant genes.

Conjugation

Gonococci also contain conjugal plasmids that can be transferred from one strain to another by pili. Nine plasmids are described throughout the world, out of which six are involved in penicillin resistance.

PATHOGENESIS

Gonococci has a predilection for columnar and cuboidal epithelium of genitourinary tract, eye, rectum and throat producing acute suppuration that may lead to tissue invasion followed by chronic inflammation and fibrosis. Cornified or stratified squamous and transitional epithelia are resistant to this organism.

The initial event of infection is adherence of gonococci to mucosal cells in a process mediated by pili, opa and other surface proteins. It is pinocytosed by epithelial cells to the sub epithelial space and is attached to nonciliated epithelial cells. Gonococcal LOS (endotoxin) impairs ciliary motility and destroys surrounding ciliary cells. This promotes further attachment of additional organisms. Progressive mucosal cell

damage and sub mucosal invasion are accompanied by vigorous polymorphonuclear leukocyte response, submucosal microabscess formation and exudation of purulent material into the lumen of infected organ.

If untreated polymorphonuclear leukocytes are gradually replaced by mononuclear cells and abnormal round cell infiltration has been reported to persist for several weeks after which gonococci can no longer be isolated.[32]

CLINICAL MANIFESTATIONS

Incubation period varies from 1 to 14 days with an average of 2-5 days.

Acute Gonorrhoea in men

It presents as acute anterior urethritis which is characterized by symptoms of dysuria and copious urethral discharge. The discharge is profuse, yellow to yellowish green purulent one. The lips of the external urinary meatus are edematous and there is associated perimeatal erythema. Tender inguinal lymphadenopathy may be present. One quarter of patients develop only scanty or minimally purulent exudates indistinguishable from non- gonococcal urethritis.

If prompt treatment is not initiated, posterior urethritis may ensue in 10-14 days, which presents as frequency of micturition, urgency,

occasional strangury and rarely tenesmus. Erection may be painful. The extend of involvement can be assessed by performing two glass test.[33] In this test the patient is asked to void urine in 2 glasses after overnight urine holding. In anterior urethritis, only the first urine specimen is hazy or cloudy and the specimen in the second glass is clear. Presence of haziness or threads in the second glass suggests posterior urethritis. A three glass test is also performed for greater accuracy, in which the anterior urethra is irrigated with a colorless antiseptic solution (such as 1: 8000 oxycyanide of mercury) until the washings in the first glass are clear. The patient then voids urine in two other glasses. Presence of haze in the first glass suggests posterior urethritis and in the second depicts involvement of the bladder.

Acute gonococcal infection can also produce acute tysonitis, phimosis, paraphimosis, littritis, inflammation of periurethral and paraurethral glands, balanitis, cowperitis, prostatitis, seminal vesiculitis, epididymitis, trigonitis, pustular lesions over penile skin associated with shallow ulceration around the frenulum.

Complicated Gonorrhoea in Men

In the absence of treatment, spontaneous resolution occurs in majority of cases after 4-6 weeks, with 95% of the cases becoming asymptomatic within 6 months. In rare instances, if the patient goes untreated or proves refractory to treatment, serious sequelae may ensue. They are urethral strictures fistulae leading to watering-can perineum,

chronic littritis, cowperitis, prostatitis, seminal vesiculitis or epididymitis. Penile lymphangitis associated with regional lymphadenitis and penile oedema (bull headed clap) are the other complications.

Acute Gonorrhoea in Women

Uncomplicated gonorrhoea in women remain asymptomatic in about 50% of cases that may act as a reservoir of infection. The primary site of infection is endocervical canal. The organism colonises the urethra, bartholin and skene's glands and spreads to involve the cervix, uterus, fallopian tubes and pelvis. The vulva, vagina, bladder and upper urinary tract are relatively spared.

Acute gonococcal infection in females presents as endocervicitis, which is characterized by purulent vaginal discharge, dysuria, intermenstrual uterine bleeding and menorrhagia. On speculum examination, copious pus emanating from the external os can be visualized. There is severe erythema and oedema of the zone of ectopy and associated mucosal bleeding induced by swabbing the endocervix.[34] Cervical erosions may also be present. Purulent urethral discharge may be expressed by massaging on the urethra from above downwards through the anterior vaginal wall. Endocervicitis results in blockade of the cervical glands and formation of retention cysts or Nabothian follicles that protrude into the vaginal portion of cervix.

Acute gonococcal vulvitis is rare and may present as edema. Erythema and tenderness of the labia. Gonococcal cystitis and trigonitis is also extremely uncommon.

Complicated Gonorrhoea in Women

Pelvic Inflammatory Disease (PID) is the most common local complication accounting for 10 to 20% of cases with acute gonococcal infection.[35] The incidence of gonococcal PID is highest during the early proliferative phase of the menstrual cycle. This is as a result of cyclical changes in both the complement function and antibacterial activity of serum secondary to changes in sex harmone levels.[36]

Acute salpingitis, tubo-ovarian abscess, pelvic abscess, pelvic peritonitis, chronic urethritis, skenitis, bartholinitis, proctitis, frozen pelvis, tubal infertility, ectopic pregnancies and chronic pelvic pain are the other complications of gonorrhoea in women.

Anorectal Gonorrhoea

Gonococcal proctitis in males usually results from anal coitus in passive homosexuals and rectum is a frequent site of infection in 40% of homosexual men.[37] In women, the rectal mucosa is infected in 35% to 50% of cases with gonococcal cervicitis consequent upon perineal contamination with infected cervical secretions. Symptoms may be pruritus, mucopurulent discharge, rectal bleeding, tenesmus, and constipation.

Pharyngeal gonorrhoea

This has been reported in about 3-7% of heterosexual men with gonorrhoea, 10-20% of infected women and 10-25% of homosexual men. Pharyngeal infection alone is present in 5% of gonococcal cases. The infection is transmitted by oro-genital contact and is more efficiently acquired by fellatio than by cunnilingus.[38] Symptoms are usually absent or mild in 90% of cases in few instances, acute pharyngitis or tonsillitis may occur. Pharyngeal gonorrhoea is a risk factor for developing disseminated gonococcal infection.[39]

Gonococcal conjunctivitis

It is a rare entity in adults and often seen in patients with concomitant anogenital gonorrhoea as a consequence of direct contamination by fingers or towels. The condition may be asymptomatic or mild infection to severe forms resulting in corneal ulcerations and panophthalmitis.[40]

Disseminated gonococcal infection (DGI)

This occur from the first day to 3 months. It is due to gonococcal bacteremia manifested by acute arthritis, tenosynovitis and dermatitis. It is common in women than in men.

