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RESEARCH ARTICLE

MICROBIOLOGICAL PROFILE OF CLINICALLY DIAGNOSED VAGINOSIS IN TERTIARY CARE HOSPITAL

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Abstract

Introduction: The Normally vaginal flora contains Lactobacillus more in numbers and Gardnerellavaginalis in low numbers. When Gardnerellavaginalisis outnumbered, it causes a condition called bacterial vaginosis. Lactobacillus species maintains the acidic pH of the adult vagina protecting from various infections. In pre-pubertal and postmenopausal vagina, lactobacilli are scanty, which predisposesto many infections. Around 75% of women in reproductive age will have at least one episode of candidiasis.

Aim & Objectives: 1. To know Nugent's score with Gram's staining in received samples. 2. To know the culture outcomes of received samples.

Material &Methods: This is a Retrospective study of one year duration (July 2021- June 2022). Vaginal swabs of patients received in Microbiology Department were processed.

Results: Nearly half of the samples based on Nugent score were categorized as normal flora, 86 (48.31%), 38 (21.34%) as having intermediate flora and 54 (30.33%) as bacterial vaginosis. A total of 178 samples were processed out of which 92(51.68%) were culture positive. Out of 178 samples 36 (20.22%) isolates were Candida spps. and 56 (31.46%) were bacterial isolates. All Gram-positive isolates were sensitive to vancomycin (100%). E. coli were most sensitive to imipenem, meropenem (88.46%).This is study concluded that microbiological profiles of vaginosisnecessary to diagnose and treat the patients.In this studyAerobic vaginitis is most commonly caused by E.coli and Vulvovaginal Candidiasis most commonly caused by Candida albicans (25%), which can be easily treated.

Introduction:-

Lactobacillipresent as normal flora in vagina of adult females, prevents the growth of pathogenic bacteria by producing lactic acid and maintain the acidic pH in vagina. Decrease in the concentrations of Lactobacilli

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andincrease in the concentrations of Gardnerellavaginalis, Mobiluncus (motile, curved, gram-variable or gram negative anaerobic rods) and rise in vaginal pH causes a condition called as Bacteria vaginosis.[1]In 2002, Donders et al. proposedthe concept of Aerobic vaginitis (AV).Displacement of the healthy vaginal Lactobacillus species with aerobic pathogens such as Enterococcus faecalis, Escherichia coli and Staphylococcus aureus causesa localized vaginal inflammation is called as aerobic vaginitis.(2-3). AV is characterised by yellow vaginal discharge, mucosal hyperaemia, itching, burning, and dyspareunia and accounts for 4.2–25.8% of all vaginal infections.(3,4)Around 75% of women in reproductive age have at least one episode of candidiasis.[4]In spite of the presence of Candida in the vagina does not necessarily indicate infection. Colonization of the vagina by candida usually precedes infection.[5,6,7]Presence of Lactobacilli and immune responses, allow the persistence of Candida species as vaginal commensals.[5,8]Lactobacillus species may protect against the development of Vulvovaginal Candidiasis (VVC), but there is limited data to support this hypothesis.[5,9]Frequent complaints of vaginosis are abnormal vaginal discharge, itching, burning sensation and discomfort. However, a number of vaginal infections present with few or no symptoms.[10,11]Though some pathologic conditions causing vaginosis are well defined as Bacterial Vaginosis, vaginal Candidiasis, Tricomoniasis, yet 7-72% of women with vaginosis may remain undiagnosed and have been termed as 'intermediate flora' and its management probably differ from that of bacterial vaginosis. It is important in pregnant females at the risk of pre-term delivery.[12,13]

Vaginal Candidiasis is commonly caused by C. albicans; however, infections due to non- albicans species of Candida appear to be increasing.[12,14]It should be important to diagnose bacterial vaginosis especially in pregnant females and start treatment as early as possible to prevent complications such as low birth weight infants, preterm births [15,16], postpartum endometritis[15,17], pelvic inflammatory disease [15,18], and infertility[15,19]. Microbiological diagnosis of bacterial vaginosisby scoring of the microbial flora in the Gram stained vaginal smear was first described by Spiegel et al.[15,20] in 1983, and thereafter, it is modified by Nugent et al.[15,21]With respect to the above, the present study was done keeping aims & objective as

1. To know Nugent's score with Gram's staining in received samples
2. To know the culture outcomes of received samples

Material & Methodes:-

This is a Retrospective study of one year duration (July 2021- June 2022).

