PREVALENCE OF MICROBIAL INFECTION OF PREGNANT WOMEN AT DIFFERENT TRIMESTERS WITH OBSTETRIC COMPLICATIONS

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ABSTRACT

Microbial infection with some species of microorganisms during pregnancy can affect the health of pregnant women, resulting in high morbidity and complication rates during pregnancy. The aim of this study was to investigate the prevalence of microbial infection in pregnant women according to trimesters and to determine whether the presence of abnormal vaginal colonization is associated with a higher risk of miscarriage, preterm labour (PTL), and premature rupture of membranes (PROM). This study was carried out on 200 pregnant women between 20 and 35 years old with obstetric complications. The clinical specimens were collected and cultured on different culture media. The isolates were subcultured on specific culture media and subjected to identification using morphological staining, cultural characteristics and biochemical tests. The total number of microorganisms isolated was 359, Gram-negative 203 (56.6%), followed by 116 Gram-positive bacteria (32.3%), and 40 fungal isolates (11.1%). The highest isolation rate of Gram negative bacteria was *E. coli* (27.3%) as shown in 1st trimester (10.3%), 2nd trimester (5%) and 3rd trimester (12%), while the lowest isolation rate was *C. diversus* (0.56%). Whereas the highest isolation rate of Gram positive bacteria was *E. faecalis* (17.8%) as shown in 1st trimester (6.4%), 2nd trimester (4.46%) and 3rd trimester (6.96%), while the lowest isolation rate was *S. pyogenes* (0.56%) and *K. kristinae* (0.28%). The highest isolation rate of fungal isolates was *C. ablicans* (7.8%) as shown in both 1st trimester and 2nd trimester (2.23%, each) and 3rd trimester (3.34%), while the lowest isolation rate was *C. tropicalis* (0.28%) and *C. parapsilosis* (0.28%). Moreover, all the isolates were subjected to an antimicrobial susceptibility test conducted using modified Kirby-Bauer disk diffusion method. The isolates showed different antimicrobial susceptibility patterns to the tested antimicrobial agents with various percentages. All the isolates of *N. gonorrhoeae* were resistant to tested antibiotics while all the isolates of *C. diversus* and *S. agalactiae* were sensitive to the tested antimicrobial agents. Analysis of phenotypic resistance of beta lactam antibiotics showed that 41 (21.93%) out of 187 isolates of Gram negative bacteria were positive for extended-spectrum β-lactamases (ESBls) production, the highest incidence was 25 (13.37%) isolates of *E. coli*, while the lowest incidence was *P. mirabilis* (one

isolate). Whereas 19 (10.16%) out of 187 isolates were positive for *Amp*C synthesis, the highest frequency was 12 (6.4%) isolates of *E. coli* while the lowest frequency was one isolate of *P. mirabilis*. In addition, only 10 (5.3%) out of 187 isolates of Gram negative bacteria that represented 10 (29.4%) out of 34 *P. aeruginosa* isolates were positive for metallo-β-lactamases (MBLs) production. On the other hand, MBLs were not detected in *E. coli, K. pneumoniae, C. diversus* and *P.mirabilis*. In conclusion, a significant percentage (97%) of the studied pregnant participants with experienced obstetric complications had microbial colonization correlated to the different trimesters. In order to prevent complications associated to childbirth, routine laboratory examinations during pregnancy, such as urine cultures and vaginal swabs are advised to be carried out.

Keywords: Microbial infection, pregnant women, obstetric complications, antimicrobial susceptibility.

INTRODUCTION

Vaginal microbiome composition changes when women become pregnant. Pregnancy is accompanied by a shift in the community of vaginal bacteria to a composition that is typically dominated by *Lactobacillus spp.* **(Nunn *et al.*, 2021).** This is associated with clinical symptoms, an elevated vaginal pH (usually ≥4.5) and presence of white adherent discharge (which contains exfoliated epithelial cells with Gram-variable polymorphic rod-shaped bacteria attached to their surfaces and a fishy odor) **(Superti and De Seta 2020)**.

The most common resident Lactobacilli of vagina include *L. crispatus, L. iners, L. gasseri*, and *L. jensenii*. Prior to culture independent techniques, *L. crispatus* was considered as the predominant species **(Mehta *et al.*, 2020)**. Lactobacilli that are better adapted to the vaginal environment of women may colonize better and protect against vaginal pathogenic bacteria. The ability of *Lactobacillus spp.* to inhibit the growth of several bacterial species, including *Gardnerella vaginalis, Peptostreptococcus spp*. and *Bacteroides spp*. is at first due to the production of a low pH and hydrogen peroxide **(Reid *et al.*, 1993*)***. The normal vaginal flora includes *S. epidermidis* and *Micrococcus spp*., other microorganisms in the vagina that may become pathogenic include *S. aureus, Enterococcus spp.,* beta haemolytic *Streptococcus spp., Neisseria spp., E. coli, Klebsiella spp., Proteus spp.* and *Candida spp.* **(Witkin *et al.*, 2021)**.

Microbial vaginosis is typically polymicrobial, characterized by the presence of mainly anaerobic microorganisms including *G. vaginalis, Prevotella spp., Bacteroides spp., Mycoplasma hominis,* and *Mobiluncus species*. Aerobic vaginitis was first characterized in 2002, as a vaginal condition distinct from bacterial vaginosis, which may require different clinical management and have distinct clinical risks **(Kaambo *et al.*, 2018)**. Aerobic vaginitis caused by bacteria like *S. aureus, E. coli*, Group B Streptococci (GBS), *Listeria spp., Mycoplasma* and *Ureaplasma species* **(Sgayer *et al.*, 2020)**. Microbial candidiasis is a vaginal mycosis infection. It is one of the most common vaginal infections in women, in the fertile period and also the most frequent

and most important fungal disease of vaginal content **(Chudzicka-Strugała *et al.*, 2024)**.

Since a susceptible bacterium can develop resistance, acquired resistance is the primary problem in clinical practice. It can happen as a result of exposure to antibiotics mutating bacterial genes or as a result of bacterial species acquiring resistance genes through three different processes: conjugation, transformation and transduction **(Okaiyeto *et al.*, 2024)**. Emergence of antibiotic resistance has been identified as a global health concern. Multiple mechanisms of antibiotic resistance are displayed by Gram-negative bacteria, which eventually lead to the establishment of multidrug resistance **(Mousavi *et al.*, 2021)**. The most public mechanisms of antibiotic resistance in Gram-negative bacteria include ESBLs, *Amp*C beta-lactamase and MBLs production. *E. coli* and *K. pneumoniae* show an increased frequency of expression of ESBLs and *Amp*C. *K. pneumoniae* and *Pseudomonas spp*. have been reported to produce MBLs frequently **(Chanu *et al.*, 2019)**.

The purpose of this study was to determine the prevalence of abnormal vaginal microorganisms in pregnant women according to the trimesters and whether the presence of abnormal vaginal colonization is linked to an increased risk of miscarriage, preterm labor (PTL), premature rupture of membranes (PROM). Also, it was designed to investigate antimicrobial susceptibility of the isolated microorganisms and the phenotypic analysis of the most resistant isolates of Gram negative bacteria to beta lactam antibiotics.

Materials and Methods

This study was conducted at the antenatal care clinic of Obstetrics and Gynecology Department of Al-Zahraa and Ain Shams University Hospitals, during the period from November 2019 till November 2022. Written informed consent was obtained from all participants after defining the nature of research. The study was approved by the research ethical committee of Faculty of Pharmacy (Boys -Cairo), Al - Azhar University. The study was done according to the code of ethics of the World Medical Association.

A. Patients:

Two hundreds of pregnant women were recruited from attendants of the antenatal care clinic of Obstetrics and Gynecology Department. The sample size was calculated according to the prevalence of the growth of organisms observed among women diagnosed with preterm premature rupture of membranes (PPROM) that was found to be approximately 85% according to **Ambalpady *et al.* (2022).** The following formula was used: sample size (N) = $(Z_{\alpha/2})^2$ *p*(1-p) / MOE^2 **(Daniel, 1999)**. One hundred ninety five patients resulted from the sample size formula.

Where Zα/2 is the critical value of the normal distribution at α/2 (for a confidence level of 95%, α is 0.05 and the critical value is 1.96), MOE is the margin of error = 5%, p is the prevalence of the growth of organisms. Accordingly, this study was conducted on 200 pregnant women with obstetric complications.

1. Inclusion criteria:

Pregnant women of age group 20-35 years old with singleton pregnancy, gestational age from 6 to 40 ws pregnancy and complicated by abortion (blighted ova, threatened abortion, missed abortion, complete abortion), PTL, PROM and PPROM, all confirmed by ultrasound examination.

2. Exclusion criteria:

Multiple gestations and medical condition predisposing to abortion as: diabetes mellitus, chronic hypertension, endocrinal diseases, autoimmune diseases, renal diseases, blood diseases, and patients who received antimicrobial therapy within 4 weeks before sampling were excluded from the study.

B. Methods:

1. For all cases, the following procedures were performed:

a) Careful history was taken in details.
b) Full general, abdominal and local examination.
c) Investigations**:** Ultrasound examination for confirmation of pregnancy, gestational age and types of complications. Complete investigation including complete blood count (CBC), random blood sugar, prothrombin time (PT), prothrombin time with international normalized ratio (PT/INR), C-reactive protein (CRP), and complete urine analysis were also performed.

2. Collection of samples:

After putting patients in dorsal lithotomy position, before local examination, sterile vaginal speculum was applied. Samples were collected from the posterior vaginal fornix and vault of vagina by sterile cotton swabs. This was done by obstetrician to ensure that the patient fulfilled the inclusion criteria for the study and two high vaginal swabs were taken from each patient. One swab was placed in a tube containing 1 mL of 0.9% NaCl (**Katila, 2016; Salinas *et al.*, 2020)** and the other swab was placed in 5 mL of Lim broth **(Jones *et al*., 1983; Wafaa *et al.*, 2019)**. The swabs ends were broken, and the swabs were left in the tubes. Finally, the tubes were closed, numbered and transferred to the laboratory. The patients were followed up by the obstetrician until end of microbiology examination. All fetal or maternal complications were documented, tabulated and statistically analyzed.

3. Culture methods:

The first swab was transferred and then incubated in 5 mL tryptone soya broth for 18 h at 35° C and then subcultured on Mannitol Salt Agar (MSA), MacConkey agar and cetrimide agar according to **Katila (2016); Salinas *et al.* (2020)**. The second swab was incubated for 18 h at 35° C and then subcultured on blood agar incubated under aerobic and anaerobic condition, chocolate agar incubated microaerophilically and

Sabouraud Dextrose Agar (SDA) for 24-48 h at 35° C **(Jones *et al*., 1983; Wafaa *et al*., 2019)**.

4. Species identification:

The isolated organisms were subjected to identification using morphological staining and cultural characteristics. The biochemical reactions for Gram negative bacteria included indole, methyl red, Simmon citrate utilization, Voges-Proskauer, catalase, urease, oxidase, motility, Triple Sugar Iron Agar (TSI), pseudomonas P agar, glucose fermentation, hydrolysis of gelatin, hydrolysis of starch, nitrate reduction and bile esculin hydrolysis tests. The biochemical reactions for Gram positive bacteria included catalase, oxidase, motility, coagulase, sodium hippurate hydrolysis, bacitracin resistance, Christie–Atkins–Munch-Peterson (CAMP), hydrolysis of starch, D-mannitol fermentation and bile esculin hydrolysis tests **(Brenner *et al*., 2005**; **Vos *et al*., 2009; Purty *et al*., 2013)**. Candida isolates grown on SDA were identified according to their colony morphology after incubation on specific chromogenic Candida agar (**Titan Biotech LTD.)** for 24-48 h at 35° C **(Nadeem *et al*., 2010)**.