Bacteremia begins from 7 to 30 days. Pregnancy and pharyngeal gonococcal infections are risk factors. Complement deficiency especially

terminal components C_5-C_8 is found in 13% of DGI. DGI are AHU negative auzotype, serotype protein I (Por) A, resistance to human serum and are penicillin sensitive. But now both plasmid mediated PPNG and chromosomally mediated antibiotic resistant strains have been isolated from these patients.[41]

Arthritis

Migratory arthralgia resolve spontaneously or progress into septic arthritis of knee, ankle, elbow and wrist are involved asymmetrically. Upper extremity more often than the lower.

Tenosynovitis

Common in dorsum of hand, wrist, ankles, or knees.

Dermatitis

75% of DGI have tiny papule, pustules, vesicles with erythematous base. Centre of the lesion becomes necrotic or haemorrhagic. Erythema nodosum and erythema multiforme lesion may also be seen.[42]

Gonococcal endocarditis

It occurs in 1 to 3% of DGI, but not common and it results in progressive valvular damage with abundant skin lesion, emboli to kidney and brain. Pericarditis with ECG changes, pericardial friction rub, purulent pericardial fluid and myocarditis suggested by tachycardia and ST-T wave changes occurs.

Gonococcal Perihepatitis

Fitz Hugh Curtis syndrome is due to extension of infection from fallopian tube by lymphangitis or via hematogenous spread. It mimics cholecystitis. Overwhelming sepsis with Water-House Friderichson syndrome or adult respiratory distress syndrome is rare.

Meningitis

Few cases were reported with typical presentation of acute bacterial meningitis without typical findings of DGI. In CSF Neisseria gonorrhoeae is indistinguishable from N. meningitidis.

Gonorrhoea in Pregnancy

Is associated with increased risk of spontaneous abortion, premature labor, early rupture of fetal membranes and perinatal infant mortality. Pelvic inflammatory disease is uncommon after first trimester.

Neonatal Gonorrhoea

A purulent to mucopurulent discharge from the eyes of an infant starting within 21 days of birth. Average incubation period is 1-4 days. The symptoms are red edematous, painful eyes with pus oozing out. Cornea may be involved. In severe cases eye may be destroyed.

Childhood Gonococcal Vulvo-Vaginitis

Usual means of acquiring infection is poor hygiene because of contact between the child's genitalia and the contaminated hands of an adult with gonorrhoea causing inflammation of vulva and vagina. Sexual assault may need to be ruled out.

Co-infection with Chlamydia Trachomatis

This commonly accompanies gonococcal infection in 10-20% of men and 20-30% of women.[43] Patients with concurrent infections are either asymptomatic or have fewer symptoms as compared to cases with gonorrhoea alone.

LABORATORY DIAGNOSIS
SPECIMENS AND SITES OF COLLECTION

Acute symptomatic cases	:	Urethral swab, Endocervial, Rectal and Anal swab.
Asymptomatic cases	:	Early morning first voided urine sample.
Chronic cases	:	Prostatic massage.
Tysonitis, Bartholin abscess, Skenitis	:	Pus
Distant infection sites	:	Conjuctival swab, Pharyngeal swab.
Metastatic Infection sites	:	Pus, CSF, Blood, Synovial fluid.

Specimen Transport

The specimen is transported in various non-nutritive Transport media such as Amie's or modified Stuart's medium. It should be processed within 6 hours. Stuart's transport medium is used with charcoal treated swabs.

DIRECT DEMONSTRATION OF N.GONORRHOEAE

Staining Methods

Methylene blue stain is simple stain used to demonstrate Morphology of the intra cellular diplococci alone.

Gram stained smear reveals gram negative diploccocci within polymorphonuclear leukocytes. In symptomatic men sensitivity is 90%, Specificity is 95%. In females with endocervical exudates, sensitivity is50% and specificity is 95%. In PID, the sensitivity is 60-70% and specificity is from 95-100%. Anorectal smears obtained from patients with proctitis have a sensitivity of 40-60%.

CULTURE

Culture is considered the 'gold standard' for the diagnosis of gonorrhoea. It is obligatory especially in the diagnosis of oropharyngeal, rectal, disseminated or asymptomatic infection in both sexes. It is also essential in determining antibiotic sensitivity pattern and treatment efficacy.

Gonococci are fastidious organisms requiring enriched media for growth. They are aerobic and capnophilic (requiring 5-10% CO_2 for growth). Optimum growth occurs at 35-36^{o} C.

No growth occurs below 25^{o} C or above 38.5^{o} C. It requires a moist environment and a pH between 7.2 and 7.6.

Selective culture Media

Thayer- Martin Medium: Chocolate agar based medium supplemented with growth factors for fastidious microorganisms and antimicrobial vancomycin and colistin that inhibit gram +ve and gram-ve bacteria and nystatin to inhibit yeasts and moulds.

Modified Thayer Martin Medium: This contains Thayer Martin Medium+ trimethoprim for recovery from rectal swabs. Trimethoprim Prevents swarming of proteus.

New York City Medium- Corn starch agar based medium containing yeast dialysate, citrated horse plasma and lysed horse erythrocytes with vancomycin, colistin, amphotericin and trimethoprim.

Other enriched media for gonococcal isolation include Chacko-Nair medium, Martin Lewis, GC-Lect medium, chocolate agar, haemoglobin yeast liver (HYL) medium and blood agar.

Vancomycin free Selective Medium (VFSM) for vancomycin sensitive strains of N.gonorrhoeae.[44]

Sensitivity of culture is reported between 80% and 95%, with false negative results attributed to poor specimen storage, transport problems and inhibition of growth by the components of selective media.

GROWTH AND CULTURAL CHARACTERISTICS

Gonococci form small, round, translucent, soft, emulsifiable and convex colonies with fine granular surface and lobate crenated margins of 1mm size. The colonical types recognizable are types T1-T4. Kelogg in 1963 described these types by growing them on a clear medium and viewing with obliquely transmitted light. T1 and T2 colonies are found in primary cultures obtained from the clinical material, while T3and T4 colonies appear on subcultures.

T1 and T2 colonies are small, lenticular and brown and possess pilated gonococci (P^{+} and P^{++}). T3 and T4 colonies are larger, flatter, granular and non pigmented and contain non-pilated organisms (P^{-} and P^{-}). Jephcott et al in 1971 reported another type of colony, i.e. T5.[45] This colony appear very shiny like T2 and as large as T3. It was dark brown in colour, granular with concentric rings on its surface and coarse, irregular margins.

Based on the expressions of an outer membrane protein called protein II (PII), the colonies may be differentiated into transparent (Tr) and opaque (Op) types when viewed through a light microscope on a clear media in appropriate lighting.