High Vaginal swabs of patients received in the Microbiology department was processed as

Microscopic examination:
Smears were prepared from swabs and were air-dried, heat fixed, and gram staining was done.

Gram's staining:[1]
Heat fixed smear was covered with crystal violet for 1 minute, washed with water, flooded with Gram's iodine for 1minute, washed with water, and then decolorized with alcohol for 10-20 seconds. The smears were rinsed under running water to stop the decolourisation and then counter stained with safranin for 30 sec.. The smear was rinsed with running tap water and air-dried. Gram reaction and number of bacteria, presence of yeast cells and hyphae, presence or absence of pus cells were noted and also assessed as per the grading systems of Nugent et al.Standardized Gram stain scoring method developed by Spiegel et al[20] and thereafter it is modified by Nugent et al[21], which is followed in this study.

According to the scores smears are classified in to three categories, scores 0–3 are normal, Score 4–6 is intermediate, and Score ≥7 is bacterial vaginosis.

Aerobic bacterial culture:
The specimens were inoculated on Blood agar, MacConkey agar plates and incubated aerobically at 37 ^{o}C.

Biochemical tests[22]:
Specific identification of bacterial isolates were done by biochemical properties as per standard laboratory guidelines.

Antibiotic susceptibility testing:
Antimicrobial susceptibility testing was performed for all aerobic bacterial isolates by modified Kirby Bauer disk diffusion method as per 2021 CLSI guidelines.

Processing for fungal isolates:
The specimens were inoculated on Saboraud's dextrose agar, Cornmeal agar (to see the chlaymadospore in Candida albicans) and CHROM agar (shows different coloured colonies according to species). Differentiation between albicans and non-albicans candida was done by germ tube test.

Result:-

Total number of 178 vaginal swab received and processed..

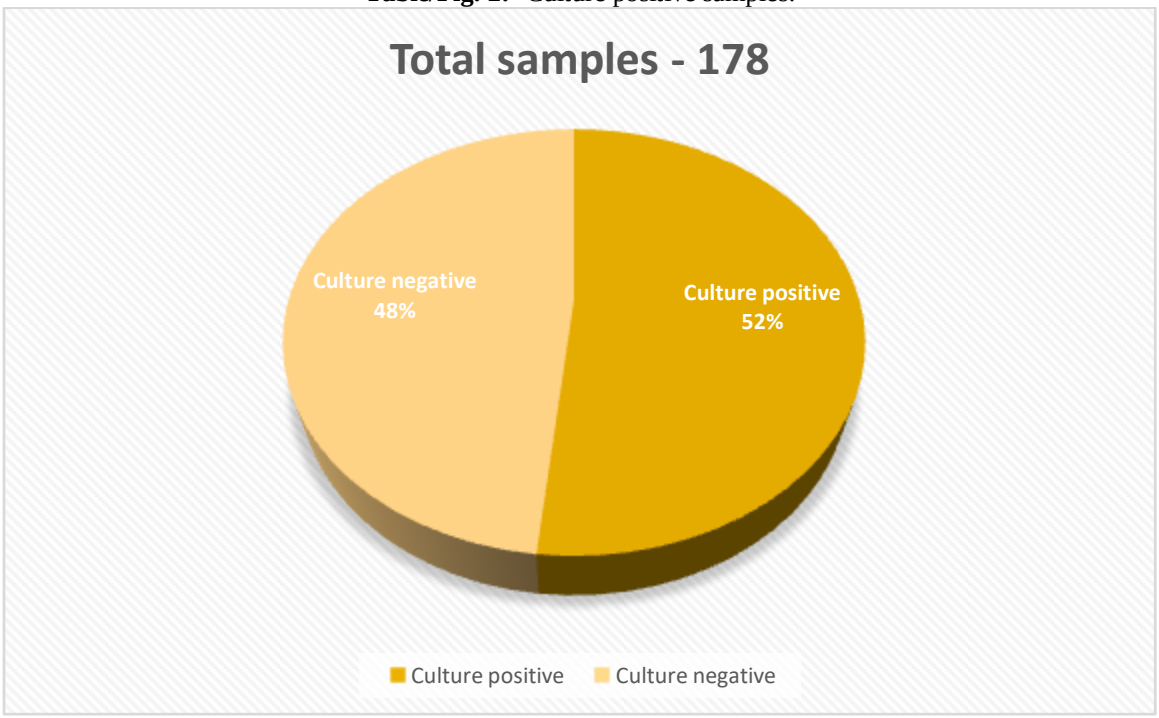
Based on Gram staining Nugent Score Nearly half of the samples86 (48.31%), were categorized as normal flora, 38 (21.34%) as having intermediate flora and 54 (30.33%) as bacterial vaginosis.

