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STUDY PROJECT:- TO STUDY HIGH VAGINAL SWABS IN 500 SEXUALLY ACTIVE WOMEN ATTENDING IN MATERNITY HOSPITAL, THAPATHALI TO FIND PREVALENCE OF N. GONORRHOEA INFECTION.

REPORT

SUBMITTED BY

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I. **Study Project:-** To Study high Vaginal Swabs in 500 sexually active women attending in Maternity Hospital, Thapathali to find Prevalence of N. Gonorrhoea infection.

II. **Objective of Study :**

1. To detect N. gonorrhoea infection in asymptomatic persons.
2. To detect N. gonorrhoea in asymptomatic person who are unlikely to seek diagnostic services.

III. **Age group: 18 – 40 years of age.**

IV. **Methodology and materials:**

1. In 16 months period from June 1992 to October 1993, Endo-cervical swabs of 500 sexually active women of age group 18 – 40 attending Gynae. O.P.D of Maternity Hospital were taken for isolation of N. gonorrhoea.
2. Endo-cervical swabs were taken from the first six patients attending the O.P.D.
3. For the purpose of screening of the women detail clinical history regarding occupation of husband and discharge from private parts, pain during micturation including clinical examination was completed.
4. Collection of sample:
   a) Without use of antiseptic and lubricant a sterile cotton swab was inserted into the cervical canal and the swab was rotated for absorption of the exudate. Gynecologist conducted this procedure.

b) Immediately after collection of the specimen the swab was inserted in Stuart transport medium and bottle top of the media was replaced tightly.

c) After proper labeling, the specimen was transported to Central Health Laboratory.

5. Laboratory processing of the specimen.

a) Soon after the specimen arrived in Laboratory inocculation was done in Thayer Martin medium. Refer annex I

b) The in-occulted plates were then incubated at 36° C in about 5% carbon dioxide under moist environment for 48 hours using a candle jar. Refer annex II

c) For Microscopic examination of the specimen, a smear is made on a new clean glass slide by rolling the swab gently over the slide. After drying and fixation the smear is stained by use of Gram stain. After staining the smear is examined microscopically for presence of pus cells – Neutrophils containing Gram – negative diplococci - N. gonorrhoea. – Annex III

d) Examination of the culture media after two days of incubation the culture plates are examined for growth of microorganisms in Thayer Martin and chocolate agar media. Any suspected colony of N. gonorrhoea is sub cultured in Thayer Martin media without antibiotics for growth of pure colonies for further identification and antimicrobial susceptibility. Suspected colonies in culture plates are also identified by fresh oxidise reagent. Growth of any other organism that N. gonorrhoea is also noted and identified. Apart from sub–culture, smear is also made for Gram stain to look for Gram negative diplococci. Refer annex IV

v. Observation and Finding

1. Age group – chart I

Age group	No of study samples
16 – 19	36
20 – 29	252
30 – 39	143
40 +	69
	500

Note: - 393 subjects out of 500 fall in age group in 20 - 39

2. No of children – chart II

No of children	No of study subjects
1	70
2	110
3	89
4	96
Total	

Note: – 430 subjects out of 500 have more than one child.

- 320 subjects out of 500 3 or more children

3. **Major symptoms/Clinical Diagnosis – chart III**

Symptoms/Diagnosis		No of study samples			
		Age Group			
		16 – 19	20 – 29	30 – 39	40 +
1.	Vaginitis	5	25	12	11
2.	Cervicitis	1	21	17	6
3.	Primary Sterility	2	17	1	x
4.	Secondary Sterility	x	1	x	4
5.	Pelvic inflammatory disease	1	14	11	x
6.	Urinary tract infection	2	1	x	x
7.	Prolapse	x	X	1	2
8.	Vulval Ulcer/Vaginitis	1	4	5	3
9.	Discharge – white		79	73	39

Note: – The predominant presenting symptoms are white discharge, vaginitis or cervicitis.