Kelogg and his coworkers observed that after repeated in vitro passages, P^+ "type 1" colonies retained their virulence whereas the P^- "type 4" colonies lost it. P^-colonies have decreased ability to cause infection in males.

BIOCHEMICAL REACTIONS

N. gonorrhoeae is oxidase, catalase positive. It ferments glucose, but not maltose, sucrose, fructose, or lactose producing acid but no gas. Carbohydrate utilisation tests are used to distinguish between N.gonorrhoeae and N. meningitidis. Serum free media like cysteine-Trypticase agar or Muller- Hinton agar with appropriate carbohydrate and indicator are commonly used.

Acidification of Carbohydrate Containing Media: are conventional cysteine tryptic digest semisolid agar- base (CTA) medium containing 1% carbohydrate and phenol red pH indicator, Rapid carbohydrate utilization test and commercial carbohydrate utilization tests like, RIM-N-Kit, Neisseria-Kwik test kit, Gonobio- Test, Bactec test, Minitek Neisseria Test and API Quad-Ferm are useful.

Definitive identification of N. gonorrhoeae

Rapid carbohydrate utilization Test (RCUT): Most frequently used rapid carbohydrate utilization test are non-growth dependent and utilizes preformed enzymes is more sensitive and specific. They combine ease of performance, rapidity and reliability with low cost.

Presence of pre-formed enzymes elaborated during growth allows degradation of the carbohydrates and results in the production of acid. The test is performed in a non-growth dependent buffered salt solution with pH indicator phenol red which changes colour from red to yellow as acid is formed.

A test for β lactamase production has been incorporated into the RCUT which can be detected by change in colour of phenol red from red to yellow,when penicillin is converted to penicillinoic acid. Ampicillin which is morestable and sensitive to TEM β lactamase is used instead of penicillin G in the test.

β Lactamase Detection

Apart from ampicillin in RCUT, methods to detect β lactamase are chromogenic cephalosporin (Nitrocefin) test, paper acidometric method and rapid tube iodometric method and paper iodometric test.[46]

β lactamase is an extracellular enzyme produced by many strains of bacteria, specifically hydrolyses the amide bond in the β lactam ring of penicillin analogues, rendering this antibiotic inactive. Penicillinoic acid is formed with a resulting colour change.

Fluorescent Antibody staining: Blind sweep of growth after 24-48 hrs of incubation or at 18 hours is taken. Polyclonal antibodies against the epitopes on the major outer membrane proteins (MOMPS) are used.

Current procedure utilise monoclonal mouse antigonococcal antibodies against epitopes on P.I of N.gonorrhoeae. The organism appear as apple-green fluorescent diplococci.

Co-agglutination

Recent modification of the Swedish monoclonal system as the Phadebact monoclonal GC test is antibody coated staphylococci used to detect those gonococci no longer identified by alternative monoclonal antibodies.

Lectin Agglutination

The binding of wheat germ a glycoprotein to the B D 1.4 N acetylglucosamine of a carbohydrate moiety is used a rapid test to differentiate cultured gonococci from other Neisseriae.

Chromogenic Enzyme Substrate Tests

Specific for gonococci only and the system must b used only on specimens isolated on selective media for pathogenic Neisseria as N.sicca and N.mucosa give false results. The enzymatic identification system uses specific biochemical substrates that, after hydrolysis by bacteria enzymes, yield a colored end product which is detected directly or after the addition of diazo dye. Gonochek-II includes β-galactosidase, γ-glutamylamino peptidase and prolyl-hydroxylprolyl amino peptidase enzymes.

Genogen II test

This test uses protein I monoclonal antibodies that are conjugated with colloidal gold as the detection reagent on a filter paper.[47] Change of

the filter paper colour to red is suggestive of N. gonorrhoeae. Culture confirmation probe test: The acuprobe N. gonorrhoeae culture confirmation test identifies the organism by detection of specific RNA sequences that are unique to gonococcus.[47] This test is found to be 100% accurate in identifying N. gonorrhoeae.

DIRECT ANTIGEN DETECTION IN CLINICAL SPECIMENS

These tests are rapid, highly sensitive and specific and a large number of specimens can be processed at one time and available for the direct detection of organism in urogenital specimens.

Solid Phase Immonoassay

ELISA is commercially available as "Gonozyme". It is useful as a rapid alternative to culture, especially in transport delays. It is useful in genital sites only.[48]

Nucleic Acid Hybridization

All nucleic acid detection methods can be carried out on dead organisms and are suitable where culture is impracticable or inconvenient. Proved to be highly sensitive and specific. Problems of non-specific inhibition by genital secretions have shown reduced sensitivity.

Ribosomal RNA Probe

Gen- Probe Pace 2 system (Gen-Probe Inc San Diego, CA, USA) uses magnetic bead separation stage and a chemiluminescent labeled DNA probe to detect gonococcal rRNA. It is very quick but requires expensive kits and equipment. In high prevalence population sensitivity is

between 94.2% and 100% with specificities between 99.8% and 99.9% for specimens from both sexes and including those from non-genital sites.

Plasmid DNA probe

Probes made complementary to the 2.6 MDa cryptic plasmid and to the 4.4 MDa penicillinase conding plasmid of gonococci have been tested. Radioactively labeled probes was designed for reference laboratory for screening of populations on a different continent. Results were comparable with culture and permitted the detection of penicillinase production.

Polymerase / Ligase Chain Reaction Techniques

Sensitive and specific system, uses a portion of the 2.6 MDa plasmid that is also presented on the gonococcal chromosome. Roche Molecular system has extended their Amplicor PCR system to include a second generation multiplex test which simultaneously detects N. gonorrhoeae and Chlamydia trachomatis. Showed 100% sensitivity and 99% specificity in clinical samples.[49]

Nested PCR

Technique is used to demonstrate gonococcal DNA in sterile synovial fluids of patients with gonococcal arthritis.

Ligase chain reaction technique detecting Opa and pilin genes was developed by Birkenmeyer and Armstrong. Examining genital, extragenital swab and first voided samples from both sexes found the overall performance superior to culture.[50]

SEROLOGICAL TESTS

Gonococcal antibodies such as pilin, P.I or P.II antibodies can be detected by complement fixation test, latex agglutination test, indirect immunofluorescence, radioimmunoassay, indirect haemagglutination test, ELISA and western blotting. The presence of cross- reactive antibodies to antigens of other Neisseria species or species of other bacterial genera and antibody persistence from past infections may be a limiting factors that alter the sensitivity and specificity of these tests.

TREATMENT

Effective treatment of gonorrhoeae is defined as the elimination of N. gonorrhoeae from all anatomic sites.[51] An ideal antimicrobial agent forthe first line of treatment of gonorrhoeae should eradicate more than 95% of the organisms in uncomplicated anogenital infection.