5. Antimicrobial susceptibility determination:

The antimicrobial susceptibility test was performed using the modified Kirby–Bauer disk diffusion method on plates of Mueller-Hinton Agar (MHA) (**Oxoid, UK**) according to the Clinical and Laboratory Standards Institute guidelines **CLSI (2018)**. Briefly, from overnight bacterial growth, 3–5 pure colonies of the isolate were emulsified in 3–4 mL of sterile physiological saline until it matched the turbidity of the 0.5 McFarland Standard. The suspension was uniformly inoculated onto the surface of MHA plates using a sterile cotton swab. Antimicrobial disks were placed manually on the inoculated MHA plates and incubated at 37° C for 16-24 h. The zones of inhibition were measured using a caliper after the period of incubation. The result was interpreted as sensitive (S), intermediate (I), and resistant (R) according to **CLSI (2023)**. The antimicrobial disks (**Bioanalyse**, **Turkey**) used are shown in **Table (1)**. The antifungal activity of amphotericin B and fluconazole against *Candida spp*. was performed in flat-bottom well microdilution plate. For the most part, MIC distributions created by the EUCAST **(Arendrup *et al*., 2020)**.

Table 1: Antimicrobial disks used.

Antimicrobial agent	Code	(μg) /Disk	Antimicrobial agent	Code	(μg) /Disk
Amikacin	AK	30	Ceftazidime	CAZ	30
Amoxicillin-clavulanate	AMC	20/10	Imipenem	IPM	10
Azithromycin	AZM	15	Cefotaxime/clavulanic acid	CTC	30/10
Aztreonam	ATM	30	Nitrofurantoin	F	300
Cefazolin	CZ	30	Ceftazidime/clavulanic acid	CAC	30/10
Cefepime	FEP	30	Mecillinam	MEC	10
Cefotaxime	CTX	30	Piperacillin	PRL	100
Cefoxitin	FOX	30	Tetracycline	TE	30
Ceftriaxone	CRO	30	Cefpodoxime	CPD	10
Cefuroxime	CXM	30	Rifampin	RA	5
Levofloxacin	LEV	5	Ciprofloxacin	CIP	5
Penicillin	P	10	Spectinomycin	STP	100
Gentamicin	CN	10	Erythromycin	E	15
Clindamycin	DA	2	Trimethoprim	TMP	5
Chloramphenicol	C	30	Linezolid	LNZ	30

6. Phenotypic analysis of β-lactams resistance of Gram negative bacteria:

6.1. Phenotypic production of extended-spectrum β-lactamases (ESBLs):

6.1.1. Screening test for ESBLs production:

The screening tests were done according to **CLSI (2023)** using standard disk diffusion method. The antibiotic disks including cefotaxime, ceftazidime, aztreonam and ceftriaxone (30 μg) were placed on MHA plates inoculated with the test organism and incubated for 16-24 h at 37° C. The indicative inhibition zones diameters for ESBLs production are ≤ 27, 22, 27 and 25 mm, respectively.

6.1.2. Phenotypic confirmatory tests for ESBL production:

6.1.2.1. Disk potentiation test:

The confirmatory test was done according to **CLSI (2023)** depending on using both cefotaxime and ceftazidime alone and in combination with clavulanic acid. The test was done by using the standard disk diffusion method on MHA plates: ceftazidime 30 μg alone and ceftazidime-clavulanate 30/10 μg as well as cefotaxime 30 μg alone and cefotaxime-clavulanate 30/10 μg. Incubation was done for 16-24 h at 3٧° C. The positive ESBLs production result was obtained if there was an increase of ≥ 5 mm in the zone diameter for either antibiotic tested in combination with clavulanate compared with the zone diameter of the antibiotic tested alone **(Sakaeda *et al.*, 2023)**.

6.1.2.2. Double-disk synergy test:

The test was performed as prescribed by **Drieux *et al.* (2008)**; **Garrec *et al.* (2011); Diab *et al.* (2018)**. The test Gram negative organism was inoculated on the surface of MHA plate from an inoculum corresponding to 0.5 McFarland as the routine susceptibility test by the disk diffusion method. An amoxicillin-clavulanate (20/10 μg) disk was manually placed at 20-20 mm, on the surface of MHA plates center to center of cefotaxime (30 μg), ceftriaxone (30 μg), cefpodoxime (10 μg), ceftazidime (30 μg), cefepime (30 μg) and aztreonam (30 μg) disks on the plate. A positive test indicating

ESBLs production was obtained when the inhibition zone around any of the 6 antibiotic disks was enhanced on the side of the clavulanic acid containing disk, resulting in a characteristically shaped zone referred to as a champagne-cork or keyhole and ellipsis or phantom image.

6.2. *Amp*C β-lactamases and ESBLs coproduction:

The test is based on the inhibitory effect of cloxacillin on *Amp*C production. The test isolates were inoculated on MHA plates. Cefoxitin (30 μg) disks either alone or supplemented with 200 μg cloxacillin were applied. An amoxicillin-clavulanate (AMC 20/10 μg) disk was manually placed at 20-20 mm, on the surface of MHA center to center of ceftazidime (CAZ 30 μg) and aztreonam (ATM 30 μg) disks. These plates were incubated and the diameters of the cefoxitin inhibition zones with and without cloxacillin were compared; if the difference in the inhibition zone was ≥4 mm, the isolate was considered positive for *Amp*C production. A positive test indicating ESBLs production was interpreted as described above **(Tan *et al.*, 2008**).

6.3. Metallo-β-lactamases (MBLs):

MBLs production was detected in isolates that have been resistant to or have an intermediate response to carbapenems using double disk combined test (imipenem-EDTA combined disk test). The test organisms were inoculated on plates of MHA as recommended by the **CLSI (2018)**. Two disks of imipenem (10 μg) were placed on the plate and 4 μL EDTA solution (18.75%) were added to one of them to obtain a desired concentration of 750 μg of EDTA. The inhibition zone of imipenem and imipenem-EDTA disks were compared after 16-24 h of incubation at 37° C. An increase in inhibition zone diameter of the imipenem-EDTA disk ≥7 mm more than the imipenem disk alone, the test was considered positive for MBLs production **(Yong *et al.*, 2002)**.

7. Statistical analysis:

Data collected were reviewed, coded, and statistically analysed by using SPSS program (statistical package of social science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Mean and standard deviation were calculated to measure the central tendency and dispersion of quantitative data, while the frequency of occurrence was calculated to measure qualitative data. Comparing groups was done using Chi-square-test (χ^2), for comparison of qualitative data. The level of significance was taken a *p*-value of <0.05 and the results were represented in different Tables and Figures.

RESULTS

This is a prospective study carried out on 200 pregnant women, 149 (74.5 %) outpatients from clinics of Obstetrics and Gynecology Department of Al-Zahraa University Hospital and 51 (25.5%) out patients from Ain Shams University Hospital **(Figure 1)**. Patients who suffered from abortion, PTL, PPROM and PROM were included in this study from November 2019 to November 2022.

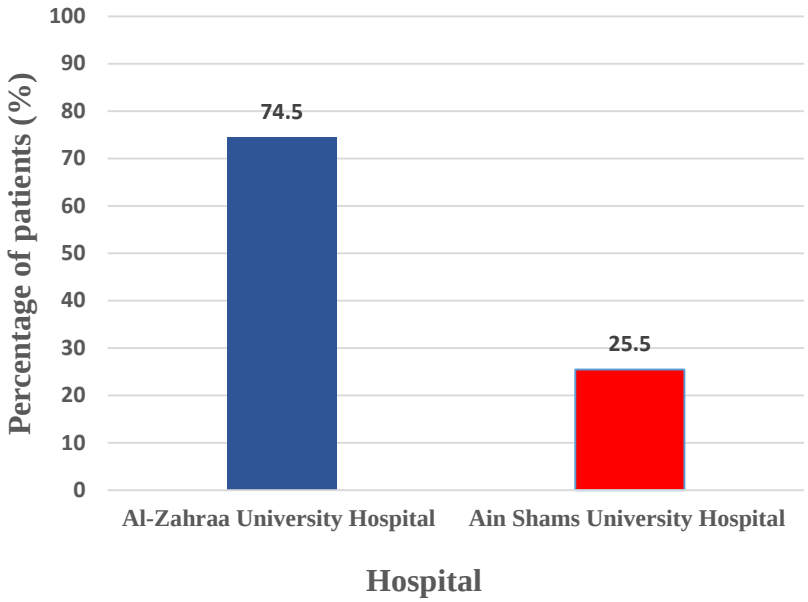


Figure 1: Distribution of patients from the two hospitals involved in the study.

1. Prevalence of different types of obstetric complications among studied patients:

The percentages of patients with abortion (blighted ova, missed abortion, inevitable abortion and threatened abortion), PTL, PPROM and PROM were 7.5%, 19.5%, 1%, 12.5%, 9%, 12.5% and 12.5%, respectively at Al-Zahraa University Hospital. While the percentages of patients from Ain Shams University Hospital were 1.5%, 7.5%, 0%, 2%, 0%, 1% and 13.5 %, respectively. The total percentages of patients were 9%, 27%, 1%, 14.5%, 9%, 13.5% and 26%, respectively **(Figure 2)**.

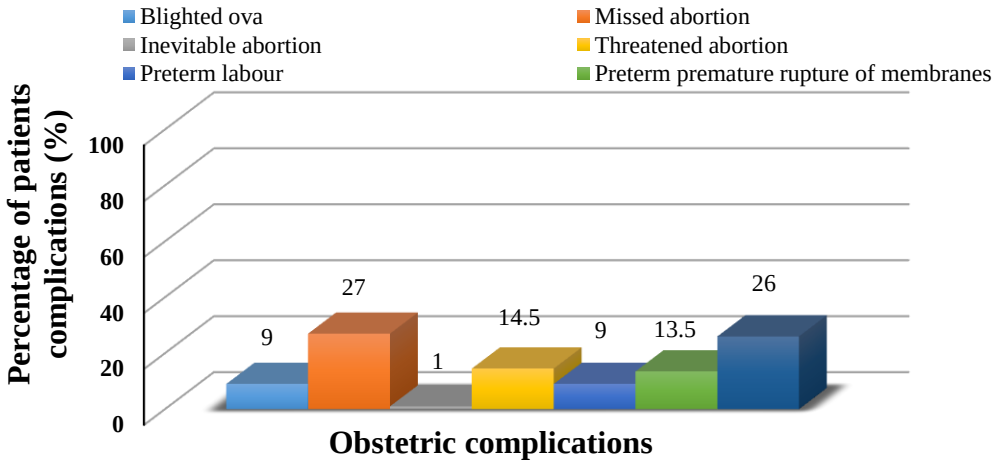


Figure 2: The frequency of different types of obstetric complications among the studied patients.

2. Distribution of obstetric complications among patients in different trimesters:

The incidence of missed abortion in the 1st trimester was 22.5% compared to 4.5% in the 2nd trimester with statistically significant difference, threatened abortion in the 1st trimester was 9% compared to 5.5% in the 2nd trimester with statistically significant difference, PTL in the 2nd trimester was 2% compared to 7% in the 3rd trimester with statistically significant difference and PPROM in the 2nd trimester was 5% compared to 8.5% in the 3rd trimester with statistically significant difference **(Tables 2 & 3)**.

Table 2: Distribution of obstetric complications (abortion) among studied patients in the 1st trimester and 2nd trimester.

Types of Obstetric Complications	1st Trimester		2nd Trimester		*P*-value
	Number	%*	Number	%*	
Blighted ova	18	9	0	0	0.001**
Missed abortion	45	22.5	9	4.5	0.002**
Inevitable abortion	2	1	0	0	0.269***
Threatened abortion	18	9	11	5.5	0.001**
Total	83	41.5	20	10	0.001**

***Percentage was correlated to total number of patients.**

****Significant difference between trimesters groups regarding complications (*P*-value <0.05).**

*****Non-significant difference between trimesters groups regarding complications (*p*-value >0.05).**

Table 3: Distribution of obstetric complications (PTL, PROM and PPROM) among patients in the 2nd and 3rd trimesters.