4. **Gram Stain Direct smear – Chart IV**

Age group	Total samples	Positive for diplococci
16 – 19	36	1
20 – 29	252	3
30 – 39	143	2
40 +	69	x
Total	500	

Note: - Out of 500 subjects only six were positive for gram negative diplococci In direct smear.

5. Culture for N. gonorrhoea (G.C.) – Chart V

Age group	Total samples	Positive for diplococci
16 – 19	36	1
20 – 29	252	2
30 – 39	143	1
40 +	69	x
Total	500	

Note: - Out of six for diplococci in gram stain only four were found to be culture positive for N.gonorrhoea.

Major findings

- About 60% of the study subjects have 3 or more children
- Vaginitis , cervicitis and white discharge were major presenting
- Symptoms of the patients attending gynae. OPD.
- 1.2 % of the study subjects were positive for N. gonorrhoea and suffering from sexually transmitted diseases
- Out of six samples positive for gram negative diplococci in direct smear only four were found by culture method – 66.6%

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9. STD Case Management Workbook – 4
   Diagnosis and Treatment.
10. STD Case Management Workbook – 5
    Educating the Patient.
11. STD Case Management Workbook – 6
    Partner Management.
12. STD Case Management Workbook – 7
    Recording and development plan

Annex I

1. The specimens received in Stuart transport medium were arranged in order. The specimens were then innoculated on Thayer Martin medium and chocolate agar medium. The inoculum was streaked by a sterile wire lop. The basic principle is the thinning out the inoculum to provide single isolated colony for identification of the pathogen. For identification and antibiotic sensitivity testing, the isolation of the pathogen is an absolute need.

2. The inoculated plates were then incubated at 36 c. in about 5% Co2.

Annex II

Procedure for Candle Jar technique :

a) In an air tight jar place a small candle along with the plates to be incubated. (The volume occupied by the plates should not be more than 1/3 of jar); ad also keep a small beaker with water (this creates humid environment).

b) Light the candle and tighten or seal the jar. The flame will use up some oxygen and then will be extinguished producing about 3.5% - 5% Co2 atmosphere.

c) It is then incubated immediately for 48 hours.

Annex III

Making of smear from swab.

1. After inoculating the plate the same swab is used for making smear. Roll the swab on a new clean slide. Rolling the swab is the method of choice to examine the intracellular organism. Rubbing of the swab is avoided because it damages the pus cells.

2. Code number is written on the slide to identify the patient and lab. record number.

Drying of smear

After preparation of smear on slide it should be protected from dust and is allowed to air dry.

Fixation of smear

The purpose of fixation is to prevent the washing out of micro-organisms from smear during the staining process.

Alcohol fixation

Fixation by this method is recommended for Gram negative intra – cellular diplococci. Fix with 2 or 3 drops of absolute methanol or ethanol. Allow the alcohol to dry on smear.

Gram stain

The Gram stain remains the stain of choice for diagnosis of N. gonorrhoeae

a. Cover the fixed smear with iodine, leave for 1 minute wash rapidly in running water.

b. Flood the slide with iodine, leave for 1 minute gently rinse with running water.

c. Decolorize with acetone – alcohol until the drops falling off the slide are no longer blue. This usually takes from 10 – 20 seconds, depending on the thickness of the smear. Excessive decolorisation must be avoided, otherwise Gram positive bacteria may appear to be Gram negative.

d. Rinse quickly in running water to stop the decolorisation and drain of excess water.

e. Counter stain with dilute carbol fuchsin for 1 minute.

f. Rinse with running water and gently blot the slide with absorbent paper.

g. Wipe the back of the slide and is then allowed to air dry.

Microscopic examination of Grams stained smear

Look for pus cells (Neutrophils) containing Gram negative diplococci because these could be N. gonorrhoeae. The size of organism 0.6 – 1 micron. They typically occur in paring with adjacent sides flattened if the Neutrophil are ruptured Gram negative cocci may be extra cellular.