Antimicrobial Options

Before the advent of sulphonamides in 1935, gonorrhoea therapy involved local genital irrigation of antiseptic solutions such as silver nitrate or potassium permanganate or passage of urethral sound. By 1944, treatment failure with sulphonamides occurred in about one third of patients and this increased to about 90% by the end of 1940's.[52]

Penicillins

Introduced for treatment of gonorrhoea by Mahoney, Arnold and Harris in 1943. Single dose treatment with penicillin was the standard regimen followed. Extended spectrum penicillins such as ampicilin, amoxycillin, ticarcillin, piperacillin, and mezlocillin also showed significant in vitro activity against non β lactamase producing N.gonorrhoeae.

Cephalosporins

Third generation preparations have proved highly efficacious. ceftriaxone, cefotaxime, cefixime are the drugs which are recommended in the treatment of gonorrhoea.

Fluoroquinolones

This group of drugs became popular in the mid 1980s. They are ciprofloxacin, ofloxacin, norfloxacin, pefloxacin and newer quinolones like fleroxacin, endofloxacin, lomefloxcin and gemifloxacin.

Other antimicrobial options are azithromycin, spectinomycin, trospectinomycin, tetracyclines, gentamicin, kanamycin.

Problem of antimicrobial resistance

Clinically there is no difference between infections caused by antibiotic resistant and antibiotic sensitive strains. In adequate therapy results in extended period of infectiousness and an increase in the number of sex partners who become infected. The sequelae of gonococcal

infection, pelvic inflammatory disease (PID), gonococcal ophthalmia, disseminated gonococcal infection (DGI) are likely to increase due to inadequate therapy.[53]

Resistant strains in the community also have an adverse impact on the costs of patient management-additional laboratory tests, added drug costs, extra clinic visits and more extensive intervention activities. The pattern of antimicrobial resistance varies widely. It is highest in countries where effective therapy is either unavailable or too expensive and facilities for diagnosis are inadequate, and often is coincident with a high prevalence of HIV infection.[54] The treatment regimens should therefore be tailored to the prevalence of antimicrobial resistance in each country or region to prevent spread of resistant strains.

From epidemiological point any method of treatment which givesmore than 5% of treatment failure must be regarded as ineffective.

Factors Contributing to Emergence of Resistant Strains

1. Natural selection

When a microbial population is exposed to an antibiotic, more susceptible organisms will succumb, leaving behind only those resistant tothe microbial onslaught. These organisms can then pass on their resistance to others by conjugation or transformation. This process is exacerbated by the abuse, overuse and misuse of antimicrobials in the treatment of human illness and in animal husbandry, aquaculture, and agriculture.

2. Drug access and resistance

Poverty and inadequate access to drugs contribute to be a major force in the development of resistance. Poor quality, counterfeit or truncated treatment courses leads to more rapid selection of resistant organisms.[55]

3. Misdiagnosis and resistance

In developing countries problems like prescribing inadequate amount of antimicrobials or unnecessary antibiotics or antibiotics of wrong choice are responsible whereas in developed countries over prescribing antibiotics contribute to resistance.

4. Counterfeit drugs

When antibiotics are prescribed at lower levels than treatment guidelines indicate, resistance can flourish. Counterfeit drugs may have weaker ingredient, wrong ingredient of no active ingredient.

5. Dubious Pay-offs and High Priced Prescriptions

Countries where health care providers earn only subsistence wages, unethical pharmaceutical companies sometimes pay a commission for recommending more expensive broad spectrum antibiotics when cheaper narrow spectrum alternatives would suffice.

6. Patient Pressure

Prescribing wrong or unnecessary antibiotics due to patient demand for antimicrobials can contribute to the development of resistance.

7. Lack of Education

In many countries the majority of patients purchase antimicrobials and other drugs without visiting a health worker first. Many drug retailers advise their consumers to buy non-essential drugs as they are under-educated and under-informed.

8. Resistance and Hospitals

Some teaching hospitals sometimes unwittingly promote the type of irrational drug dispensing without proper investigations that contribute to resistance.

9. Globalization and Resistance

International travel and trade can contribute to development of resistance. Lack of political will by some governments and little enforcement of effective guidelines within health care institutions can contribute to resistance.

10. Practice of Chemoprophylaxis

Prophylactic administration of antibiotics immediately or soon after sexual exposure clearly reduces the risk of infection. This practice is likely to select and facilitate transmission of antibiotic resistant strains. A history of self-initiated antimicrobial prophylaxis was found associated with infection with ciprofloxacin-resistant gonococci.[56]

11. Other factors

Use of selective media containing vancomycin in the laboratory diagnosis of gonorrhoea is associated with emergence of PPNG. Certain biologic characteristics of gonococcal infection short incubation period and ability to produce asymptomatic infections favour spread of resistant strains.

Mechanism of antibiotic Resistance in Neisseria gonorrhoeae

Antibiotic resistance in N.gonorrhoeae can be controlled by plasmid (PPNG or TRNG) or chromosomal DNA (CMRNG). The knowledge about genetic control of antibiotic resistance can help in understanding the spread of clinically significant resistant strains.

Penicillin Resistance

Chromosomal- mediated resistance

Gonococcal strains that require 2μg or more/ml of penicillin for inhibition and do not produce β-lactamase are designed as chromosomally mediated penicillin resistant N. gonorrhoeae (CMRNG).

This type of resistance is as a result of the additive effect of mutations at multiple loci, including pen A (encodes for penicillin binding protein 2), mtr (controls antibiotic susceptibility by an efflux system which actively removes the antibiotic from the cell) and pen B (closely linked to porin protein of gonococcus) [57]

Plasmid Mediated penicillin resistance

This type of resistance is attributed to the production of β lactamase via single step acquisition of plasmids. β lactamase or Penicillinase acts on the β lactam ring of the penicillin molecule and destroys its antibiotic activity by converting it into inactive penicillinoic acid.

The biological characteristics of PPNG stains isolated from Asia and Africa are different.[58] The Asian strains are proline dependent and carry the 4.4. MDa plasmid. There is an additional conjugate 24.5 MDa large plasmid associated with it. The presence of the associated conjugate plasmid in Asian strains is responsible for rapid spread of resistance to other gonococci.

The African strains are arginine dependent and carry the 3.2 MDa plasmid, which is not associated with the large conjugative plasmid, hence dissemination of these strains occurs more slowly. Both the plasmids encode for the TEM-1 β lactamase.