Types of Obstetric Complications	2nd Trimester		3rd Trimester		*P*-value
	Number	%	Number	%	
PTL	4	2	14	7	0.014**
PPROM	10	5	17	8.5	0.001**
PROM	0	0	52	26	0.001**
Total	14	7	83	41.5	0.001**

***Percentage was correlated to total number of patients.**

****Significant difference between trimesters groups regarding complications (*P*-value <0.05).**

3. The frequency of various microbial isolates collected from pregnant women with obstetric complications:

A total of 359 microbial isolates were isolated from the study group of pregnant women as follow; 203 Gram-negative (56.6%) with high incidence of *E. coli* (27.3%)

and a lower incidence *C. diversus* (0.56%), followed by 116 Gram-positive bacteria (32.3%) with high incidence of *E. faecalis* (17.8%) and a lower incidence *K. kristinae* (0.28%), and 40 fungal isolates (11.1%), mostly *C. albicans* (7.8%) and a lower isolation rate of *C. tropicalis* and *C. parapsilosis* at percentage of 0.28%, each (**Figures 3 & 4)**.

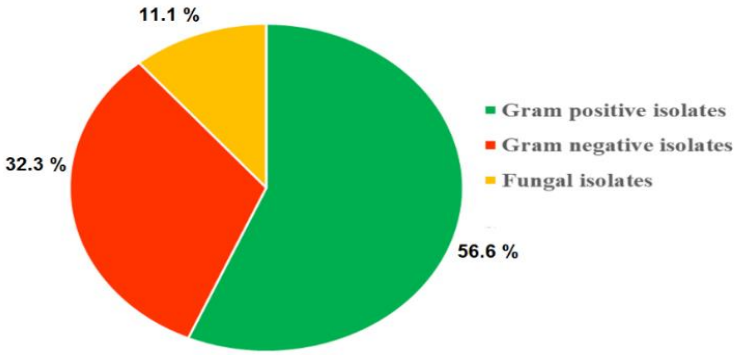


Figure 3: Distribution of different groups of microorganisms isolated.

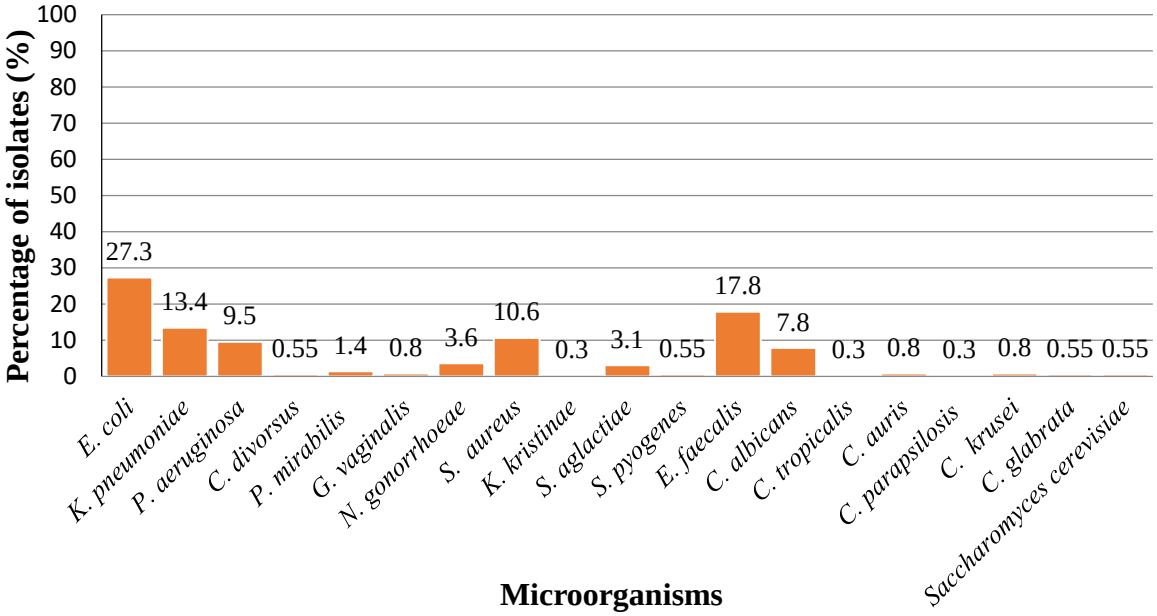


Figure 4: Distribution of various microbial isolates collected from pregnant women with obstetric complications.

Regarding obstetric complications, this study showed higher isolation rate of Gram negative bacteria in case of missed abortion (13.14%) followed by PROM (12.84%) while the least isolation rate was for inevitable abortion (0.84%). The higher incidence of bacterial species was 7% for *E. coli* in PROM while the least incidence of *E. coli* was 0.28% in inevitable abortion. On the other hand, the higher isolation rate of Gram positive bacteria was in case of missed abortion (10.3%) while they not detected

in inevitable abortion. The higher incidence of bacterial species was 5.29% for *E. faecalis* in missed abortion while they not detected in inevitable abortion.The higher isolation rate of fungi was in case of PROM (3.07%), followed by PPROM and missed abortion (2.23%, each) while they not detected in inevitable abortion. The higher incidence of *C. albicans* was found in missed abortion and PROM (1.95%, each) while they not detected in inevitable abortion **(Table 4).**

Table 4: Prevalence of microbial isolates among patients with different obstetric complications.

Pathogen	Total		Blighted ova		Missed abortion		Inevitable abortion		Threatened abortion		PTL		PPROM		PROM	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Gram-negative bacteria	**203**	**56.6**	**17**	**4.74**	**47**	**13.14**	**3**	**0.84**	**34**	**9.48**	**17**	**4.73**	**39**	**10.87**	**46**	**12.84**
E. coli	98	27.3	9	2.51	23	6.4	1	0.28	18	5	7	1.95	15	4.18	25	7
K. pneumoniae	48	13.4	6	1.67	10	2.8	1	0.28	6	1.7	5	1.4	9	2.51	11	3.06
P. aeruginosa	34	9.5	1	0.28	8	2.28	0	0	5	1.39	2	0.55	13	3.62	5	1.4
C. diversus	2	0.56	0	0	0	0	0	0	1	0.28	1	0.28	0	0	0	0
P. mirabilis	5	1.38	1	0.28	2	0.55	0	0	0	0	0	0	0	0	2	0.55
G. vaginalis	3	0.83	0	0	0	0	0	0	0	0	2	0.55	1	0.28	0	0
N. gonorrhoeae	13	3.6	0	0	4	1.11	1	0.28	4	1.11	0	0	1	0.28	3	0.83
Gram-positive bacteria	**116**	**32.3**	**6**	**1.66**	**37**	**10.3**	**0**	**0**	**20**	**5.58**	**13**	**3.61**	**23**	**6.39**	**17**	**4.7**
S. aureus	38	10.6	0	0	17	4.73	0	0	5	1.4	2	0.55	6	1.67	8	2.23
K. kristinae	1	0.28	0	0	0	0	0	0	0	0	0	0	0	0	1	0.28
S. aglactiae	11	3.04	2	0.55	0	0	0	0	3	0.84	2	0.55	2	0.55	2	0.55
S. pyogenes	2	0.56	0	0	1	0.28	0	0	0	0	0	0	1	0.28	0	0
E. faecalis	64	17.8	4	1.11	19	5.29	0	0	12	3.34	9	2.51	14	3.89	6	1.67
Fungi	**40**	**11.1**	**1**	**0.28**	**8**	**2.23**	**0**	**0**	**6**	**1.67**	**6**	**1.67**	**8**	**2.23**	**11**	**3.07**
C. albicans	28	7.8	1	0.28	7	1.95	0	0	4	1.11	4	1.11	5	1.39	7	1.95
C. tropicalis	1	0.28	0	0	0	0	0	0	0	0	0	0	0	0	1	0.28
C. auris	3	0.84	0	0	0	0	0	0	0	0	2	0.55	0	0	1	0.28
C. parapsilosis	1	0.28	0	0	0	0	0	0	0	0	0	0	0	0	1	0.28
C. krusei	3	0.84	0	0	0	0	0	0	1	0.28	0	0	1	0.28	1	0.28
C. glabrata	2	0.56	0	0	1	0.28	0	0	0	0	0	0	1	0.28	0	0
S. cerevisiae	2	0.56	0	0	0	0	0	0	1	0.28	0	0	1	0.28	0	0
Total	359	100	24	6.7	92	25.6	3	0.9	60	16.7	36	10	70	19.5	74	20.6

***Percentage was correlated to total number of isolates.**

4. Prevalence of microbial isolates according to trimesters of pregnancy:

The present study illustrated a higher isolation rate of Gram negative bacteria. The incidence of *E. coli* (12%) in the 3rd trimester was higher than the 1st (10.3%) and 2nd (5%) trimesters. The isolation rate of Gram positive bacteria, mostly *E. faecalis* (6.96%) was isolated in a high incidence rate in the 3rd trimester followed by the 1st (6.4%) and 2nd (4.46%) trimesters. Fungal isolates mostly *C. albicans* were isolated in the 3rd trimester (3.34%) more than the 1st and 2nd (2.23%, each) trimesters **(Table 5)**.

Table 5: Prevalence of microbial isolates according to trimesters of pregnancy.

Microorganisms	Number of Isolates		1st Trimester		2nd Trimester		3rd Trimester		*p*-value
	No.	%	No.	%	No.	%	No.	%	
Gram-negative bacteria	**203**	**56.6**	**73**	**20.33**	**40**	**11.1**	**90**	**25.07**	**0.417****
E. coli	98	27.3	37	10.3	18	5	43	12	0.498**
K. pneumoniae	48	13.4	15	4.2	11	3.06	22	6.1	0. 882**
P. aeruginosa	34	9.5	12	3.34	6	1.7	16	4.5	0.771**
C. diversus	2	0.56	1	0.28	0	0	1	0.28	0.735**
P. mirabilis	5	1.4	2	0.56	2	0.56	1	0.28	0.503**
G. vaginalis	3	0.83	0	0	0	0	3	0.83	0.136**
N. gonorrhoeae	13	3.6	6	1.7	3	0.8	4	1.1	0.6**
Gram-positive bacteria	**116**	**32.3**	**42**	**11.7**	**30**	**8.34**	**44**	**12.26**	**0. 362****
S. aureus	38	10.6	16	4.5	10	2.8	12	3.34	0. 315**
K. kristinae	1	0.28	0	0	0	0	1	0.28	0.516**
S. aglactiae	11	3.1	3	0.8	3	0.8	5	1.4	0.861**
S. pyogenes	2	0.56	0	0	1	0.28	1	0.28	0.498**
E. faecalis	64	17.8	23	6.4	16	4.46	25	6.96	0.751**
Fungal	**40**	**11.1**	**10**	**2.79**	**11**	**3.06**	**19**	**5.25**	**0. 414****
C. albicans	28	7.8	8	2.23	8	2.23	12	3.34	0.67**
C. tropicalis	1	0.28	0	0	1	0.28	0	0	0.176**
C. auris	3	0.8	0	0	0	0	3	0.8	0.136**
C. parapsilosis	1	0.28	0	0	0	0	1	0.28	0.516**
C. krusei	3	0.83	0	0	1	0.28	2	0.55	0.452**
C. glabrata	2	0.56	1	0.28	0	0	1	0.28	0. 735**
S. cerevisiae	2	0.56	1	0.28	1	0.28	0	0	0.429**
Total	359	100	125	34.9	81	22.5	153	42.6	

No.: Number; %: Percentage.

5. Antimicrobial susceptibility testing:

5.1. Antimicrobial susceptibility of *Escherichia coli*:

In the present study the most isolates of *E. coli* were resistant to ceftriaxone (84.7%) and cefotaxime (87.7%). Regarding cefazolin, mecillinam, cefuroxime and tetracycline, 78.6%, 77.5%, 77.5% and 75.5% of isolates were resistant, respectively. The resistance to ciprofloxacin and nitrofurantoin was 4٠.٨% and 12.2%, respectively. In contrary, only one *E. coli* isolate showed resistance to amikacin and no isolate was resistant to imipenem **(Figure 5)**.