Table/Fig.1:- Grading of Nugent's score in gram stained vaginal smear.

Nugent's score	No. of samples (n=178)	Percentage (%)
< 4	86	48.31%
4-6	38	21.34%
≥7	54	30.33%
Total	178	100%

A total of 178 samples were processed out of which 92(51.68%) were aerobic culture positive. [Table/Fig.2]

Table/Fig. 2:- Culture positive samples.



Result outcome of these 2 methods (Nugent's Score and culture) shown in Table/ Fig.3.

Table/ Fig. 3:- Correlation by Nugent score and culture.

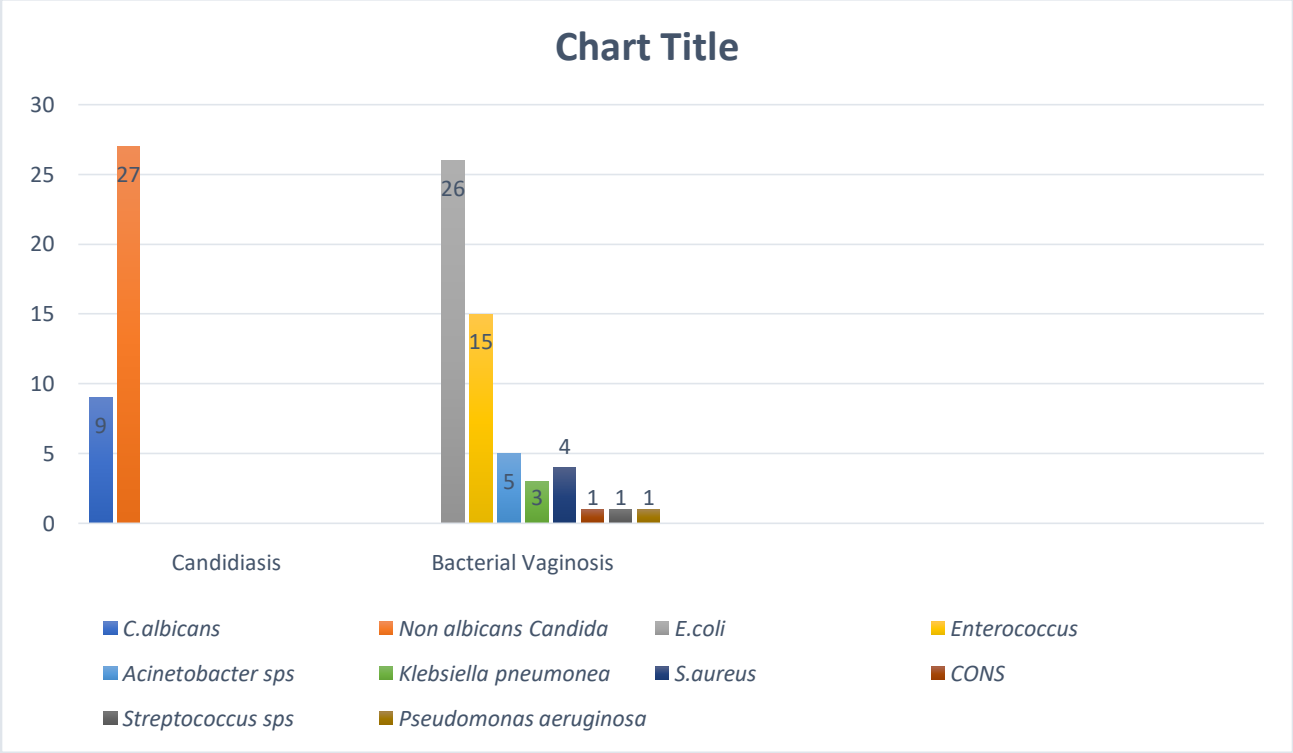
Nugent's score	Candidiasis	Vaginosis	Normal flora
< 4 (86)	1	1	84
4-6 (38)	30	7	1
≥7(54)	05	48	1

Total (178)	36	56	92

The difference was found to be statistically significant. (The chi-square statistic is 251.1165. The p-value is < 0.00001. The result is significant at p < .05).