Tetracycline-resistant N. gonorrhoeae (TRNG)

TRNG are attributed either to multistep chromosomal mutations or the acquisition of a tetracycline resistant plasmid. (TC^1). Gonococcal isolated requiring more the 2μg/ml of tetracycline for inhibition are defined as chromosomally mediated TRNG. Chromosomal loci mediating low level tetracycline resistance have been designated mtr, penB and tet of which two loci (mtr and penB) are also involved in mediating penicillin resistance.[59]

Plasmid mediated resistance is due to the acquisition of the tet M. determinant by the conjugate plasmid (24.5 MDa) resulting in a TC^1 plasmid of 25.2 MDa. Plasmid mediated TRNG strains are likely to spread more rapidly than PPNG strains because of the presence of tet M. plasmid in other flora found in the genital tract including G. vaginalis, U. urealyticum and M. hominis.

Fluoroquinolone resistant N. gonorrhoeae (QRNG)

The national Committee for Clinical Laboratory Standard (NCCLS) in USA has defined strains of N. gonorrhoeae resistant to fluoroquinolones if they have minimal inhibitory concentrations (MICs) of more than 1μg/ml for ciprofloxacin or more than 2μg/ml for ofloxacin.

Resistance to fluoroquinolones involves number of separate chromosomally mediated mechanisms manifesting as sequential increase in MIC. It results from mutations in the DNA gyrase genes of changes in

the cell wall permeability. Moderate level of resistance results from aminoacid changes in gyr A whereas mutants with high level resistance have been found to have changes both in gyr A and the topoisomerase IV gene, par C.

Cephalosporin resistance

Analysis of mutations in a strain with reduced susceptibility to ceftriaxone minimum inhibitory concentration (MIC) = 0.5 microg/mL) showed the N. gonorrhoeae strain contained a significantly different sequence of the penA gene from that of the ceftriaxone-susceptible strains.

This strain also included a ponA mutation that is associated with high-level resistance to penicillin, mtrR mutations that mediate overexpression of the MtrCDE efflux pump responsible for resistance to hydrophobic agents such as azithromycin, and penB mutations that reduce porin permeability to hydrophilic agents such as tetracycline.

Moreover, this strain contained gyrA and parC mutations that confer high-level resistance to ciprofloxacin. These results indicate the emergence of a N. gonorrhoeae strain with reduced susceptibility to ceftriaxone, which also showed a multidrug-resistant phenotype.[60]

Spectinomycin resistance

Spectinomycin resistance results from chromosomal mutations in the 16S rRNA genes resulting in decreased binding of the drug to its ribosomal target.[61] High level resistance occurs in a single step.

Azithromycin resistance

Azithromycin resistance is due to over expression of the mtrCDE-encoded efflux pump mediated by mutations in the mtrR gene, which encodes a transcriptional repressor that modulates expression of the mtrCDE operon.[62] Those strains which did not have the mutation in the mtrR promoter region, were found to have a C2611T mutation (Escherichia coli numbering) in the peptidyltransferase loop in domain V of the 23S rRNA alleles.[63]

Susceptibility testing

Susceptibility testing may be performed by disk diffusion, agar dilution (full MIC or breakpoint) or by commercially available E-test. In disk diffusion, a small disk impregnated with a given concentration of antibiotic is placed on the surface of an agar plate inoculated with a bacterial suspension. After overnight incubation the size of the zone of inhibition indicates the susceptibility of the organism.

In agar dilution the antibiotic to be tested is serially diluted, and each at several concentrations this when added to an appropriated volume of medium, will give required concentration. A standardized suspension of each strain is inoculated onto the series of agar plates using a multipoint inoculator. Appropriate sensitive and resistant control organisms should be included with the series of test isolates. After overnight incubation the lowest concentration of antibiotic which inhibits the growth is taken as the MIC.

The E-test is a novel method of determining the MIC. The E-test consists of a thin, plastic strip, the lower side of which contains a continuous concentration of gradient of stabilized and dried antibiotic while the upper side is marked with a MIC scale. The strip is placed on an agar plate inoculated with a suspension of the test organism and after overnight inoculation the intersection between the value printed on the edge of the strip and the inhibition zone is the MIC.

Comparability of results

The data collected should be comparable between laboratories, and therefore it is necessary to have uniform methodology for isolation and susceptibility testing of N. gonorrhoeae.

Interpretation of the results

This depends on proper performance of the test. For example in disk diffusion method, the variables that influence the outcome include the depth, pH, cation content, supplements, and source of the agar; the age and turbidity of the bacterial inoculum; the way in which the inoculum is spread on the plate; the temperature, atmosphere, and duration of incubation, the method of reading results; the antimicrobial content of the disks, their age and storage conditions; and more. For these reasons, performance of the test must strictly adhere to the guidelines and the performance monitored by periodically testing the quality control strains (ATCC 49226) of known susceptibility.

Surveillance programmes

Several studies done worldwide provide data about the prevalence of antibiotic resistant strains. Regional programmes for monitoring gonococcal susceptibility have been developed in industrialized countries such as the USA (Gonococcal Isolate surveillance Project GISP), Australia (Australian Gonococcal surveillance programme), SEAR GASP (South East Asia Region- Gonococcal Antimicrobial Surveillance Programme), Canada and the Netherlands.

A global surveillance network, the gonococcal antimicrobial Surveillance programme (GASP) coordinated by the WHO, has been established with an aim to create a series of networks of laboratories based on WHO regions that will monitor gonococcal antimicrobial susceptibility and disseminate information on trends in susceptibility and emergence of resistance.[54]

GLOBAL SCENARIO

Chromosomal-mediated penicillin resistance

First reported in USA from north California in 1983.[64] Since then increasing number of CMRNG strains have been reported from USA. Australian surveillance programme detected CMRNG in 8.5% isolates in 1995.

Highest percentage of CMRNG strains has been reported in the south-east Asian countries including Philippines, Thailand and Indonesia. In a study in Bangkok in 1995 CMRNG was exhibited by 36% of strains.

In Japan in 2002 CMRNG is present in 12.6% of isolates. In arecent study from Bombay 3% of the isolated strains were CMRNG.

Plasmid-mediated penicillin resistance

The first PPNG strain emerged in the far east and west Africa in 1975.[65] In USA in 1999 2.1% of the isolates were PPNG strains. The prevalence of PPNG is the highest in south East Asia and Sub-Saharan Africa.

In India PPNG was first reported by Vijayalakshmi et al from the Institute of Venereology, Madras in 1980. In a study conducted in 2001 in Chennai from the Institute of Venereology showed 35.8% of isolates were PPNG. 35.3% of isolates were found resistant to penicillin in New Delhi in 2003. A study from Bombay revealed 51.7% of isolates to be PPNG.

Report from China in 1999 showed 32.65% of strains showed penicillin resistance.[67] In Rwanda 70.5% of strains were PPNG in 2000. [66] In New Zealand 9% were penicillin resistant in 2002.Annual report of the Australian Gonococcal Surveillance Programme, 2005 showed 29.5% penicillin resistant strains.

