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Identification and antibiotic susceptibility patterns of vaginal microbiome isolated from pregnant and non-pregnant women

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ABSTRACT

Background and objective. An abnormal vaginal discharge and an increase in intestinal aerobic bacteria indicate a vaginal infection. The aim of this study was to assess the antimicrobial susceptibility profiles of bacterial species present in the vaginal microbiota of both non-pregnant and pregnant women.
Material and method. A total of 211 vaginal swabs were collected from 120 pregnant and 91 non-pregnant women. The VITEK 2 Compact Automated System validates bacterial isolate diagnosis and antibiotic susceptibility.
Results. Out of 120 vaginal samples of pregnant women, 105 samples were identified as Gram-positive (GP) (79) and Gram-negative (GN) (26) while 15 samples were unidentified by VITEK®. The 91 nonpregnant samples comprised identified GP (53), GN (23) and unidentified samples (15). The GP bacteria were highly resistant to Oxacillin (OX1) in both groups, while the GN bacteria were resistant to Ceftazidime (CAZ) and ticarcillin (TIC) in non-pregnant group and TIC in the pregnant groups. The GP bacteria in both groups were sensitive to Tigecycline (TGC). Pregnant and non-pregnant GN bacteria were sensitive to Meropenem (MEM) (61.5%) and Piperacillin/Tazobactam (TZP) (91.3%), respectively.
Conclusion. The current study showed that TZP and MEM were both groups' most effective antibiotics against GN isolates. GP bacteria were significantly TGC-sensitive.

Keywords: microbial diversity, antimicrobial susceptibility profile, vaginosis, delivery ward, antibiotic resistance

INTRODUCTION

The human body has several organs that are normally colonized by bacteria which play a protective role against the growth of pathogenic bacteria [1]. One of these organs is the vagina which accounts for about 9% of the total human microbiota. The vagina is normally colonized by numerous different types of bacteria reaching up to 10^9 colony-forming units (CFU) per milliliter of vaginal fluid and comprising both aerobic and anaerobic bacteria [2]. The vaginal microbiota is usually dominated by lactobacilli which produce lactic acid, with the potential ability to prevent the colonization of diseases or undesirable bacteria. For instance, lactic acid keeps an acidic vaginal pH between 3.5-4.5, which protects against parasitic *(Trichomonas vaginalis)*, bacterial *(Neisseria gonorrhoea)*, and viral (HIV) agents [3-4]. However, the vagina is a common site of urogenital infection worldwide [5-6] as it is directly exposed to the external environment and due to its position near the anal canal and urethra which are also normally colonized by bacteria.

The bacterial communities of the vagina might be influenced by several factors including gestational status, contraceptive usage, menstrual cycle, and sexual activity [3]. In pregnancy, the beneficial rising of the vaginal flora is expected due to hormonal changes, immune system changes, and metabolic changes [7]. In addition to that, there is a dramatic increase in estrogen concentration from additional

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production by the placenta. This elevated estrogen levels lead to the maturation of vaginal epithelium resulting in glycogen accumulation which favors *Lactobacillus spp.* abundance [8]. As a result of their rise during pregnancy, vaginal pH drops, creating a barrier against pathogenic bacteria and viruses as well as an increase in vaginal secretion [7].

The abuse & disuse of antibiotics locally or systematically is associated with an increased risk of imbalance of growth of vaginal flora and can aid in the development of multidrug resistance [9]. It might be challenging to prescribe antibiotics during pregnancy since infections must be treated while also protecting the unborn fetus from potential side effects of the drugs [10]. Antibiotic resistance is when bacteria acquire or develop the ability to get through the defenses of drugs used to treat them [11]. This has led to antibiotic-resistant genes have been widely dispersed throughout the environment as a result of the long-standing misuse and overuse of antibiotics [12]. Without an effective action plan, the yearly mortality rate is anticipated to exceed 10 million by 2050, exceeding the cancer death rate [13]. This problem is known as antimicrobial resistance, and it is a major threat to global public health. Antimicrobial resistance can lead to longer-term illnesses and even death, as well as increased healthcare costs. Therefore, it is important to use antimicrobial agents responsibly [14]. The aim of the study was to determine the types of vaginal bacteria in pregnant women and non-pregnant women as well as to know the prevalence rate and resistance profile of bacteria in vaginal swabs.

