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A STUDY ON DIAGNOSIS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF AEROBIC BACTERIA ISOLATED FROM ABNORMAL VAGINAL DISCHARGE

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ABSTRACT **Introduction:** Diagnosing exact cause of bacterial vaginosis is a challenging task microbiologically, as coinfections may exist and also many organisms are responsible for vaginal and cervical infections. We have undertaken this study to create awareness among clinicians about various emerging pathogens responsible for bacterial vaginosis and their resistance patterns. **Materials and Methods:** During the study period of one year (January 2019 to December 2019), a total of 146 vaginal discharge samples were collected and processed for culture and sensitivity in Department of Microbiology. Standard Quality Control strains were used as a part of testing. All the results of culture and sensitivity were entered into spread excel sheet and evaluated. **Results:** On assessment of culture study, out of 146 organisms isolated, 58 (39.7%) were Klebsiella species, 34 (23.2%) were Staphylococcus aureus, 29 (19.8%) were Escherichia coli, 13 (8.9%) were Candida species, 6 (4.1%) were Pseudomonas species, 3 (2.05%) were Coagulase Negative staphylococci, 2 (1.3%) were Citrobacter species and one (0.6%) isolate was Streptococcus viridians. More than 50% of isolates showed sensitivity to colisin, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, piperacillin+tazoabactum, ceftazidime+tazobactum, amoxyclav. 50% of Staphyloccocus aureus were MRSA. **Conclusion:** Now-a-days, bacterial infections are emerging and also their resistance patterns differs, so there is a greater need to treat patients based on culture and sensitivity report. This will definitely aid us to provide accurate treatment, to avoid emergence of resistant bugs and to alleviate health care costs.

KEYWORDS : Abnormal vaginal Discharge, Bacteria, Sensitivity pattern

INTRODUCTION

Abnormal vaginal discharge is a common outpatient clinical condition in reproductive age group; women won't seek medical attention at early stage, as it is a usual physiological phenomenon. Most of these cases have been diagnosing and managing clinically and also most neglected problem. Clinicians are fails to provide accurate management if they won't opt for laboratory tests [1].

Bacterial vaginosis is the most common cause of abnormal vaginal discharge. Abnormal vaginal discharge includes three clinical presentations - bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis. It is the replacement of good normal flora of vagina by bad or pathogenic bacteria such as Anaerobes, Escherichia coli, Klebsiella,Gardenerella, Mycoplasma etc.; usually associated with group of anaerobic pathogens [2].

Bacterial vaginosis is most prevalent in reproductive age group worldwide [3], presents with abnormal vaginal discharge, features of it described as timing, colour, consistency, smell, itching or abdominal pain association, fever, dyspaerunia, pelvic tenderness. To aid in the management of STD patients in rural areas where there are minimal facilities of testing, WHO has developed a set of syndromic management guidelines for treatment of symptomatic men and women.

Diagnosing exact cause of bacterial vaginosis is a challenging task microbiologically, as coinfections may exist and also many organisms are responsible for vaginal and cervical infections. Clinicians are fails to provide accurate management if they won't opt for laboratory tests [1]. Misdiagnosis of bacterial vaginosis may lead to severe complications including high HIV risk, urinary tact infection, secondary infections due to scratching, persistent abdominal pain, infertility, abortion, chorioamnionitis, endometritis after miscarriage, pelvic inflammatory disease [4,5].

Clinically Bacterial vaginosis can be diagnosed by appearance of discharge, a pungent odor. Discharge can be investigated for the presence of clue cells, whiff test and pH >4.5. The gold standard diagnosis is gram stain and culture. We have undertaken this study to create awareness among clinicians about various emerging pathogens responsible for bacterial vaginosis and their resistance patterns. The aim of this study is to know the incidence of bacteria responsible for abnormal vaginal discharge and their antibiotic susceptibility patterns.

MATERIALS AND METHODS

This is a prospective cross sectional study conducted in the department of Microbiology, Government Medical College, Kurnool. Reproductive age group patients presenting with abnormal vaginal discharge to department of Obstetrics and Gynaecology were included in this study.

INCLUSION CRITERIA:

Patient's in the age group of 20-50 years
Patient's willing to get tested by vaginal discharge culture and sensitivity
Patient's who gave consent for further study.

EXCLUSION CRITERIA:

Trichomoniasis and Gardenerella vaginosis
Patient's with abnormal uterine bleeding
Patient's with cervical or vaginal mass
Patient is in Menstruating period

Study Period and Sample processing:

During the study period of one year (January 2019 to December 2019), a total of 146 vaginal discharge samples were collected and processed for culture and sensitivity. Two high vaginal swabs have been collected during Gynaecological examination using Sim's speculum under aseptic precautions. Vaginal discharge type, wet mounts, pH, amine test was done and findings noted at the OPD itself. One vaginal swab collected was used for gram staining and another swab was inoculated on Nutrient agar, Mac conkey Agar, Sheep blood Agar and Sabourads dextrose Agar and incubated at 370c for 24-72 hours.