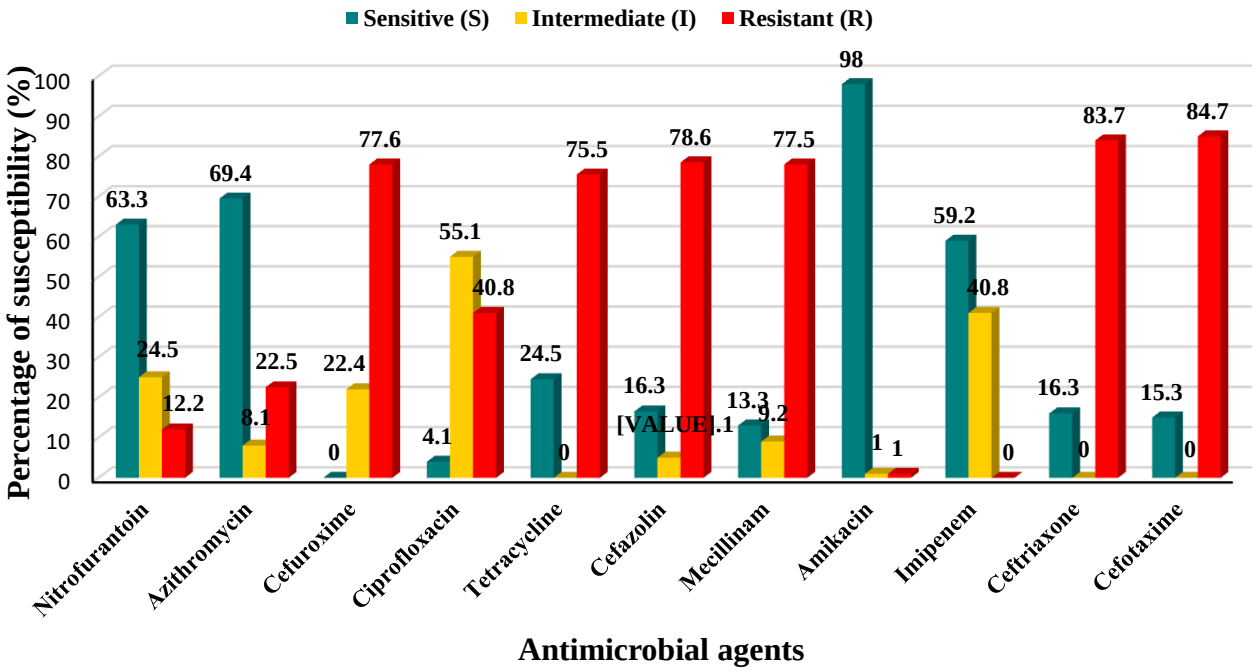


Figure 5: Antimicrobial susceptibility of *Escherichia coli.*

5.2. Antimicrobial susceptibility of *Klebsiella pneumoniae*:

All *K. pneumoniae* isolates (48) were resistant to cefuroxime (100%). Most of these isolates were resistant to cefotaxime, mecillinam and tetracycline; 89.6%, 85.4% and 83.3%, respectively. They showed resistance to azithromycin, ciprofloxacin, cefazolin and nitrofurantoin of 77.1%, 75%, 70.8% and 45.8%, respectively. No isolate resistant to amikacin and imipenem was detected **(Figure 6)**.

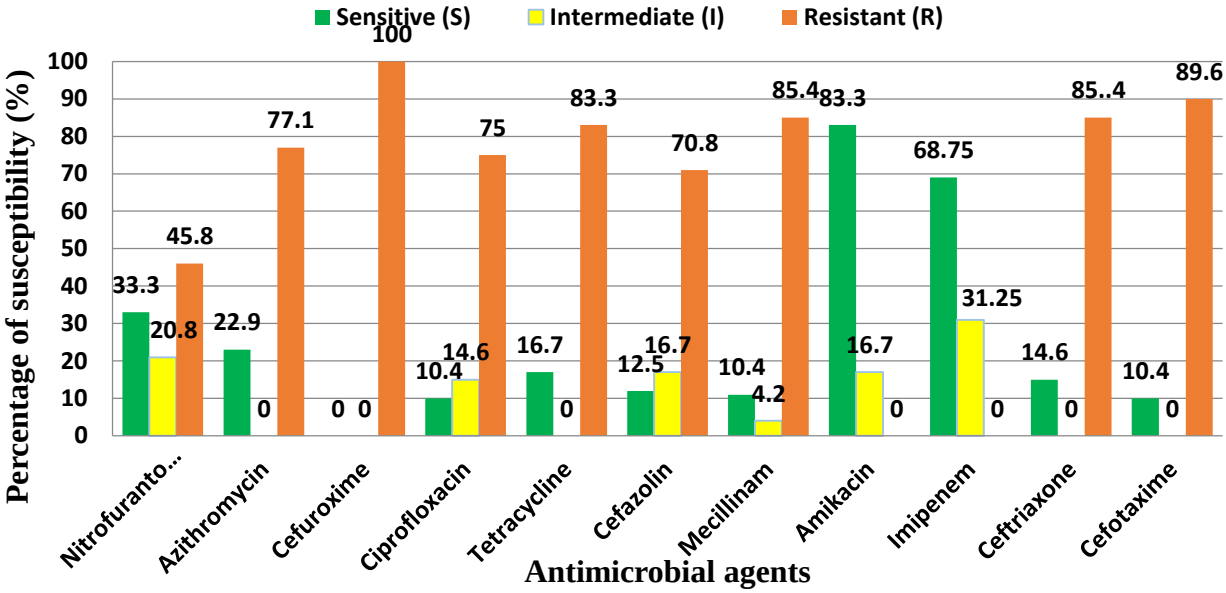


Figure 6: Antimicrobial susceptibility of *Klebsiella pneumoniae*.

5.3. Antimicrobial susceptibility of *Pseudomonas aeruginosa*:

In the present study, *P. aeruginosa* isolates were resistant to levofloxacin (50%), piperacillin (47.1%), ceftazidime (44.1%), imipenem (38.2%), cefepime (38.2%) and ciprofloxacin (32.4%). No resistance was found to amikacin **(Figure 7)**.

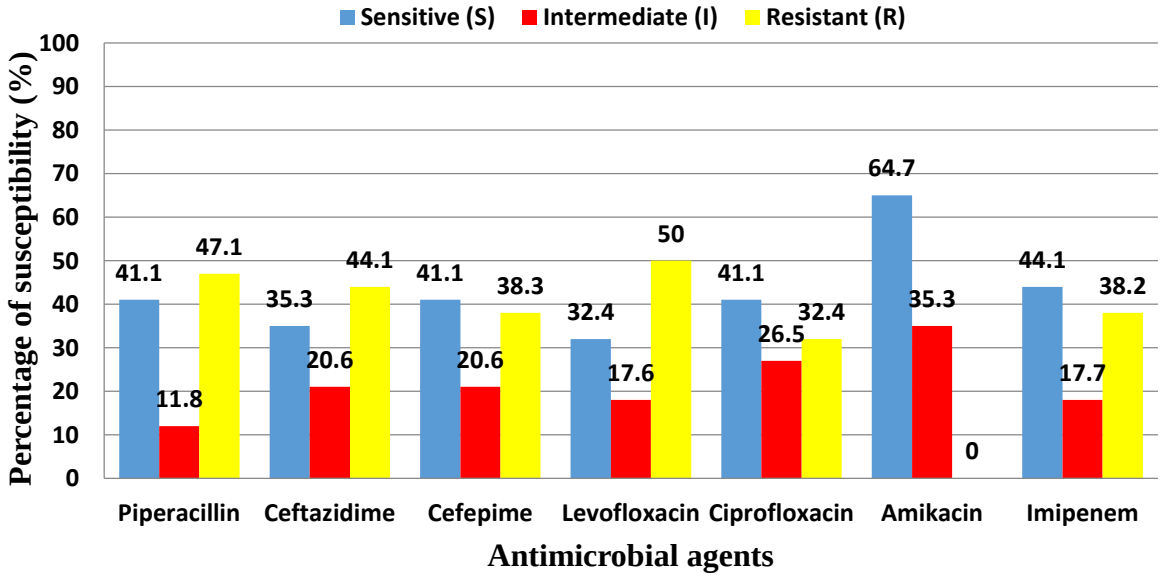


Figure 7: Antimicrobial susceptibility of *Pseudomonas aeruginosa*.

5.4. Antimicrobial susceptibility of *Proteus mirabilis*:

The antimicrobial susceptibility of the five isolates of *P. mirabilis* showed that three (60%) were resistant to cefotaxime and ceftriaxone whereas only two isolates (40%) were resistant to cefuroxime, mecillinam, tetracycline and cefazolin. No resistance was found to nitrofurantoin, azithromycin, ciprofloxacin, amikacin and imipenem **(Figure 8)**.

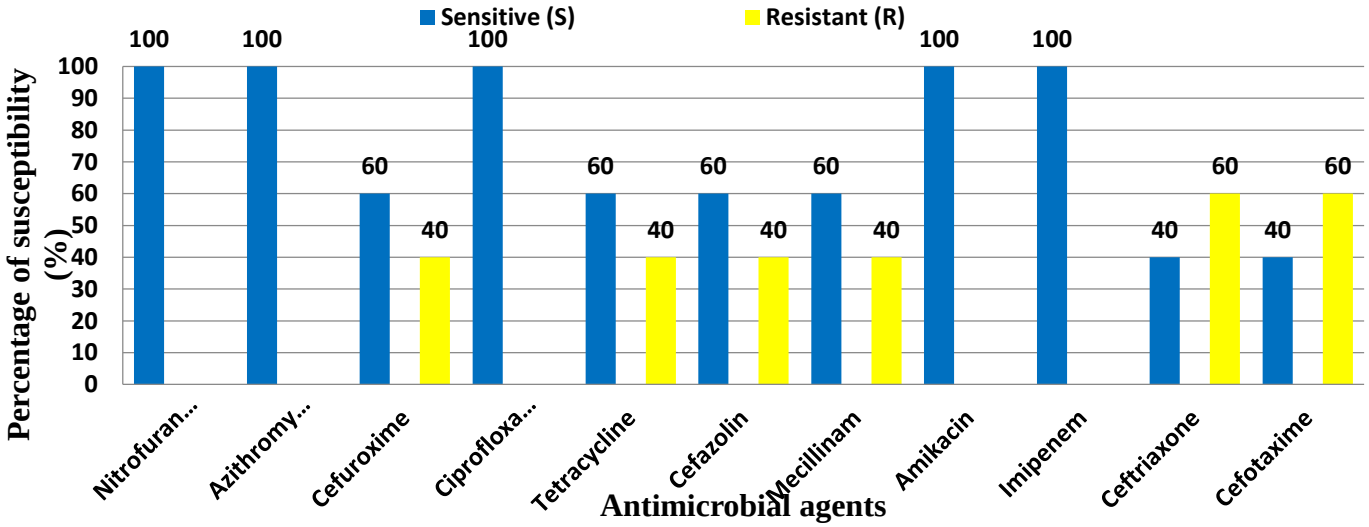


Figure 8: Antimicrobial susceptibility of *Proteus mirabilis*.

5.5. Antimicrobial susceptibility of *Citrobacter diversus* and *Neisseria gonorrhoeae*:

In the present study the two *C. diversus* isolates were sensitive to all tested antimicrobials and showed no intermediate activity or resistance **(Figure 9)**. In contrast, all *N. gonorrhoeae* isolates (13) were resistant to all tested antibiotics **(Figure 10)**.