Out of 178 samples56 (31.46%) were bacterial isolates and 36 (20.22%) isolates were candida spps. Among 36 candida isolates 9 (25%) were Candida albicans and 27 (75%) were Non-albicans Candida spss. In 56 bacterial isolates 26 were Escherichia coli, 5 were Acinetobacter, 15 were enterococcus, 4 were S. aureus, 3 were Klebsiellapneumoniae, 1 were CONS, 1 were pseudomonas, 1 were streptococci spps. [Table/ Fig. 4]

Table/ Fig. 4:- Various isolates from received samples.



All Gram-positive isolates were sensitive to Vancomycin (100%). S. aureus was most sensitive to linezolid (100%), followed by Levofloxacin, Ampicillin sulbactum, Cotrimoxazole, Ciprofloxacin (75%) and Clindamycin, Erythromycin, Teicoplanin, Gentamicin, Cefoxitin (50%) and least sensitive to Penicillin (25%). CoNS were most sensitive to linezolid, Erythromycin, teicoplanin, Gentamycin, Cefoxitin (100%), and totally resistant to clindamycin and ampicillin. Enterococcus spp. was most sensitive to Gentamicin (100%) followed by ampicillin/ sulbactam (80%) and levofloxacin (86.66%).

Table/ Fig. 5:- Antibiotic susceptibility pattern of Gram-positive isolates.

Organisms	**Ampicillin/sulbactam**	**Trimethoprim-sulfamethaxasole**	**Ciprofloxacin**	**Gentamicin**	**Vancomycin**	**Clindamycin**	**Erythromycin**	**Linezolid**	**Levofloxacin**	**Teicoplanin**	**Cefoxitin**	**High level Gentamicin**	**Penicillin**
S.aureus (4)	3 (75%	3 (75%)	3 (75%)	2 (50%)	4 (100%)	2 (50%	2 (50%	4(100 %)	3 (75%	2 (50%	2 (50%	-	1(2 5%)

	)					)	)		)	)	)		
CoNS (1)	0	0	1 (100%)	1 (100%)	1 (100%)	0	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	-	1 (10 0%)
Streptococci (1)	1 (100 %)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	-	-	1 (10 0%)
Enterococcus (15)	12 (80%)	3 (20%)	5 (33.33 %)	15 (100%)	15 (100%)	-	-	12 (80%)	13 (86.6 6%)	11 (73.3 3%)	-	6 (40 %)	6 (40 %)

E. coli was most sensitive to Imipenem, Meropenem (88.46%) followed by Amikacin& Gentamicin (76.92%) but least sensitive to Ciprofloxacin (30.76%). Klebsiella spp. was most sensitive Ciprofloxacin (75%) followed by imipenem, meropenem&amikacin (50%) and least sensitive to Trimethoprim-Sulfamethoxazole (25%) and totally resistant to Ceftriaxone, Piperacillin- Tazobactam, Cefepime. Pseudomonas sp. were most sensitive to Imipenem, Piperacillin- Tazobactam, Gentamicin, Ciprofloxacin (100%) [Table/ Fig.6]

Table/ Fig. 6:- Antibiotic susceptibility pattern of Gram-negative isolates.

Organisms	Ampicillin/sulbactam	Trimethoprim-Sulfamethaxasole	Ciprofloxacin	Gentamicin	Ceftriaxone	Imipenem	Piperacillin-Tazobactam	Amikacin	Tetracycline	Amoxyclav	Cefepime	Levofloxain	Meropenem	Tobramycin
E.coli (26)	18 (69. 23%)	10 (38.4 6 %)	8 (30 .76 %)	20 (76. 92%)	16(61 .53%)	23(88 .46%(	22(84 .61%)	20 (76. 92%)	13(5 0%)	10 (38. 46%)	8(30. 76%)	8(30. 76%)	23(88 .46%(	10 (38. 46%)
Acinet obacter (4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsi ella(4)	1(25 %)	1(25 %)	3(7 5%)	2(50 %)	0	2(50 %)	0	2(50 %)	0	2(50 %)	0	1(25 %)	2(50 %)	2(50 %)
Pseudo monas (1)	0	0	1 (10 0%)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	0	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)