MATERIALS AND METHODS

Samples collection

The study was conducted in three hospitals, Basra Teaching Hospital, Basra Hospital for Women and Children, and Al-Mawanai Teaching Hospital, during the period from January to June 2022. Samples were taken from non-pregnant and pregnant women (those of reproductive age 18-45 years) during a gestation period of 38-40 weeks. Samples were taken using sterile swabs by the physician or a nurse from the posterior fornix of the vagina of pregnant women inside the delivery ward it was then gently rubbed for 20 seconds against the mid-vaginal wall with the swabs. The swabs were then placed in a sterile container with a preservative and sent to the laboratory for further testing. Additionally, litmus paper was used to measure the pH of the vagina [15]. It was assured that all women included in the study neither received antibiotics nor used a vaginal wash in the last two weeks prior to the sampling.

Ethical consideration

Ethical approval for the study was obtained from the Basra Health Department; the Research and development department gave permission. Vaginal swabs were collected under the supervision of a gynecologist after obtaining the patient's consent.

CFU determination

The bacteria suspensions were serially diluted to 10-3, and 100 µl of dilutions 10-1 to 10-3 were plated out on Nutrient Agar and incubated for 24 h at 37°C. Colony-forming units (CFU/ ml) were determined by counting [16].

Bacterial identification and antibiotic susceptibility assessment

The automated VITEK 2 compact system (bioMérieux, France) was used to identify bacteria and assess the antibiotic susceptibility of GP and GN bacteria utilizing AST-N222 and AST-P580 cards. Before applying the VITEK system, isolates of clinical significance performed a subculturing process to ensure purity. These isolates were then inoculated on a specific plate, such as nutrient agar, blood agar, and MacConkey agar, and subsequently incubated under aerobic conditions at 37°C overnight. Bacterial isolates were classified using colony morphology and Gram staining. Following overnight incubation, the pure bacterial colonies were used to get a standard saline inoculum for the suitable VITEK identification (ID) card. The following ID cards were used to identify bacteria: Gram-positive ID card: (GP reference 21 342); Gram-negative ID card, (GN reference 21 341).

Specific sensitivity cards, also known as AST cards, were utilized in order to determine both the minimum inhibitory concentrations (MICs) and the antimicrobial susceptibility tests (ASTs). The VITEK 2 system and the advanced expert system (AES) were used to interpret the susceptibility tests in accordance with the criteria established by the Clinical and Laboratory Standards Institute (CLSI). The AST cards included the GP sensitivity card (AST-P580) and GN sensitivity card (AST-N222). The manufacturer's instructions were followed throughout all processing processes. VITEK 2 Compact logged and loaded ID and AST cards. The data obtained from the VITEK 2 Compact system were automatically reported and printed using the VITEK 2 Systems software, specifically version 06.01.

The vaginal GP bacterial were tested against AST-P580, including Oxacillin (OX1), Gentamicin (GM), Tobramycin (TM), Nitrofurantoin (FT), Fusidic acid (FA), Vancomycin (VA), Clindamycin (CM), Erythromycin (R), Linezolid (LNZ), Levofloxacin (LEV), Moxifloxacin (MXF), Rifampicin (RA), Tetracycline (TE), Tigecycline (TGC) and Trimethoprim/Sulfamethoxazole (SXT).

The vaginal GN bacterial were tested against AST-N222, including Ticarcillin (TIC), Ticarcillin/clavulanic (TCC), Piperacillin (PIP), Piperacillin/Tazobactam (TZP), Ceftazidime (CAZ), Cefepime (FEP), Aztreonam (ATM), Imipenem (IPM), Meropenem

(MEM), Amikacin (AN), Gentamicin (GM), Tobramycin (TM), Ciprofloxacin (CIP), Minocycline (MNO) and Trimethoprim/Sulfamethoxazole (SXT).