Fluoroquinolone resistance

Resistance to ciprofloxacin and ofloxacin emerged in the late 1980s and early 1990s from patients in South East Asia In USA 16% of isolates were QRNG in 1994. In 1994 21.8% and 10.4% strains were resistant to ciprofloxacin from Thailand and Hong Kong respectively. In Philippines 72.6% of isolates in 1996-97 were QRNG. In China 82.65% of strains were QRNG in 1999. In New Zealand in 2002 6.8% were QRNG. In Australia 30.6% QRNG isolates were found in 2005.

In India 15.2% isolates showed resistance to ciprofloxacin in Bombay in 1999. In Chennai 24.5% of strains were QRNG in 2001. In 2003 67.3% of isolates were found to be QRNG in New Delhi.

Cephalosporin resistance

In 1999 13.27% of isolates were found resistant to ceftriaxone in China.[67] Multidrug-resistant Neisseria gonorrhoeae with decreased susceptibility to cefixime isolated in Hawaii, 2001. 9.2% of isolates were resistant to cefixime in Japan in 2002. 17.5% of strains were resistant to ceftriaxone in Sverdlovsk in 2003.[68] Slightly more than 1% showed resistance to ceftriaxone in Australia in 2005.

In Chennai 3.7% of isolates were resistant to ceftriaxone in 2001.In 2003 5.9% of the isolates were found less sensitive to ceftriaxone in New Delhi.

Spectinomycin resistance

In China in 1999 8.16% of isolates showed spectinomycin resistance. In Trinidad in 2001 9% of strains exhibited resistance to spectinomycin.

In Chennai 22.2% of isolates were resistant to spectinomycin in 2001.

Azithromycin resistance

28.3% of isolates from Manaus, Brazil, 1998 showed reduced susceptibility to Azithromycin. . 9.8% of isolates were resistant to Azithromycin in Cuba, 1995-1998. Isolates from Nepal in 2003, 19% exhibited reduced susceptibility.

ACO guidelines for the treatment of gonorrhoea:

Uncomplicated gonococcal infection

This includes anterior urethritis and proctitis in males; cervicitis, urethritis and proctitis in females and pharyngitis in both.

Recommended regimens

Azithromycin, 2g orally as a single dose * or

cefixime, 400 mg orally as a single dose or

ceftriaxone, 250 mg intramuscular (IM) as a single injection

* Will treat both gonococcal and chlamydial infections.

FOLLOW UP

Patient has to be followed on 3, 7, 14, 30th day. Direct gram stained smears of centrifuged urine deposit and discharge done. If smear is positive and the patient had complaints, culture was repeated and treated as failure. In females endocervical swab taken and culture done.

Epidemiological treatment

All sex partners exposed within 2 weeks prior to the onset of symptoms in patients with gonorrhoea has to be treated. For patients with asymptomatic gonococcal infection, however extending contact tracing to 1 month may be useful.

AIMS AND OBJECTIVES

1. To identify the gonococcal infection in patients attending the STD clinic.

2. To study the antimicrobial susceptibility of gonococcus.

3. To study the prevalence of Penicillinase producing N. gonorrhoeae.

4. To study the associated sexually transmitted infections.

5. To modify the disease intervention activities and therapy recommendations accordingly.

Purpose of the study

The knowledge of the prevalence of antibiotic resistant strains is essential for instituting disease control and preventive measures. In India we do not have enough data about the prevalence of antibiotic resistant strains. Efforts are being made to widen the SEAR GASP (South East Asia Region Gonococcal Antimicrobial surveillance programme) network in India. Treatment recommendations has to be changed depending upon the surveillance data.

WHO no longer recommends a single, first line treatment for gonorrhoea. Instead each nation must make decisions according to its own resistance situation as resistance levels vary from one nation to the next indeed from clinic to clinic.[55]

MATERIALS AND METHODS

Study design

Prospective observational study.

Sample

43 patients with gram stained smear or culture positive for gonococcus who attended the Institute of Venereology, Government General Hospital, Chennai from February 2005 to September 2006.

Methodology

Routine history and clinical examination was done for all patients. Appropriate specimens were collected according to the duration of illness and site of gonococcal infection. Data regarding the age, sex, risk factors associated with clinical illness and transmission factors were obtained.

All patients were treated as per NACO guidelines with either ceftriaxone or azithromycin. Follow up was done on 3, 7, 14 and 30 days. Screening for other sexually transmitted diseases was done and treated according to the Institute guidelines

SPECIMEN COLLECTION

In Males

1. Urethra : Patient were instructed not to urinate for 1 to 2 hours before specimen collection. Specimens were collected by wearing sterile gloves. In cases of urethral discharge (figure 1) the prepuce was retracted, area around the urethral meatus was cleaned with sterile normal saline and urethral discharge was collected in two sterile platinum loops. Direct plating was done at bed side. The second loop was used for gram staining.

If there is no discharge or only minimal discharge present, the urethra was milked towards the orifice to express the discharge and then the specimen was collected. Even on milking there was no discharge then a sterile charcoal treated swab was inserted 2-3 cms into the urethra and gently rotated for 5-10 seconds to scrape the mucosa. A wet mount examination was also made from the discharge to rule out trichomoniasis and candidiasis.

2. Urine : 5-10 ml of first voided urine was collected in a sterile test tube and centrifuged deposit was used for direct plating and gram staining.

In females

1. Endocervix: A sterile Cusco's self retaining vaginal speculum was inserted into the vagina (lubricant not used) and the endocervix was visualized (figure 2). Cervix was cleaned with clean sterile cotton wool swab to remove cervical mucosal plug. Specimen was collected with two swabs. The first sterile charcoal treated swab was inserted into the endocervical canal for 2 cms and rotated for 5-10 seconds to collect the specimen.

Specimen was inoculated into the culture medium. Second sterile swab was used for gram staining. A wet mount preparation was done by mixing one drop of discharge from the posterior fornix of the vagina and one drop of normal saline over a clean glass slide and placing a coverslip over it.

MICROSCOPY

Wet Mount Examination:

The slide was examined first under 10x magnification and then under 40x magnification, to look for trichomonads and polymorphs. (figure 3)

Gram stain

A thin smear was prepared on a clean glass slide by rolling the swab on the slide. After air drying and heat fixing the smear, it was

stained with gram stain. It was examined under 100x magnification, to look for the presence of epithelial cells, polymorphonuclear leucocytes and the location of gram negative diplococci whether intracellular or extra cellular(figure 4,5).