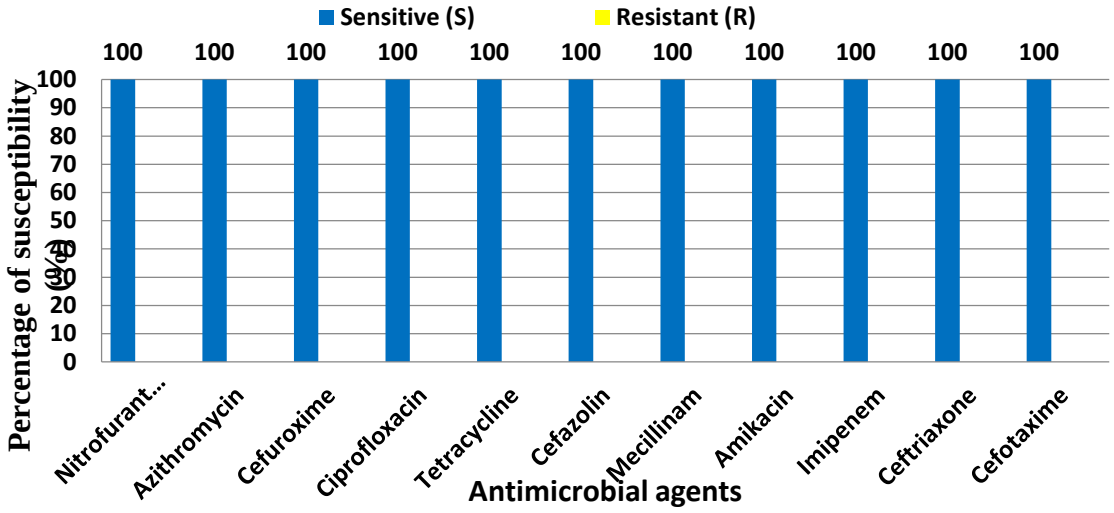


Figure 9: Antimicrobial susceptibility of *Citrobacter diversus*.

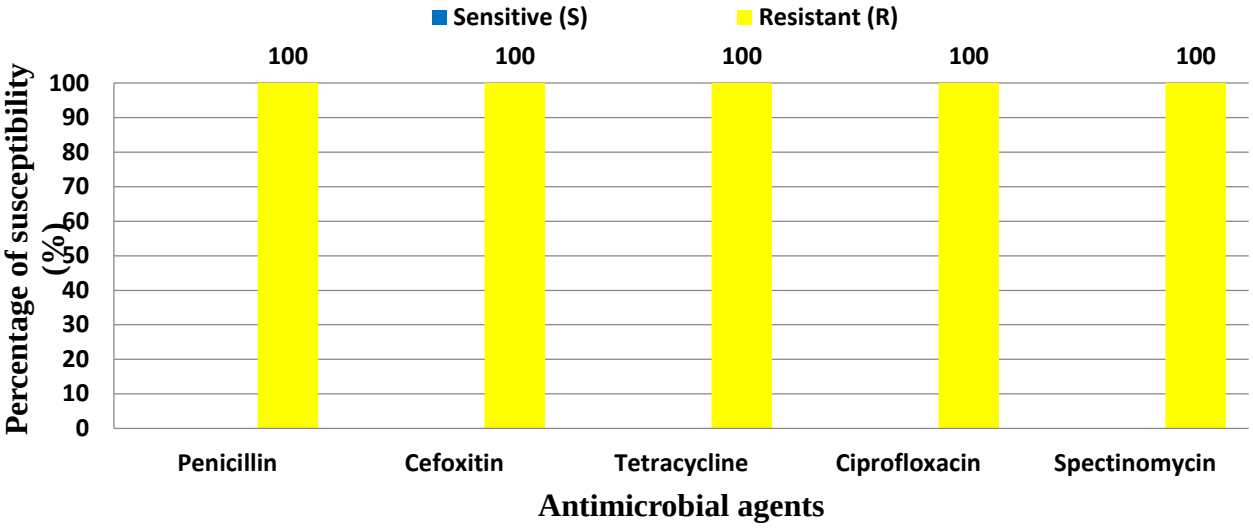


Figure 10: Antimicrobial susceptibility of *Neisseria gonorrhoeae*.

5.6. Antimicrobial susceptibility of *Staphylococcus aureus*:

All of the *S. aureus* isolates (38) were sensitive to levofloxacin, nitrofurantoin, clindamycin, trimethoprim, chloramphenicol, rifampin and cefoxitin. Only 10.5% of the isolates were resistant to each of gentamicin and erythromycin, 23.7% and 26.3% of the isolates were resistant to linezolid and tetracycline, respectively. While 73.7% of the isolates were resistant to each of penicillin and azithromycin **(Figure 11)**.

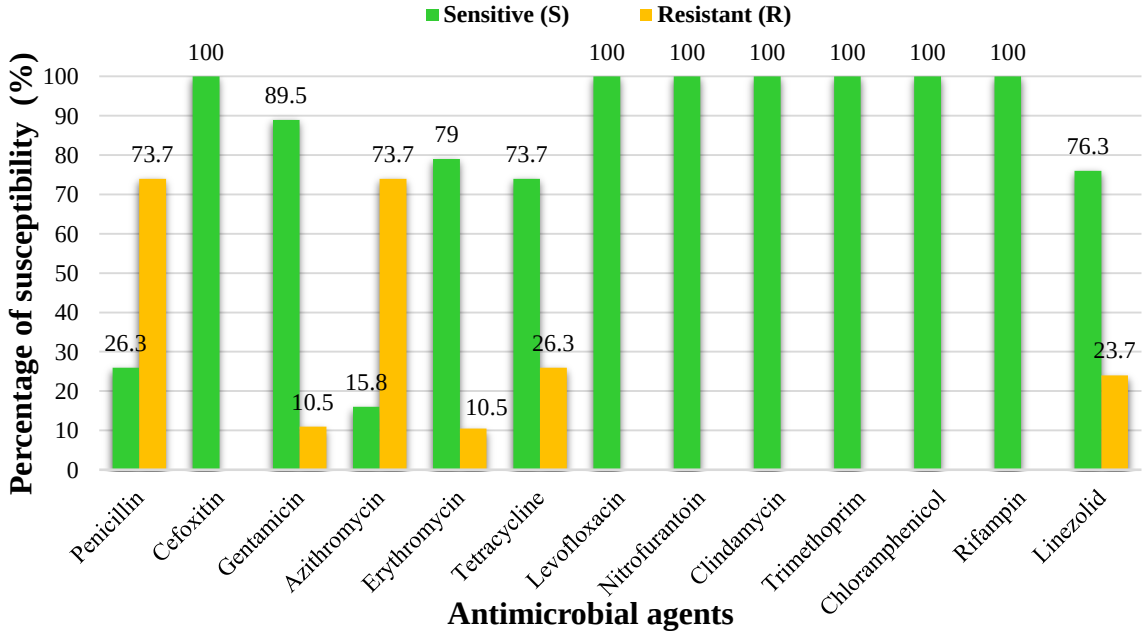


Figure 11: Antimicrobial susceptibility of *Staphylococcus aureus*.

5.7. Antimicrobial susceptibility of *Enterococcus faecalis*:

Most of the *E. faecalis* isolates were resistant to tetracycline (67%), followed by ampicillin (36%), chloramphenicol (25%), rifampin (21%), and linezolid (20%). While the least resistance percentages were against vancomycin (16%), nitrofurantoin (14%) and teicoplanin (12%) **(Figure 12)**.

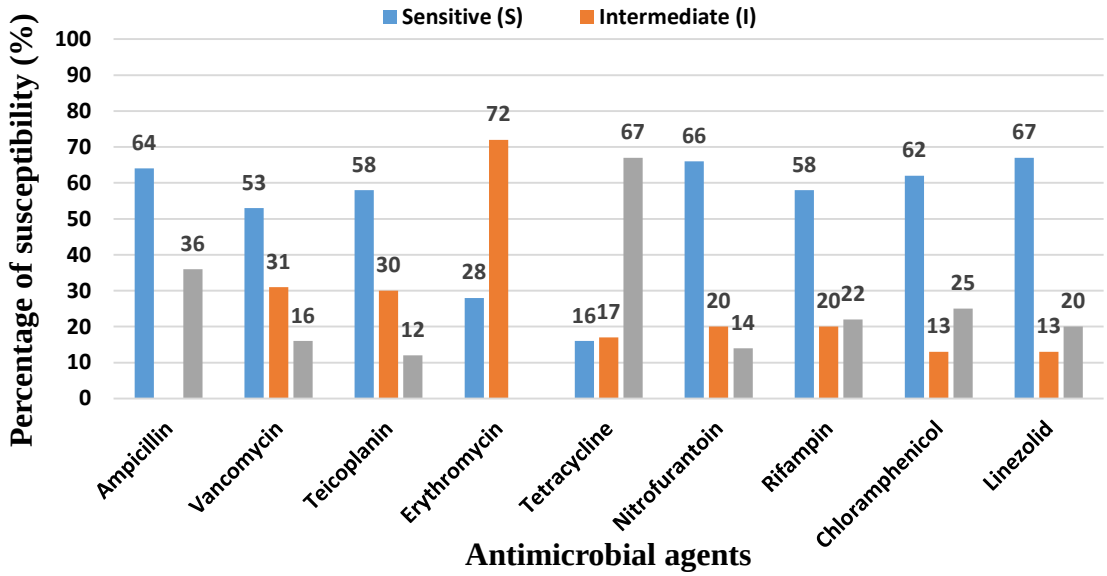


Figure 12: Antimicrobial susceptibility of *Enterococcus faecalis*.

5.8. Antimicrobial susceptibility of *Streptococcus agalactiae*:

The 11 isolates of *S. agalactiae* were sensitive to all tested antibiotics (ampicillin, ceftriaxone, vancomycin, azithromycin, tetracycline**,** levofloxacin, chloramphenicol**,** clindamycin and linezolid).

5.9. Antimicrobial susceptibility of *Candida spp.*:

In the present study, testing the antifungal activity of amphotericin B and fluconazole against *Candida spp.* isolates illustrated that *C. auris*, *C. glabrata* and *C. albicans* showed 100%, 50% and 21% resistance to both drugs, respectively **(Figure 13).**

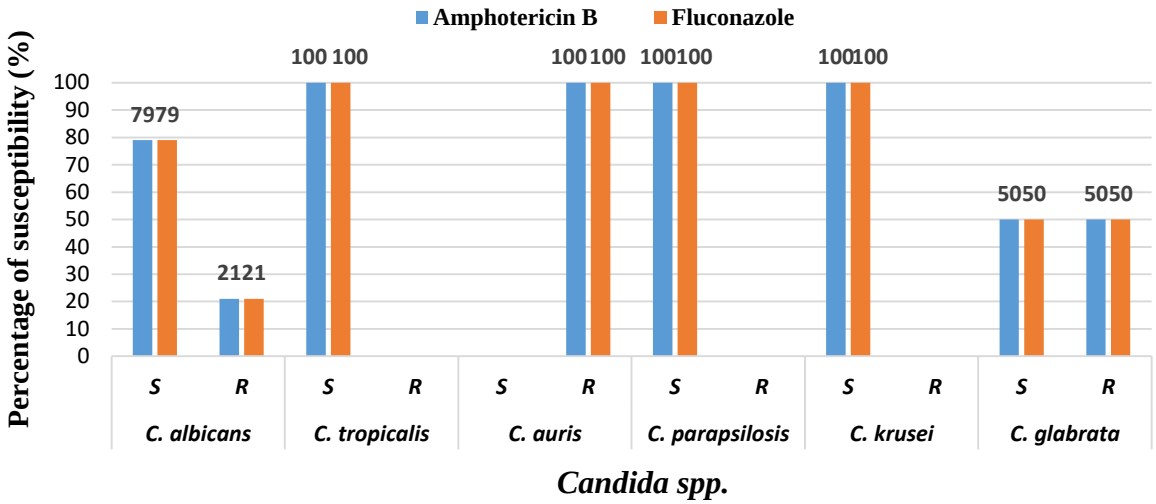


Figure 13: Antifungal susceptibility of different *Candida spp.*

6. Phenotypic analysis of β-lactams resistance:

6.1. ESBLs production of Gram negative bacteria:

In this study, ESBLs were detected in 41 (21.93%) out of 187 isolates of Gram negative bacteria as follow 13.37% of *E. coli*, 6.95% of *K. pneumoniae*, 1.07% of *P. aeruginos*a and one isolate of *P. mirabilis* **(Figure 14)**. These results were confirmed by disk potentiation test **(Figure 15, a & b)** and double-disk synergy test showing ellipsis or phantom image **(Figure 16, a & b),** and showing champagne-cork or keyhole **(Figure 16, c & d)**.