Data Analysis

The data was submitted and analyzed utilizing Microsoft Excel (2021) and the Unweighted Pair Group Method with Arithmetic mean (UPGMA) Clustering method and the similarity index (Dicecoefficient).

RESULTS

Participants in the study

From January to June 2022, High vaginal swabs (HVS) from 211 women (120 pregnant women and 91 non-pregnant women) were examined at the Laboratory of Microbiology in the College of Pharmacy at the University of Basra.

The vaginal pH values of non-pregnant and pregnant women during labour

The vaginal pH range was (3.5-5) and (4-8) in non-pregnant and pregnant women during labour, respectively. with a statistically significant difference ($P < 0.05$).

The abundance of the cultivable bacterial community

The mean values of cultivable bacteria in non-pregnant women were 3.28 logs whereas those for pregnant were 3.78 logs with a statistically significant difference ($P < 0.05$) (Figure 1).

Microbial diversity

Our results show that out of 120 vaginal pregnant samples, 15 vaginal samples (12.5% of pregnant women) were unidentified bacteria, while in 91 vaginal non-pregnant samples, 15 vaginal samples (16.4%) were unidentified bacteria. So that the total number of unidentified samples was 30, comprising a percentage of (14.2%) of the total collected samples.

Data from the VITEK® 2 compact system identify 79 GP and 26 GN bacteria in the 105 vaginal samples from pregnant women, whereas the 76 non-pregnant samples comprise 53 GP and 23 GN bacteria as illustrated in Tables 1 & 2.

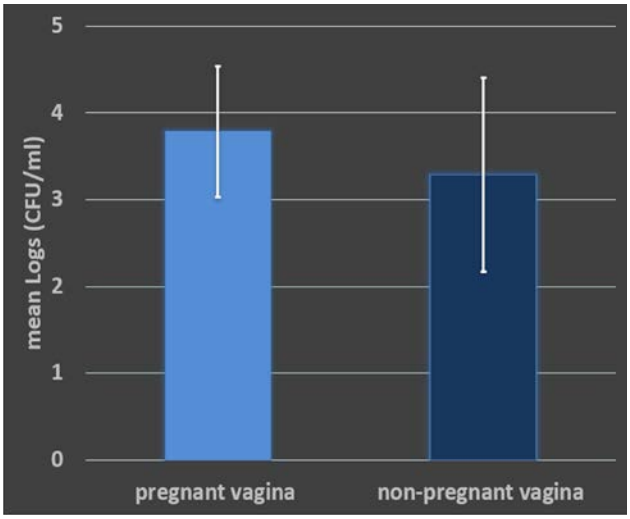

FIGURE 1. Distribution of total cultivable bacterial communities expressed in mean Logs (CFU/ml) in the non-regnant and pregnant women during labour. Whisher: mean ± standard deviation

In samples of pregnant women, GP bacteria include 11 different bacterial species of which *Staphylococcus hemolyticus* was the more prevalent species (23 samples (21.9%)) and *Kocuria rhizophilia* was the least identified bacteria (2 (1.9%)). Regarding GN species, 12 species were identified in pregnant's samples of which *Echerichia coli* was the most frequent (6 (5.7%)) as demonstrated in Table 1.

In non-pregnant samples, 13 GP species and 7 GN species were identified, however, they had the same prevalent species as pregnant's samples but of different percentages (*Staphylococcus hemolyticus* 15 (19.7%)) and *E. coli* (14 (18.4%)) as seen in Table 2.

On the other hand, thirteen species were found in both groups of our study (nine were GP and four were GN species) as demonstrated in Figure 2.