Culture

The specimen collected for the culture is immediately inoculated on the selective medium (Modified Thayer Martin Medium with VCNT) and non selective chocolate agar. The culture plate is incubated at 35-36^{o} C in moist humid air containing 5-10% Co_2 in a candle jar, humidity was obtained by using sterile moist cotton ball (figure 6). The plates were examined after 24 hrs and if there were no growth the plates were further incubated and read after 48 hours. Colonies which showed gram negative diplococci in Gram stain, oxidase positive were identified presumptively as N. gonorrhoeae and sub cultured on to chocolate agar for further tests like sugar degradation test, β-lactamase test and antibiotic sensitivity.

Colony characters

Thayer- martin agar: Small pinpoint colonies circular glistening with entire edge raised, convex, grayish, translucent/ opaque colonies were seen (figure 7,8).

Chocolate agar: Medium sized colonies, convex, greyish, translucent / opaque colonies were seen.

Identification of N. gonorrhoeae from culture

1. Gram staining

The smear prepared from colonies grown, is stained with grams stain to look for gram negative diplococci.

2. Oxidase test

A drop of freshly prepared aqueous solution of 1% tetra methyl paraphenylene diamine dihydrochloride is added on a colony and immediate development of purple colour (within 5-10 seconds) indicates a positive test.(figure10)

3. Sugar degradation test

N. gonorrhoeae utilizes glucose only but not maltose or lactose or sucrose. The tube containing glucose changes from red to yellow. The control tube remains red.(figure11).

β Lactamase test

Rapid tube Iodometric test for detection of penicillinase producing Neisseria gonorrhoeae (PPNG) was used. Several colonies from 24 hours pure culture were taken in a loop and a heavy turbid suspension is made in 0.1 ml of penicillin solution (Penicillin G powder in phosphate buffer) in a small test tube. 2 drops of starch solution is added to the suspension of bacteria and penicillin and mixed. 1 drop of iodine reagent is added with a pasteur pipette. A control tube without the organism was also

included. The solution will immediately turn blue because of reaction of the iodine and starch. Rapid decolourisation indicates the production of β lactamase. (figure12).

ANTIBIOTIC SENSITIVITY TESTING

The antibiotic sensitivity testing in N. gonorrhoeae was done by Kirby-Bauer disk diffusion method. Sensitivity for Penicillin G(10 units), Ciprofloxacin (5mcg), Ceftriaxone (30mcg), Cefixime (5mcg), Spectinomycin (100mcg) and Azithromycin (15mcg) were tested.

2 ml of gonococcus suspension (10 colonies from a pure subculture plate mixed with 5 ml of phosphate buffered saline) was pipetted out on a dry sterile culture plate (GC agar with haemoglobin and isovitalex supplements). The Plate was tilted in all directions so that the whole plate surface becomes wet. The remaining fluid is removed with a pasteur pipette and the plate was dried in the incubator in inverted position at 37^{o}C for 30 minutes. The antibiotic disks were placed on the agar plate using sterile forceps maintaining a gap of 2.5 cms between two disks. The plate was incubated in a candle extinction jar at 37^{o}C in a incubator for 18-24 hours. If the organism is sensitive there is a clear zone around the disk (figure13,14).

The diameter of the clear zone is measured using a ruler and interpreted accordingly. The zone size for interpretation are given in the appendix.

RESULTS

Specimens from 43 patients (40 male, 3 females) were collected. The number of patients diagnosed by smear and culture are given in table 1. 91% of patients were in the age group of 20-40 years (chart 1). 45% of males and 100% of females were married (chart 2). 90% of males and 66% of females were literate (chart 3). 90% of males were employed (chart 4). 85% of males had heterosexual orientation, 12.5% of males had bisexual orientation and 2.5% had homosexual orientation (chart 5). 52.5% of males had last sexual exposure with CSWs and 32.5% of males with known contacts (chart 6). All male patients had urethral discharge. 42.5% of males had dysuria. 33% of females had no symptoms (chart 7).

Antibiotic sensitivity tests were carried out on 40 isolates of Neisseria gonorrhoeae obtained in pure culture. 70% of isolates were resistant to penicillin and 30% were less sensitive to it. 52.5% of the isolates were PPNG. 57.5% of isolates were resistant to ciprofloxacin and 42.5% were less sensitive to it. 7.5% were resistant to ceftriaxone, 12.5% were resistant to cefixime and 15% were resistant to spectinomycin. All the isolates were sensitive to azithromycin (Table 2).

All the 43 patients were treated according to NACO guidelines, 20 patients with Injection Ceftriaxone 250 mg IM single dose and 23 patients with Tab. Azithromycin 2 grams single dose and were

followed up. Out of 43 patients 30 turned for follow up and all showed signs of cure. No treatment failures were noticed with ceftriaxone or azithromycin.

Associated STDs

Three male patients had HIV (6.9%). Three male patients had Syphilis and one had Genital Wart. One female patient had Trichomoniasis. (Table 3)

Table-1: Smear and culture results

	Positive		**Negative**	
	Male	**Female**	**Male**	**Female**
Smear	40	2	0	1
Culture	37	3	3	0

Table-2: Anti Microbial Sensitivity of Gonococcus

Name of the drug	Sensitive		Moderately sensitive		Resistant	
	No	%	No	%	No	%
Penicillin	0	0	12	30	28	70
Ciprofloxacin	0	0	17	42.5	23	57.5
Ceftriaxone	37	92.5	-	-	3	7.5
Cefixime	35	87.5	-	-	5	12.5
Spectinomycin	34	85	0	0	6	15
Azithromycin	40	100	0	0	0	0

Table-3: Associated STDs

	Male (No. of Patients)	Female (No. of Patients)
Syphilis	3	0
HIV	3	0
Genital Warts	1	0
Trichomoniasis	0	1

DISCUSSION

A total 43 patients with gonococcal infection were studied in detail. The analysis of age incidence in the present study showed 67.4% of patients were in the age group of 20-30 yrs which is the sexually active group where other STD are also common. The next predominant age group was 31-40 yrs. This analysis correlates with the study by Mehta Swami D et al.[69]

In our study 47.5% of the males were unmarried. Bjekic M.Vlajinac et al[70] showed that both sexes gonorrhoea infection was commonest in populations who has never married and the present study is similar.

According to our study 52.5% of males had contact with the high risk group CSW who form the core group in the transmission of STD. Jaiswal AK et al[71] reported that the main source of infection (74.5%) was female commercial sex workers. In our study also CSW played a major role in the transmission of gonorrhoea.

In our study 2.5% of males had homosexual orientation and 12.5% of males had bisexual orientation. Dafferty WF[72] reported 8.4% gonorrhoea in homosexuals which is higher than our present study. A Mcmillan et al[73] reported that unprotected receptive anal sex was most risky factor favoring transmission of gonorrhoea and HIV. Hence anoreceptive contact tracing is an essential criteria to prevent transmission.