Discussion:-

Bacterial vaginosis is the prevalent infection in women of reproductive age group. It is simple to treat but if left untreated can lead to complications.As the microbiological profile of vaginal infection is changing, early diagnosis will help to plan appropriate and effective therapy.In the present study out of 178 swabs, nearly half of the specimens based on Nugent score were categorized as normal flora, 86 (48.31%), 38 (21.34%) as having intermediate flora and 54 (30.33%) as bacterial vaginosis [Table/Fig:2]. A study conducted by SwapnaMuthusamyet al[23] categorized 52.30% as normal flora, 33.07% as intermediate flora and 14.61% aa bacterial vaginosis, which is comparable to present study. A study conducted by Madhivanan et al.[24] showed 65.4% had normal flora, 15.4% had intermediate flora and 19.1% had bacterial vaginosis. Dr.AnandalakshmiPonnaluri et al.[25] 46.92% cases had Bacterial vaginosis, intermediate in 38.07%, and Normal in 15.76% cases were observed.

In our study we performed aerobic culture only to know the Microbiological profile of clinically diagnosed vaginosis. In our study bacterial isolates were 31.46% and candida isolates were 20.22% [Table/fig:3].Vaidehi J Mehta et al [12]reported vaginal candidiasis is highest 54% followed by Aerobic vaginosis 38%. Mumtaz S et al[13]

reported vaginosis 76%.Chaudhary V et al[26] reported vaginosis 33.68% and candidiasis 32.6% , Mahakal N et [27] reported vaginosis 5% and candidiasis 46%, Afroz N et al[28] reported vaginosis 24% and candidiasis 24%.

Culture positivity was 31.46% and Nugent's score positivity was 30.33%in our study. The positivity of candidiasis was 20.22%[Table/Fig. 4]. Detection of positivity by culture was more than the Nugents score in our study. Which correlates well with the study by SwapnaMuthusamy, Selvielangovan et al[23] where culture positivity was 21 % and Nugent's score positivity was 19%, and krohn et al[29] where culture sensitivity was 92% and Nugent's score sensitivity was 62%. A study by udayalaxmi et al[30] showed difference in order of positives, culture as the least sensitive method.

This study showed an aerobic bacterial vaginosis contributes 56 (31.46%). Out of which maximum isolates were Escherichia coli 26(46.42%), followed by Enterococci 15(26.78%), Acinetobacter5(8.92), Staphylococcus aureus 4(7.14%), Klebsiellapneumoniae 3(5.35%), Pseudomonas 1(1.78%), CONS 1(1.78%) and Streptococci spps were 1 (1.78%) [Table/fig.5].Dr.AnandalakshmiPonnaluri et al showed[25]E.coli (21.53%), Coagulase negative staphylococcus (24.23%), Enterococcus (19.23%) Staphylococcus (18.46%), Acinetobacter (8.46%), Pseudomonas aeruginosa (4.23%), Klebsiella(3.84%).Mahaseth BK, Malla TB et al[32] reported that E.coli and staphylococcus aureus contributing 7(38.88%) cases which was followed by Klebsiella 2(11.11), acinetobacter and streptococcus 1(5.55%). Pal K et. al,[32] reported that E.coli (24.92%) and Klebsiellapneumoniae (23.50%) were the most common followed by S.aures (16.52%). Mumtaz S et al[13]., found S. aureus in (46.07%) followed by E.coli (13.7%) 8.Tansarli et al[33]. and Zarbo et al[34]. also reported a high incidence of S.aureus(41.7%) and (27.9%) respectively. Sangeetha KT et al[2] reported that enterococcus faecalis (32.26%) was the most prevalent organism isolated which was followed by E. coli (25.8%) and staphylococcus an aureus (22.6%)3. Whereas the study conducted by Khan and Khan et al[35] reported, Enterococcus faecalis (31%) as the most frequently isolated vaginal pathogen.