Annex IV

Looking for the colony characteristic after two days incubation.

1. Thayer Martin medium N. gonorrhoea appear small, raised grey coloniesabout 1 – 2 mm in size.

2. In chocolate agar plate , the colonies appear small , raised and colourless.

3. The suspected colonies are sub – cultured in Thayer Martin medium without antibiotics to get pure growth which is required for further tests and for antimicrobial susceptibility testing. Suspected colonies can also be detected in a mixed culture by adding fresh oxidase reagent (1% solution of tetramethyl _ p_ phenylene diamine dihycrochloride)
   Note: - Oxidase positive colonies must be subcultured within 1- 2 minutes, Otherwise the organisms may not be viable.

Microscopic examination of Gram stained smear from culture plate N. gonorrhoea. N. gonorrhoea is non-motile, non-sporing, Gram negative diplococci with their joining sides flattened. When in clusters it is often difficult to see that the organisms are joined in pairs.

Identification of Neisseria gonorrhoea:

1. Routine tests (Screening test)
   The Gram stain and oxidase reaction are the screening tests employed to establish any colonies suspected of Neisseria gonorrhoea.

   Method of oxidase test :
   With use of stick or glass rod, a colony of the test organism is picked up and is then transferred on the filter paper soaked with fresh oxidase reagent (1% solution of tetramethyl – P Phylene diamine dihydrochloride)

Result:

Oxidase producing organisms e.g. N. gonorrhoea shows blue – purple colour within 10 seconds.

2. Carbohydrate degradation test has been considered the definite means of identifying N. gonorrhoea.

Non – Culture Method:

This method, developed by kellong & Turner and modified by Brown, is recommended by WHO because this is more sensitive and specific. This method which utilises preformed enzymes takes only 4 – 5 hours for reporting.

1. Sub culture the isolate on CA and incubate for 24 hours.

2. Two loopful of growth from 3 mm size loop (flame sterlised) is transferred into tube containing 0.5 ml. of BSS (Buffered Balanced Salt indicator solution.)

   Note: - Do not culture older than 24 hours which may give false negative results.

3. Arrange five tubes (70 mm x 10 mm size) for each test. Add 0.05 ml. 20% sterile Glucose, Maltose, Sucrose and Lactose to each tubes followed by 0.1 ml. BSS. The fifth tube without sugar will serve as a control.

4. 0.05 ml of the bacterial suspension is transferred to each of the five tube and mixed well.

5. Incubate them in a water bath of 37 c for 4 hours.

   Result : A colour change from red to yellow is positive, control tube has to remain red.

	Glucose	Maltose	Lactose	Saccharose
N. gonorrhoeae	+	-	-	-

Beta – Lactamase test :

This test has become a routine test for the rapid detection of ampicillin and penicillin resistance in beta lactamase producing strain of N. gonorrhoea.

Paper acidomertic method :

Oxid Beta – Lactamase papers contain benzyl penicillin and a pH indictor. Resistant strains hydrolyse the penicillin to penicilloic acid with a consequent pH change.

TECHNIQUES :

Place one drop of distilled water on a clear microscope slide and cover with a beta lactamase test paper so that the paper is moitened but notover saturated. Using a wire loop or wooden stick, take several colonies and streak on to the dampened strip. The presence of a Beta-Lactamase producing strain is indicated by the streaked portion of the strip changing colour from violet to yellow after approximately 5 minutes. Neisseria strains that do not produce beta – lactamase enzymes do not effect the colour of the paper. Known positive and negative controls should be applied to each strips.

Minimal Identification Features of N. gonorrhoea.

N. gonorrhoea Gram – negative diplococci with concave opposing edges and long axes parallel.

Oxidase test – positive.

Growth on selective GC media, No growth on nutrient agar , Maltose not fermented, Only Glucose fermented.