After inoculation daily observation of media was made, colonies were examined and identified by Biochemical reactions and other tests according to CLSI guidelines. Antibiotic susceptibility testing was done on Muller Hinton Agar by modified Kirby bauer disk diffusion method using clinical and laboratory standard institute guidelines (CLSI). Antibiotic disks used for Gram positive organisms testing were Penicillin (10U), Gentamicin (10µg), amikacin (30µg), ciprofloxacin (5 µg), erythromycin (5µg), clindamycin (2µg), cotrimoxazole (1.25 µg/23.75 µg), cefoxitin (30 µg), linezolid (30 µg), vancomycin (30µg) and teicoplanin (30µg), amoxyclav (30 µg). Gram negative isolates antibiotics were: amoxyclav (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefixime (30 µg), Gentamicin (10µg), amikacin (30µg), ciprofloxacin (5 µg), imipenem (10 µg), Ceftazidime+clavulanic acid

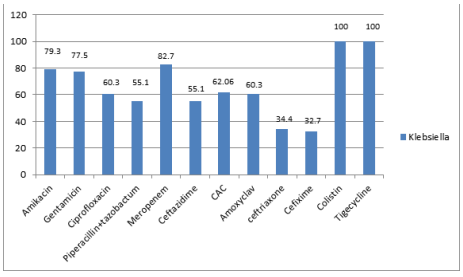
(30/10 µg), meropenem (10 µg), amikacin (30 µg), colistin (10 µg), tigecycline (15 µg). Standard Quality Control strains were used as a part of testing. All the results of culture and sensitivity were entered into spread excel sheet and evaluated.

RESULTS

On assessment of culture study, out of 146 organisms isolated, 58 (39.7%) were Klebsiella species, 34 (23.2%) were Staphylococcus aureus, 29 (19.8%) were Escherichia coli, 13 (8.9%) were Candida species, 6 (4.1%) were Pseudomonas species, 3 (2.05%) were Coagulase Negative staphylococci, 2 (1.3%) were Citrobacter species and one (0.6%) isolate was Streptococcus viridians.

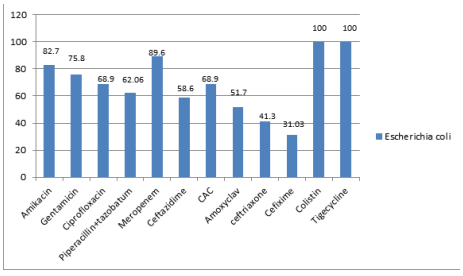
Out of 58 Klebsiella species, all the 58 (100%) isolates showed sensitive towards colistin, tigecycline, around 45 (77%) isolates showed sensitivity towards amikacin, gentamicin, meropenem, around 35 (60%) isolates showed sensitive to ciprofloxacin, ceftazidime+clavulanic acid, amoxyclav, piperacillin+tazobactum, ceftazidime and below 20 (34%) isolates were sensitive to cefixime and ceftriaxone.

Fig 1. Antibiotic susceptibility pattern of Klebsiella species



Out of 29 Escherichia coli isolates, all the 29(100%) isolates showed sensitive towards colistin, tigecycline, 26 (89.6%) isolates were sensitive to meropenem, around 22 (75%) isolates showed sensitivity towards amikacin, gentamicin, ciprofloxacin, around 18 (62%) isolates showed sensitive to ceftazidime+clavulanic acid, piperacillin+tazobactum, ceftazidime and ≤ 15 (51%) isolates were sensitive to amoxyclav, cefixime and ceftriaxone.

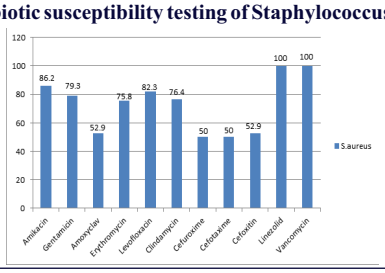
Fig 2. Antibiotic susceptibility pattern of Escherichia coli



Out of 6 Pseudomonas isolates, 100% isolates were sensitive to colistin, 83.3% were sensitive to imipenem, meropenem, 66.6% isolates were sensitive to amikacin, gentamicin, ceftazidime+ clavulanic acid, piperacillin+tazobactum, ciprofloxacin, 50% isolates were sensitive to ceftazidime, cefipime and 33.3% were sensitive to piperacillin.

Out of 34 Staphylococcus aureus isolates, 100% were sensitive to linezolid, vancomycin, 80-70% were sensitive to levofloxacin, erythromycin, clindamycin, around 50% isolates were sensitive to cefoxitin, ceftriaxone, cefuroxime, amoxyclav and around 20% isolates showed sensitive to amikacin, gentamicin.