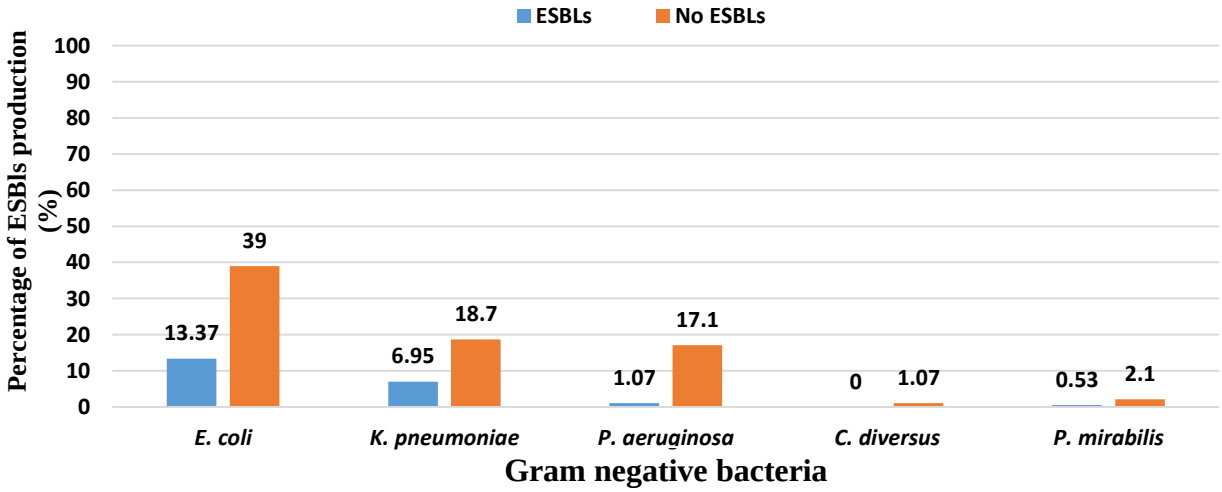
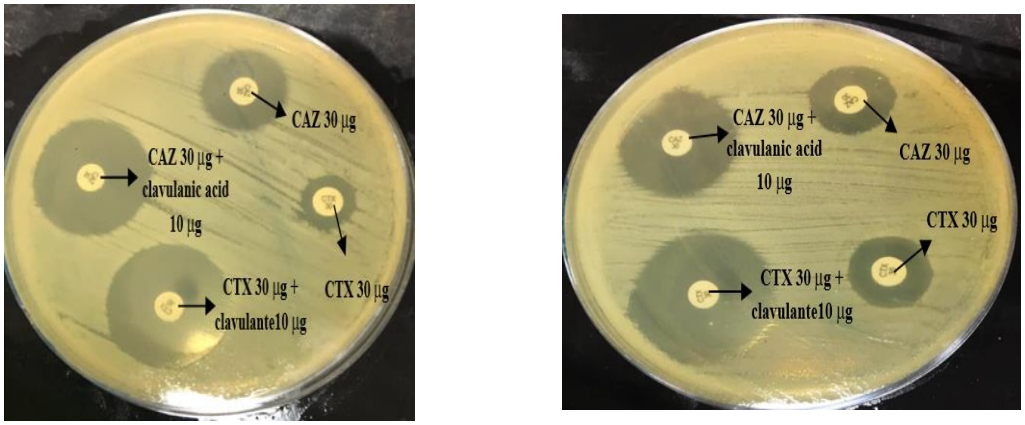


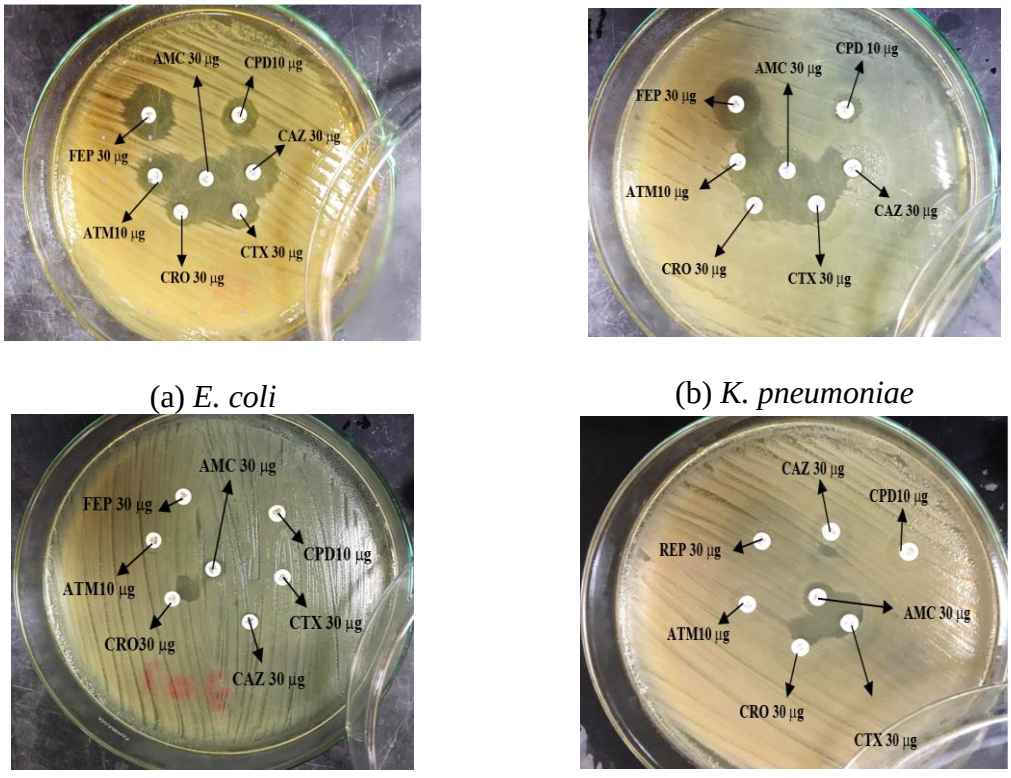
Figure 14: Phenotypic detection of ESBLs among Gram negative bacteria.



(a) *E. coli* (b) *K. pneumoniae*

Figure 15: Phenotypic detection of positive ESBLs production by disk potentiation test.

CAZ: ceftazidime; CTX: cefotaxime.



(a) *E. coli* (b) *K. pneumoniae*

(c) *E. coli* (d) *K. pneumoniae*

Figure 16: Phenotypic detection of positive ESBLs production by double disk synergy test.

AMC: amoxicillin-clavulanate, CPD: cefpodoxime, CAZ: ceftazidime, CTX: cefotaxime, CRO: ceftriaxone, ATM: aztreonam and FEP: cefepime.

6.2. *Amp*C production among Gram negative isolates:

*Amp*C production was detected in 19 (10.16%) out of 187 Gram negative isolates. *Amp*C was produced by 12 (6.4%) *E. coli* and 5 (2.7%) *K. pneumoniae* isolates, in addition to one isolate of *P. aeruginosa* and *P. mirabilis*. All the 19 isolates showed co-production of *Amp*C and ESBLs enzymes **(Figures 17 and 18)**.

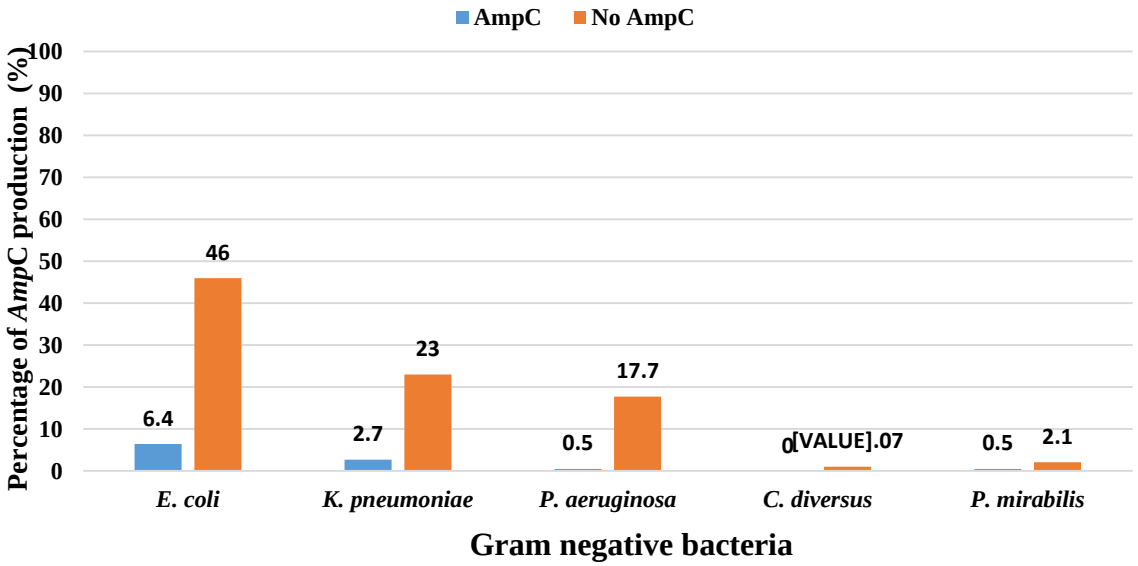
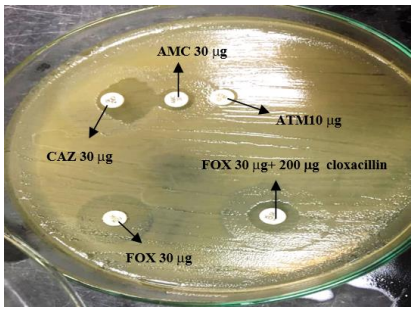


Figure 17: Phenotypic detection of *Amp*C among Gram negative bacteria.



(b) *K. pneumoniae*

Figure 18: Phenotypic detection of positive *Amp*C and ESBLs co-production by *K. pneumoniae* isolates.

6.3. Metallo-β-lactamases production (MBLs):

The production of MBLs was detected in 10 (5.3%) out of 187 isolates of Gram negative bacteria that represented 10 (29.4%) out of 34 *P. aeruginosa* isolates. These isolates showed positive results for synergistic effects of imipenem with EDTA and considered metallo-β-lactamases producers. Whereas MBLs were not detected in *E. coli*, *K. pneumoniae*, *C. diversus* and *P.mirabilis* isolates. Only one isolate of *P. aeruginosa* produced ESPLs and MBLs and one isolate produced ESPLs, *Amp*C and MBLs **(Figures 19 and 20)**.

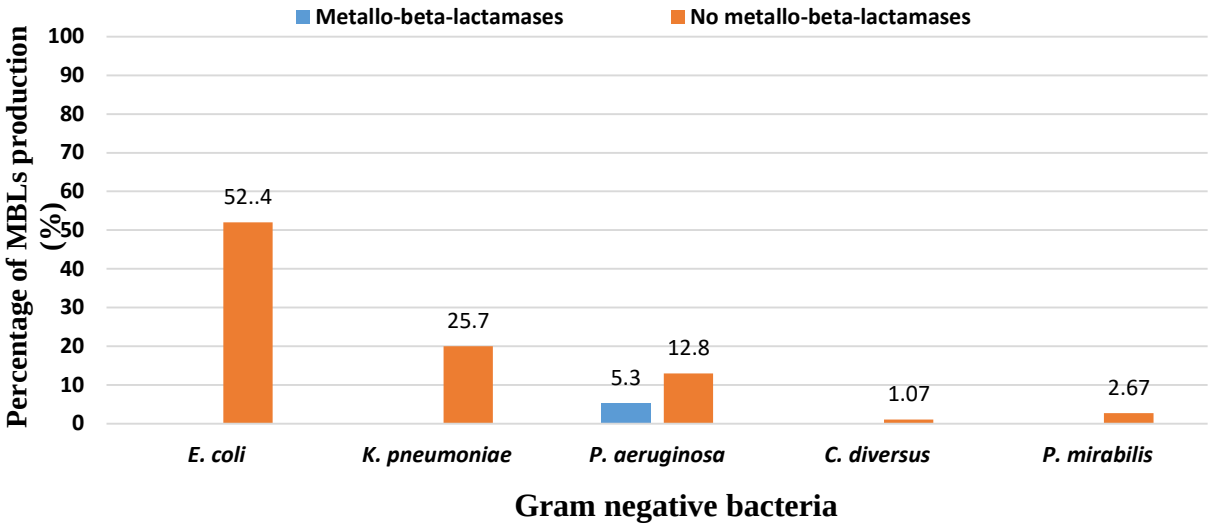


Figure 19: Phenotypic detection of MBLs production among Gram negative bacteria.