As per the present study 100% of males presented with urethral discharge and 33% of females were asymptomatic this correlates with Peter Leone[74] who reported 90% of newly acquired infection were symptomatic and 50% of women with endocervical infection were asymptomatic. Symptomatic males report earlier for treatment, but females who are asymptomatic do not take treatment and come with later complications like pelvic inflammatory diseases and infertility.

According to our study Gram stained smear were positive in 100% of males and 66% of females. This correlates with the study by Sherrard. J and Barlow-D[75] which showed urethral gonorrhoea was diagnosed by microscopy in 94.4% of gonorrhoea in men. Gram staining of genital secretions still remains the only widely accepted routine procedure for making an 'on the spot' diagnosis of gonococcal infection.

For interpretation of results of disk diffusion tests, three categories of results are proposed.[53]

1. A susceptible result implies a < 5% likelihood of treatment failure.

2. An intermediate result indicates predictable failure rates of 5% - 15% if the patient is treated with the tested antibiotic in the standard dosage.

3. A resistant result is associated with clinical treatment failure rates of > 15%.

Treatment failure may result from variety of causes, and patients may experience failure of therapy even when infected with isolates that manifest in vitro susceptibility. Test result must be used as an adjunct to not in place of clinical evaluation.[53]

In this study 70% of the isolates were resistant to penicillin. In 1987, 74% of isolates were found resistant to penicillin from India.[76] In a recent study 35.3% of isolates were found resistant to penicillin in New Delhi in 2003.[9]

52.5% of isolates in this study were PPNG. In a study conducted in the Institute of Venereology in 2001 the prevalence of PPNG was 35.8%. The prevalence of PPNG in different studies varies from 8-70% in South and South East-Asia.(8% in New Delhi, 15% in Dhaka, 17.8% in Thailand, 51.7% in Bombay, 52% in Indonesia and 70.7% in Philippines)

Ciprofloxacin resistance was found in 57.5% of isolates. In a study in New Delhi in 2003, 67.3% of the isolates were resistant to ciprofloxacin. This is comparable to our results.

Not even a single isolate was sensitive to neither penicillin nor ciprofloxacin in our study. Penicillin is not recommended for treatment either by WHO or by CDC. Continuing treatment with penicillin would contribute to treatment failures and prolonged period of infectivity and in turn spread of resistant strains in the community. Resistance to ciprofloxacin showed by all isolates was disturbing.

Resistance to ceftriaxone and cefixime were present in 7.5% and 12.5% of the isolates respectively. This is a matter of concern. The previous study from this Institute in 2001 showed 3.7% resistance to ceftriaxone. In 2003 5.9% of the isolates were found less sensitive to ceftriaxone in New Delhi. Out of the 3 isolates taken from patients who showed resistance to ceftriaxone 2 were treated with Inj. ceftriaxone 250 mg im single dose. But these 2 patients responded well to treatment and there was no treatment failure. (In vitro resistance).

Resistance to spectinomycin which is least used was 15% in our study.

All the 40 isolates were sensitive to azithromycin in our study. This looks promising since NACO recommends azithromycin as one of the drugs in the treatment of gonorrhoea. It also has the added advantage of covering Chlamydial infection.

Since no control strains (reference strains) were used in the study, all results must be verified by disk-diffusion or preferable by agar dilution method which also incorporates reference strains. Quality control is necessary for comparing results from different laboratories done under different conditions.

Going by the results of the study azithromycin and ceftriaxoneappears to offer hope. Further studies using agar dilution method are to be done before treatment recommendations are to be made.

Among the associated infections in this study, HIV infections were 6.9% of the total gonococcal cases. Co infection with Syphilis was present in 3 cases. All the three were latent Syphilis. One patient had Genital Wart. Out of 3 female patients one 33% had Trichomonas vaginalis infection. Vazquez-F et al[77] reported Trichomonas vaginalis isolated in gonorrhoea was 40.4% which correlates with our present study.

Surveillance for antibiotic resistant strains is essential for providing data for disease intervention.

Treatment of male patients diagnosed by gram-stained smear at Primary care level should be done according to prevailing guidelines. At tertiary care levels it is better to treat patients based on sensitivity reports at least for cases with treatment failures.

Surveillance work should include both Government and private sectors. Prompt analysis and review of the data should be done so that appropriate adjustments can be made in disease intervention activities and therapy recommendations. Appropriate funds should be allotted under STD control programmes for establishing laboratory facilities for surveillance. Training laboratory personnel and quality control procedures should be incorporated in surveillance programmes. Gonococcal Antimicrobial Surveillance Programme (GASP) started by the WHO in 1990 with an aim to establish a network of laboratories for surveillance based on WHO regions. Adoption of uniform methodology

by training programmes and quality control programmes are a prerequisite for involvement in GASP.

In 1987, the Centers for Disease Control and Prevention (CDC) in collaboration with other organizations implemented the Gonococcal Isolate Surveillance Project (GISP) to monitor antimicrobial resistance in the United States.

WHO has proposed wisely and widely points of action for over-coming antimicrobial resistance. These include adopting WHOs policies and strategies, educating health workers and the public on the use of medicines, measures to contain resistance in hospitals, increasing research for new drugs and increasing availability of essential drugs. WHO recommends that surveillance data be analyzed and distributed to health care workers in order to assist them in prescribing drugs appropriately.

Educating the public and health care workers on the wiser use of antimicrobial drugs is imperative to halt the spread of resistance. Governments, Professional societies and teaching institutions must keep health care priorities upto date by supplying necessary information on the selection of correct drugs, dosages and optimum treatment durations necessary for effective for patient management.

Educating consumers and the community on the judicious use of antimicrobials is also critical in tackling the problem of drug resistance.

Patients need to recognize the value of antimicrobials, how to use them and how not to use them, the importance of taking them as required, and avoiding them when unnecessary.

WHO encourage hospitals to form drugs and therapeutics committees aimed at establishing treatment guidelines. These encourage drug-use monitoring and infection control thereby preventing the transmission and spread of resistant organisms.

Encouraging the research community to develop new compounds is essential as once-effective treatment become inefficient in the face of ever evolving resistant microbes. Research into dosage regimens calculated to minimize the likelihood of selecting for resistance is also important.

CONCLUSION

1. **The results of the study indicate that multidrug resistant Neisseria gonorrhoeae is prevalent in this region.**

2. **The value of Penicillin and Ciprofloxacin as drugs for the treatment of Gonorrhoea is doubtful as all the strains were either less sensitive or resistant to these drugs.**

3. **Azithromycin looks promising with no drug resistance.**

4 **Associated STDs has to be investigated to prevent the transmission of HIV and further complications.**

5. **Need for establishing a national surveillance programme for antibiotic resistance becomes clear.**

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