Fig 3. Antibiotic susceptibility testing of Staphylococcus aureus



Among 3 isolates, 2 were MRCoNS. All the isolates of Citrobacter species (2) were sensitive strains, no resistance patterns detected. One isolate of Streptococus viridans was a sensitive strain.

DISCUSSION

Bacterial vaginosis is a common infection caused due to disturbance in normal flora, need to evaluate properly, as it may leads to increase in antimicrobial resistance mechanisms development with in the microbial population. Vaginal discharge composition, amount, quantity, cells varies from person to person, it is produced by the cells of vagina and cervix. Bacterial vaginosis is not considered under sexually transmitted infections.

The main reason behind bacterial vaginosis is decrease in lactobacilli (normal vaginal flora) and increase in a multitude of anaerobic bacteria and pathogenic aerobic bacteria with the most predominant organism being Gardenerella vaginalis, but the absolute concept of alteration of bacterial flora in vagina is not fully known. Factors most associated with Bacterial vaginosis are unprotected sex, antibiotic use, douching, intrauterine device.

In this study, out of 146 organisms isolated, 58 (39.7%) were Klebsiella species, 34 (23.2%) were Staphylococcus aureus, 29 (19.8%) were Escherichia coli, 13 (8.9%) were Candida species, 6 (4.1%) were Pseudomonas species, 3 (2.05%) were Coagulase Negative staphylococci, 2 (1.3%) were Citrobacter species and one (0.6%) isolate was Streptococcus viridians.

A study by Gopalan U et al [6] in 2016 from Tamilnadu on vaginal microbiota among symptomatic women observed that 18.2% Escherichia coli, 9% Klebsiella pneumoniae, 7.3% Klebsiella oxytoca, 6% Staphylococcus, 4.7% Pseudomonas aeruginosa, 4.4% Acinetobacter, 2% enterococcus faecalis, 1.8% Citrobacter and Streptococcus pyogenes each, 1.4% Candida, 0.8% Proteus species, 0.2% Streptococcus pneumoniae and Enterobacter aerogenes each, 0.05% of Citrobacter freundii, Citrobacter koseri, Pseudomonas putida, Morganella morganii.

More than 50% of isolates showed sensitivity to colisin, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, piperacillin+tazoabactum, ceftazidime+tazobactum, amoxyclav in this study. Gopalan U et al [6] stated that 73.2% of the isolates were sensitive to Imipenem, 70.4% were sensitive to amikacin, 65.7% were sensitive to gentamicin and 47.2% sensitivity towards ciprofloxacin. 12.3% of Staphylococcal isolates were MRSA.

Lakshmi K et al [7] did a study in pathogens responsible for bacterial vaginosis in pre and post menopausal women, stated that 15.2%, 8.7% and 19.6% of Escherichia coli, Staphylococcus aureus and Candida species were isolated in premenopausal women. Whereas Escherichia coli, Staphylococcus aureus and Candida species of 14.8%, 9.3% and 13% were isolated from post menopausal women.

Narayana-Swami N et al [8] observed most pathogen causing vaginal infections was Escherichia coli followed by Streptococcus agalactiae and diphtheroids with equal incidence. Among the antibiotics tested, isolated pathogens were completely resistant to nalidixic acid and highly sensitive to meropenem and imipenem.

Hamidi Ahsan et al [9] isolated 28%, 24%, 18%, 11%, 5%, 4%, 3%, 2%, 2%, 1% of Escherichia coli, S.aureus, Klebsiella pneumoniae, Enteorbacter aerogenes, Streptococcus agalactiae, Citrobacter fruendii, Providencia rettgeri, Streptococcus pyogenes, Citrobacter diversus, Proteus mirabilis from abnormal vaginal discharge.

The incidence of vaginal infections was 27%, in which 15 samples were micrococci and 2 samples yielded candida, as per Kumar G et al [10].

Most of the studies have shown that penems showed better sensitivity, but these are reserved antibiotics and also very expensive. Accurate diagnosis with culture and sensitivity is most important to treat vaginal infections. Newer therapeutic approaches include the development of new drugs, phage therapy (bacterial viruses can be robust anti-bacterial agents in vitro), photodynamic inactivation of micro-organisms and immunomodulators [11].

CONCLUSION

In conclusion we noted Klebsiella species, Escherichia coli and Staphylococcus aureus are predominant pathogens responsible for

bacterial infection in vagina. ESBL strains were noted in 38% and 31% in Klebsiella species and Escherichia coli respectively. 50% of MRSA isolates were noted. Abnormal uterine discharge is a under diagnosed condition among reproductive age group women. Most of the women won't seek medical attention and also clinicians usually treat on OPD basis as a gardenerella vaginalis or a candida infection. Now-a-days, bacterial infections are emerging and also their resistance patterns differs, so there is a greater need to treat patients based on culture and sensitivity report. This will definitely aid us to provide accurate treatment, to avoid emergence of resistant bugs and to alleviate health care costs.

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