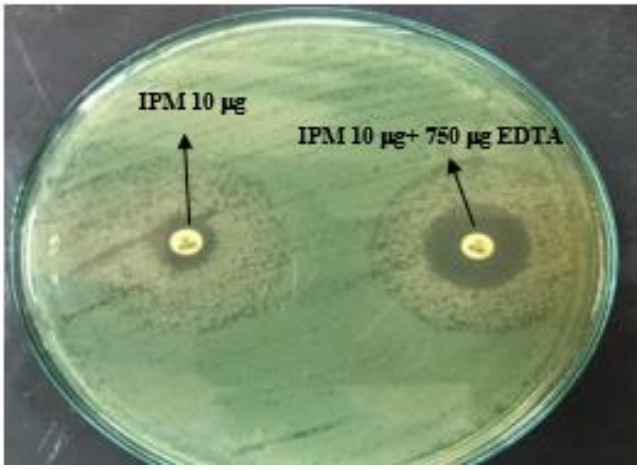


Figure 20: Phenotypic detection of metallo-β-lactamases in *P. aeruginosa* isolates.

IPM: imipenem**; EDTA:** ethylenediaminetetraacetic acid.

DISCUSSION

Most pregnancies progress without incident, but approximately 8% of all pregnancies involve complications that, if left untreated, may harm the mother or the baby. While some complications relate to health problems that existed before pregnancy, others occur unexpectedly and are unavoidable **(Magoma *et al.*, 2010)**.

High rates of morbidity and complications during pregnancy can be caused by vaginal colonization with certain species of bacteria during pregnancy. By examining the prevalence of abnormal vaginal microorganisms in pregnant women according to trimesters, **Schuster *et al.* (2020)** reported that the presence of abnormal vaginal colonization is linked to an increased risk of miscarriage, preterm labour (PTL), preterm

premature rupture of membranes (PPROM) and premature rupture of membranes (PROM).

The current study showed that the distribution of complications of pregnancy among patients according to trimester was 41.5% abortion in the 1st trimester compared to 10% in the 2nd trimester. Missed abortion in the 1st trimester was 22.5% compared to 4.5% in the 2nd trimester with statistically significant difference, threatened abortion in the 1st trimester was 9% compared to 5.5% in the 2nd trimester with statistically significant difference. **Moustafa (2023)** focused particularly on the 1st trimester of pregnancy and found a highly significant association between infection and abortion occurrence ($P < 0.001$). In the later stages of pregnancy, the incidence of PTL in the 2nd trimester was 2% compared to 7% in the 3rd trimester with statistically significant difference, and PPROM in the 2nd trimester was 5% compared to 8.5% in the 3rd trimester with a statistically significant difference. In the same context high rates of morbidity and complications during pregnancy can be caused by vaginal colonization with certain species of bacteria during pregnancy (**Schuster *et al.*, 2020**).

The current study showed that the most frequently observed microorganisms were Gram negative bacteria (56.6%) with increased incidence of *E. coli* (27.3%), followed by Gram positive bacteria (32.۴%) with increased incidence of *E. faecalis* (17.8%) and the fungal isolates (11.1%) mostly *C. albicans* (7.8%). Comparable results were reported by **Musaba *et al.* (2017)**, a higher prevalence of Gram positive aerobic bacteria (63%); **Singh *et al.* (2016)** highlighted *E. coli* as the most common isolates (34%), followed by *Candida spp*. (21%), *Enterococcus spp.* (10%), *Staphylococcus spp*. (8%), *Gardnerella vaginalis* (7%), and group B *Streptococci* (GBS) (5%).

In the current study, the fungal isolates were found in 11% of patients and the most common one was *C. albicans* (7.8%). Comparable results were reported by **Rasti *et al.* (2014)** who found that the prevalence of vaginal candidiasis in pregnant women was 32.7 %. The patients with PTL (31.8%) were infected with *C. albicans* and the pregnant women with PROM (33.3%) showed positive results of *C. albicans* infection. The change in the incidence of infection in this study is due to change in sample size. These findings highlight the clinical significance of *C. albicans* infections during pregnancy.

There are several important clinical findings regarding the vaginal microbiome in pregnant women. Firstly, the current study found that the prevalence of vaginal microorganisms detected was *E. coli* (98, 27.3%), *E. faecalis* (64, 17.8%), *K. pneumoniae* (48, 3.4%), *S. aureus* (38, 10.6%), *P. aeruginosa* (34, 9.5%), *C. ablicans* (28, 7.8%), *N. gonorrhoeae* (13, 3.6%), *S. aglactiae* (11, 3.1%), *P. mirabilis* (5, 1.4%), *G. vaginalis* (3, 0.8%), *C. auris* (3, 0.8%), *C. krusei* (3, 0.8%), *C. diversus* (2, 0.6%)., *C. glabrata* (2, 0.6%), *S. cerevisiae* (2, 0.6%), *S. pyogenes* (2, 0.6%), *K. kristinae* (1, 0.3%), *C. tropicalis* (1, 0.3%) and *C. parapsilosis* (1, 0.3%). These microorganisms were associated with different obstetric complications. These results agree with the findings of **Lajos *et al.* (2008)** who reported that the most frequent pathogens were *E. coli* (24.2%), *Candida spp*. (11.7%) and just 3 cases of GBS, **Saghafi *et al.* (2018)** who reported (24.2%), *E. coli* and *Candida spp*. (11.7%). Whereas **Ghaddar *et al.* (2020**)

reported 42% isolation rate of *C. albicans* while Non- *C. albicans* Candida (NCAC) were isolated 58%. In conversely to findings of the present study, **Li *et al.* (2019)** reported Gram-positive bacteria of 18.4%, among which GBS was the most common (14.6%) and Gram-negative bacteria of 12.8%, among which the most common species was *E. coli* (8.0%); **Zeng *et al.* (2014)** reported that the positive vaginal culture results showed 4.7% of GBS and the most common organisms were *Candida spp*. (36%), *S. aureus* (8%) and *Enterococcus spp.* (8%). However **Tang *et al.* (2020)** reported no isolation of *S. agalactiae*, this may be due to the change in the number and trimesters of patients in this study. The diversity of the vaginal microbiome observed in this study reflects its complexity and underscores the need for targeted microbial screening in pregnant women to prevent adverse maternal and neonatal outcomes.

The most common pathogen isolated in the current study was *E. coli* which agreed with previous researches conducted by **Lee *et al.* (2020)**; **Dube *et al.* (2022)** but in contrast to findings achieved by **Jeong *et al.* (2015)** who found that the most common microbiome was GBS (4.0%) followed by *E. coli* (3.8%), **Sangeetha *et al.* (2015)** reported the *E. faecalis* (32.2%) as the most prevalent Gram positive bacteria, **Lajos *et al.* (2008)** reported *Enterococcus spp.* (11.7%), **Saghafi *et al.* (2018)** reported coagulase negative *Staphylococci* (27.2%) and *Enterococcus spp.* (11.7%). Also, **Kerur *et al.* (2006)**, reported that *E. coli* and *Klebsiella spp.* were the most common pathogens (38.2% and 4.9%), respectively. This is due to change in the number and obstetric complications of patients in these studies. The prominence of *E. coli* across multiple studies, including the current research, underscores its significant role in obstetric infections, such as urinary tract infections, chorioamnionitis, and neonatal sepsis.

In the present study most of the isolated *E. coli* were resistant to ceftriaxone 83/98 (84.7%) and cefotaxime 82/98 (87.7%). Regarding cefazolin, mecillinam, cefuroxime and tetracycline, the resistance percentages were 77/98 (78.6%), 76/98 (77.5%), 76/98 (77.5%) and 74/98 (75.5%), respectively. Resistance of ciprofloxacin was 40/98 (40.8%) and nitrofurantoin was 12/98 (12.2%), while only one *E. coli* isolate showed resistance to amikacin and no other isolate was resistant to imipenem. All *E. coli* isolates were susceptible to imipenem which is in line with a report from **Nanayakkara *et al.* (2018)**. Moreover, the majority of *E. coli* were resistant to ciprofloxacin (54.2%) by **Abdelaziz *et al.* (2014) and** 56% by **Nanayakkara *et al.* (2018).** While **Ravishankar and Prakash (2017)** showed that *E. coli* isolates were resistant to ciprofloxacin (63%), cefuroxime (89%) and ceftriaxone (69%). In contrast, **Seni *et al.* (2019)**; **Emami *et al.* (2020)** found that 22% of *E. coli* were resistant to nitrofurantoin. The preserved susceptibility to imipenem and nitrofurantoin may reflect their more restricted use in clinical settings.

All *K. pneumoniae* isolates (48, 100%) were resistant to cefuroxime. Most of these isolates were resistant to cefotaxime, mecillinam and tetracycline (43/48, 89.6%), (41/48, 85.4%) and (40/48, 83.3%), respectively. They were resistant to azithromycin, ciprofloxacin, cefazolin and Nitrofurantoin (37/48, 77.1%), (36/48, 75%), (34/48, 70.8%) and (22/48 45.8%), respectively. No resistant isolates were detected to amikacin and imipenem. In opposition to this study findings, **Ravishankar and Prakash (2017)** showed that *K. pneumoniae* isolates were resistant to ciprofloxacin (51%). These high resistance percentages to antimicrobial agents further limit treatment options

particularly in settings where alternative therapies may not be readily available, due to few effective first line antibiotics.

In the present study *P. aeruginosa* isolates were resistant to levofloxacin 17/34 (50%), piperacillin 16/34 (47.1%), ceftazidime 15/34 (44.1%), imipenem 13/34 (38.2%), cefepime 13/34 (38.2%) and ciprofloxacin 11/23 (32.4%). While no resistance was detected to amikacin. In contrast to this study, **Bertrand *et al.* (2001)** reported that *P. aeruginosa* resistance rates were 21.5% and 38.3% to ceftazidime and ciprofloxacin, respectively. **Fitzroy and Orrett (2004)** found that 20% of isolates were resistant to ceftazidime, **Karlowsky *et al.* (2003)** showed that more than 10% of the isolates were resistant to amikacin, 10-20% of isolates were resistant to ceftazidime while 30% of the isolates were resistant to ciprofloxacin, and **Revathy *et al.* (1998)** found that *Pseudomonas spp*. was resistant to ceftazidime (17%). The resistance to amikacin however suggests that it remains a key therapeutic option for *P. aeruginosa* infections. This may be due to its unique mechanism of action**,** low resistance rates, and its effectiveness against multidrug-resistant isolates.

The antimicrobial susceptibility of *P. mirabilis* showed that 3 out of 5 (60%) isolates were resistant to cefotaxime and ceftriaxone. In addition, 2 isolates (40%) were resistant to cefuroxime, mecillinam, tetracycline and cefazolin. No resistance was found to nitrofurantoin, azithromycin, ciprofloxacin, amikacin and imipenem. In the same context of the findings of the present study, **Marami *et al.* (2019); Ejerssa *et al.* (2021)** found no resistance with amikacin and nitrofurantoin. These findings underscore the need for continuous surveillance of *P. mirabilis* resistance patterns, as well as the prudent use of antibiotics to mitigate the development of resistance.