Uut of 178 samples vaginal candidiasis were seen among 36 (20.22%) patients.Out of this 27 (75%) were Non-albicans Candida spss.In contrast to present study low percentage has been reported byDr.anadalakshami et al[25] 6.92% and Renu Mathew [36] 4.2% and hig percentage has been reported by VarshaChaudhary[26]reported Candida in 32.66% patients.

Antibiotic susceptibility pattern of isolates:

1. S. aureus: It develops resistance rapidly to different antibiotics by production of either plasmid encoded beta-lactamase and modification of target site -penicillin binding proteins.[37] In present study S.aureus were most sensitive to Vancomycin, linezolid (100%), followed by Ampiciilin-sulbactum, Trimethoprime-sulfamethaxasole, ciprofloxacin, levofloxacin (75%) and Gentamycin, Clindamycin, Erythromycin, Teicoplanin, Cefoxitin (50%) and least sensitive to penicillin (25%).Vaidehi J. Mehta et al[12] reported S.aureus were most sensitive toVancomycin (100%) followed by Linezolid (83.3%), Levofloxacin, Ampicillin/ sulbactam(AS) and Trimethoprim sulfamethoxazole 66.6%. S.aureus shows least sensitivity to Clindamycin and Gentamicin (50%).Mumtaz S et al[13] has reported sensitivity of S.aureus to Vancomycin, Trimethoprim sulfamethoxazole and Gentamicin 93.6%, 23.6% and 67.6% respectively.

2. **CONS:** In present study CONS were sensitive to almost all drugs.Vaidehi J. Mehta et al[12] shows Coagulase negative Staphylococci were most sensitive to Vancomycin (100%) followed by Ciprofloxacin (66.6%) and AS (33.3%) and least sensitive to Cefoxitin (16.66%). Mumtaz S et al(13) has shown 90.7%, 94.4%, 78.7% and 36.7% sensitivity to Vancomycin, PT, Ciprofloxacin and Gentamicin for CONS.

3. **Enterococcus sps:** Enterococci sp are resistance to commonly use antibiotics but can be best treat with vancomycin.[38] However, emergence of high level aminoglycoside resistance and acquired glycopeptides resistance (vancomycin resistant Enterococci sp. VRE) has necessitated the need of sensitivity.(22) In present study, Enterococcus sp. were most sensitive to Vancomycin and Gentamicin (100%) and least sensitive to Cotrimoxazole (12.5%) which is comparable with Vaidehi J. Mehta et al[12]Mumtaz et al.[13]

4. **Streptococcus sps:**Snsitive to most of the drugs.WondemagenMulu et al [11] revealed 20 % resistance rate against ciprofloxacin, norfloxacin, erythromycin and clindamycin.

5. **E.coli:**In present study, E.coliwere most sensitive to Imipenem and meropenem (88%) followed by PT (84%)Amikacin (76%).Which is comparable with Vaidehi J. Mehta et al[12]Mumtaz et al.[13]

6. **Klebsiellasp:** In present study, Klebsiella spp. were most sensitive to Ciprofloxacin (75%) followed by Imipenem, Meropenem (50%),and resistant to Cotrimoxazole (0%). Vaidehi J. Mehta et al[12] shows sensitivity to Imipenem 93.3% followed by Ciprofloxacin (98%) and Cotrimoxazole (200%). Mumtaz S et al[13] has shown 100%, 95.8%, 80.2% and 61.8% sensitivity to PT, Imipenem, Ciprofloxacin and Cotrimoxazole for Klebsiella sp.

respectively. Sangheeta et al[2]have reported 100% sensitivity towards Meropenem and 60% to Ciprofloxacin and Cotrimoxazole too.

7. **Pseudomonas sps:** in this study sensitive to almost drugs. Sangheeta et al [2] shows sensitivity to piperacillin 100%

8. **Acinetobactersps**: In our study resistant strain were isolated. Mahaseth BK, Malla TB et al[32]reported 100% sensitivity to Ciprofloxacin, Cefteriaxone, Doxycycline.

Conclusion:-

This study conclude that Nugent score is specific but needs microbiology expertise and culture has its roleto know about the causative agentand therefore helpful in the selection of empiric antimicrobial therapy of aerobic vaginosis.E.coli and Candidasps were predominant isolates found in present study followed by Enterococcus, klebsiella, acinetobactersps.

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