All *S. aureus* isolates were sensitive to levofloxacin, nitrofurantoin, clindamycin, trimethoprim, chloramphenicol, rifampin and cefoxitin. Only 4/38 (10.5%) isolates were resistant to both gentamicin and erythromycin, 9/38 (24%) and 10/38 (26%) isolates were resistant to linezolid and tetracycline, respectively. While 28/38 (74%) isolates were resistant to each of penicillin and azithromycin. This result agreed with **Marami *et al.* (2019)**; **Ejerssa *et al.* (2021)** who found no resistance with nitrofurantoin. **Stanley *et al.* (2013)** reported that isolates were resistant to penicillin (74%), azithromycin (34%), cefoxitin (5%), ciprofloxacin (5%), tetracycline (4%), and trimethoprim (1%), but sensitive to gentamicin and rifampin. **Johnson *et al.* (2021)**; **Chelkeba *et al.* (2022)** found that all isolates were sensitive to ceftriaxone, cefotaxime, gentamicin, ciprofloxacin, nitrofurantoin and cefuroxime of 82.9%, 81.4%, 79.3%, 78.6%, 66.4% and 65.7%, respectively. **Onanuga *et al.* (2018)** showed that *staphylococcus spp*. exhibited 90% and 85% resistance to cefoxitin and vancomycin, respectively. The overall sensitivity to critical antibiotics like levofloxacin, rifampin, and cefoxitin suggests that these agents remain viable treatment options, particularly for methicillin-sensitive *S. aureus* (MSSA) infections.

The antimicrobial susceptibility of *E. faecalis* showed that the most resistant isolates were resistant to tetracycline 43/64 (67%), followed by ampicillin 23/46 (36%), chloramphenicol 16/64 (25%), rifampin 14/64 (21%), and linezolid 13/64 (20%) isolates, while the least resistance rates for vancomycin, nitrofurantoin and teicoplanin were 10/64 (16%), 9/64 (14%) and 8/64 (12%), respectively. In other findings, **Sujatha**

and Nawani (2014) reported a resistance rate to ampicillin (22%); **Celen *et al.* (2011)** found 100% of isolates were sensitive to ampicillin, and **Bhola *et al.* (2020)** reported a high resistance to ampicillin (92.9%).

In the present study the antimicrobial susceptibility of *S. agalactiae* showed that all isolates were sensitive to all the used antibiotics. In the same context, **Rosana *et al.* (2020); AlZuheiri *et al.* (2021); Balachandran *et al.* (2022);** showed that GBS isolates were sensitive to ampicillin and vancomycin. In contrast, **Emaneini *et al.*** (2014) reported that *S. agalactiae* showed that all the tested isolates were susceptible to penicillin but were resistant to clindamycin (35%), chloramphenicol (45%), erythromycin (35%), linezolid (1%) and tetracycline (96%).

In this study antifungal activity against different *candida spp.* isolates was performed. Only 10/40 (25%) isolates were resistant to both amphotericin B and fluconazole. Where *C. auris* 3/3 (100%), *C. glabrata* 1/2 (50%) and *C. albicans* 6/28 (21%) isolates were resistant to both amphotericin B and fluconazole. This finding disagreed with the result of **Mukasa *et al.* (2015)**; **Khan *et al.* (2018)** who showed that fluconazole resistance of *C. krusei* was 71.43%; **Khan and Baqai (2010)** showed that fluconazole resistance was 63.8% of *Candida spp.* isolates, while **Dharmik *et al.* (2013)** showed that *candida spp*. (97.2%) were sensitive to fluconazole, **Brandolt *et al.* (2017)** showed that fluconazole resistance was 42% of the *Candida spp*., **Tsega and Mekonnen (2019)** reported that *candida spp*. were resistant to fluconazole (62%). On the other hand, the results of the present study agreed with **Yesudhason and Mohanram (2015)** who showed 100% sensitivity of *C. tropicalis* isolates to amphotericin B.

In this study ESBLs were produced in 41/187 (21.9%) isolates of Gram negative bacteria including 25 (13.4%) of *E. coli*, 13 (7%) of *K. pneumoniae*, 2 (1.1%) of *P. aeruginosa* and one isolate of *P. mirabilis.* The overall magnitude of ESBLs producing Gram negative bacteria in the present study (21.9%) is in disagreement with previous researches (38.8%) by **Tekele *et al.* (2020)**, 33.3% by **Seid and Asrat (2005)**, 34.5% by **Nepal *et al.*, (2017)**, 42.8% by **Gomara-Lomero *et al.* (2018)** and 44.0% by **Shivanna and Rao (2017)**. In the contrary, findings of this study were higher than other studies findings, 15.8% by **Yusuf and Haruna (2013)** and 6.3% by **Spanu *et al.* (2002)**.

*Amp*C producing organisms in the present study were 19 out of 187 (10.1%) isolates of Gram negative bacteria, 12 (6.4%) isolates of *E. coli*, 5 (2.7%) isolates of *K. pneumoniae*, one isolate (0.5%) of each *P. aeruginosa* and *P. mirabilis.* The overall magnitude of *Amp*C beta-lactamase producing Gram negative bacteria in the present study (10.1%) disagreed with previous studies, 15.2% by **Ogefere *et al.* (2016)**, 14.2% by **Gomara-Lomero *et al.* (2018)** and 37% by **Shivanna and Rao (2017)** who stated a higher number of *Amp*C-producing Gram negative bacteria. In contrary, findings of this study were higher when compared with some other studies findings, 1.5% by **Farrokhnazar *et al.* (2016)**, 2.6% by **Gazouli *et al.* (1998)** and 8% by **Singhal *et al.* (2005)**.

The metallo-β-lactamases production was not detected in *E. coli*, *K. pneumoniae*, *P. mirabilis* and *C. diversus.* While 10 (5.3%) out of 187 isolates of Gram

negative bacteria bacteria that represented 10 (29.4%) out of 34 *P. aeruginosa* isolates were positive for MBLs production. This indicates that those isolates harbor MBLs genes. A higher incidence was found by **Ejikeugwu *et al.* (2018)** who reported that 34.9 % isolates of *P. aeruginosa* were MBLs producing organisms.

CONCLUSION

The present study can infer that there is relation between the presence of microorganisms and obstetric complications in pregnant women. High vaginal swabs culture should be performed to all pregnant women during 1st trimester to help in prevention of the obstetric complications due to vaginal infection and to improve neonatal outcome. The study revealed that an increase in the prevalence of ESBLs, *Amp*C and MBLs may become an important public health issue. Therefore, there is a vital need for surveillance of the spread of these clinical isolates. The study illustrated the importance of phenotypic surveillance to guide control of vaginal colonization in women with obstetric complications at different trimesters of pregnancy. The choice of a definite antibiotic treatment should be based on susceptibility testing balancing the expected clinical success rate against the risk of the development of antibiotic resistance and the risk of severe side effects. Further clinical research is obviously needed to find ways and means to reduce microbial vaginal colonization at different trimesters of pregnancy and the infectious morbidity associated with it.

Limitation during study

- Coronavirus disease (COVID-19).
- Difficult during collection of specimens.
- Difficult in follow up of the patients.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of Faculty of Pharmacy (Boys-Cairo), Al-Azhar University. The ethical approval Number is 014-2024.

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إنتشار العدوى الميكروبية للنساء الحوامل في مختلف مراحل الحمل الثلاثة مع مضاعفات الحمل و الولادة

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تؤدي العدوى الميكروبية ببعض أنواع الجراثيم أثناء الحمل إلى التأثير على صحة المرأة الحامل ويؤدي هذا إلى ارتفاع معدلات الإصابة بالأمراض والمضاعفات أثناء الحمل. الهدف من هذه الدراسة هو معرفة إنتشار العدوى الميكروبية لدى النساء الحوامل أثناء مراحل الحمل الثلاثة وتحديد وجود الاستعمار المهبلي الغير طبيعي وارتباطها بارتفاع خطر الإجهاض والولادة المبكرة وتمزق الأغشية المبكر. وقد أجريت هذه الدراسة على ٢٠٠ امرأة حامل تعاني من مضاعفات الحمل تتراوح أعمارهن بين ٢٠و ٣٥ عامًا. تم جمع المسحات المهبلية وزراعتها على البيئات الغذائية المختلفة. وتم فصل العزلات الميكروبية وتم التعرف عليها باستخدام خصائص الشكل و الزراعة على البيئات الغذائية المتخصصة و الاختبارات البيوكيميائية.

العدد الإجمالي للكائنات الحية الدقيقة التي تم عزلها هو ٣٥٩ عزلة تشمل ٢٠٣ (٥٦.٦٪) بكتيريا سالبة الجرام، تليها بكتيريا موجبة الجرام ١١٦ (٣٢.٣٪)، و ٤٠ (١١.١٪) عزلة من الفطريات. وكانت أعلى نسبة عزل للبكتيريا سالبة الجرام هي الإشريكية القولونية (٢٧.٣٪) كما هو موضح في الثلث الأول من الحمل (١٠.٣٪) والثلث الثاني (٥٪) والثلث الثالث (١٢%)، بينما كانت أقل نسبة عزل هي سيترو باكتر دايفيرسوس (٠.٥٦٪). بينما كانت أعلى نسبة عزل للبكتيريا موجبة الجرام هي المكورات المعوية البرازية (١٧.٨٪) كما هو موضح في الثلث الأول من الحمل من الحمل (٦.٤٪) والثلث الثاني (٤.٤٦٪) والثلث الثالث (٦.٩٦٪) ، بينما كانت أقل نسبة عزل هي المكورات السبحية (٠.٥٦٪) و كوكوريا كريستينا (٠.٢٨٪). وكان أعلى معدل عزل من الفطريات هي المُبْيَضَّةُ البَيْضَاء (٧.٨٪) كما هو موضح في كل من الثلث الأول والثاني من الحمل (٢.٢٣٪ لكل منهما) والثلث الثالث (٣.٣٤٪) ، في حين كان أقل معدل عزل هو المبيضة المدارية (٠.٢٨٪) المبيضة نظيرة الغمدية (٠.٢٨٪).

علاوة على ذلك، خضعت جميع العزلات لاختبار الحساسية للمضادات الميكروبية بإستخدام اختبار إنتشار القرص بطريقة كيربي باور. أظهرت العزلات أنماط حساسية مختلفة للمضادات الميكروبية المختبرة و ذلك بنسب متباينة. في حين كانت جميع عزلات النَيْسَرِيّة البُنِّيّة مقاومة لجميع المضادات الحيوية المختبرة بينما كانت جميع عزلات سيترو باكتر دايفيرسوس و المكورات العقدية القاطعة للحليب حساسة للمضادات الحيوية المختبرة.

أظهر تحليل المقاومة الظاهرية لمضادات البيتالاكتامز إنتاج إنزيمات البيتا لاكتاميز بواسطة ٤١ عزلة (٢١.٩٪) من أصل ١٨٧عزلة من البكتيريا سالبة الجرام ، وكان أعلى معدل إجابية هو ميكروب الإشريكية القولونية ٢٥ عزلة (١٣.٣٧٪) بينما كان أقل معدل حدوث هو عزلة واحدة من بكتيريا بروتيوس ميرابيليس .بينما كان إنتاج السيفالوسبورينات من الفئة ج في ١٩ (١٠.٢٪) عزلة، وكان أعلى معدل إجابية لبكتريا الإشريكية القولونية ١٢ (٦.٤٪) بينما كان أقل معدل هو عزلة واحدة من بروتيوس ميرابيليس، وكما تم اكتشاف إنزيمات ميتالو بيتا لاكتاميز في ١٠ (٥.٣٪) عزلات فقط من ميكروب السودوموناس ايروجينوزا بينما لم يتم اكتشافها في الإشريكية القولونية و الكلبسيلا الرئوية و سيتروباكتر دايفيرسوس و بروتيوس ميرابيليس. ولمنع المضاعفات المرتبطة بالحمل والولادة ينصح بإجراء فحوصات مخبرية دورية أثناء الحمل، مثل مزارع البول وفحص عينات المسحات المهبلية.

الكلمات المفتاحية: العدوى الميكروبية، النساء الحوامل، مضاعفات الحمل و الولادة، الحساسية للمضادات الميكروبية