TABLE 1. Frequency distribution and percentages of GP and GN bacteria isolates of high vaginal swabs of pregnant women

Pregnant women (N=105)			
Genus or Species (Gm $^{+vi}$)	Frequency (%)	Genus or Species (Gm $^{-vi}$)	Frequency (%)
Staphylococcus haemolyticus	23 (21.9%)	*Escherichia coli*	6 (5.7%)
Staphylococcus hominis	15 (14.3%)	*Sphingomonas paucimobilis*	5 (4.8%)
Enterococcus faecalis	9 (8.6%)	*Pantoea sv.*	4 (3.8%)
Staphylococcus epidermidis	8 (7.6%)	*Proteus mirabilis*	2 (1.9%)
Staphylococcus aureus	5 (4.8%)	*Klebsiella pneumoniae*	2 (1.9%)
Kocuria Kristinae	5 (4.8%)	*Pseudomonas stutzeri*	1 (0.9%)
Staphylococcus sciuri	3 (2.9%)	*Burkholderia pseudomallei*	1 (0.9%)
Staphylococcus lentus	3 (2.9%)	*Enterobacter cloacae*	1 (0.9%)
Staphylococcus warneri	3 (2.9%)	*Aeromonas hydrophilia*	1 (0.9%)
Kocuria rosa	3 (2.9%)	*Aeromonas sobria*	1 (0.9%)
Kocuria rhizophila	2 (1.9%)	*Acinetobacter junii*	1 (0.9%)
		Acinetobacter haemolyticus	1 (0.9%)

TABLE 2. Frequency distribution and percentages of GP and GN bacteria isolates of high vaginal swabs of non-pregnant women

Non-Pregnant women (N=76)			
Genus or Species (Gm $^{+ve}$)	**Frequency (%)**	**Genus or Species (Gm $^{-ve}$)**	**Frequency (%)**
Staphylococcus haemolyticus	15 (19.7%)	*Escherichia coli*	6 (5.7%)
Enterococcus faecalis	7 (9.2%)	*Serratia sp.*	3 (3.9%)
Staphylococcus epidermidis	7 (9.2%)	*Pantoea sv.*	2 (2.6%)
Staphylococcus aureus	5 (6.6%)	*Proteus mirabilis*	1 (1.3%)
Staphylococcus hominis	4 (5.3%)	*Acinetobacter haemolyticus*	1 (1.3%)
Staphylococcus sciuri	3 (3.9%)	*Pseudomonas fluorescens*	1 (1.3%)
Kocuria Kristinae	3 (3.9%)	*Sphingomonas sp.*	1 (1.3%)
Staphylococcus lentus	2 (2.6%)		
Micrococcus luteus	2 (2.6%)		
Kytococcus sp.	2 (2.6%)		
Staphylococcus warneri	1 (1.3%)		
Staphylococcus uberis	1 (1.3%)		
Leuconostoc sp.	1 (1.3%)		

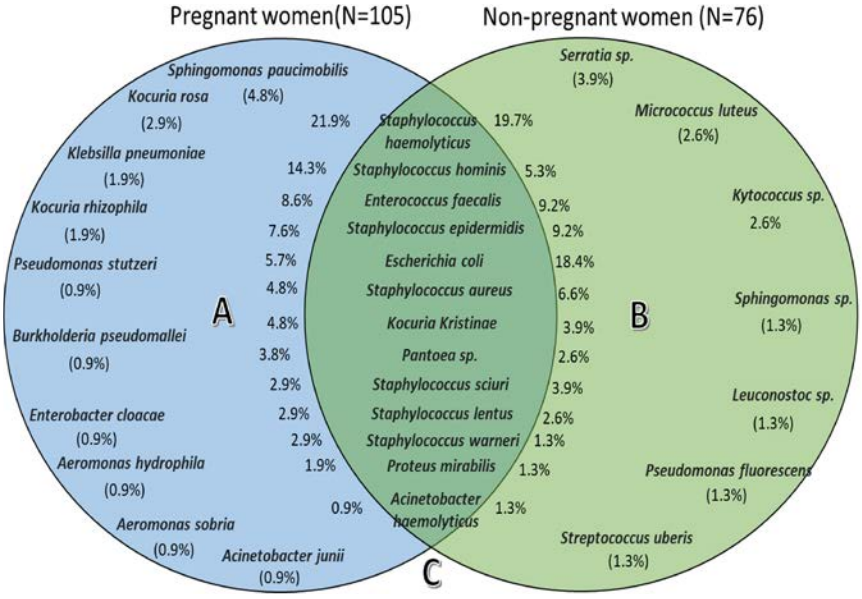

FIGURE 2. Venn diagram showing the distribution of bacterial species percentages in pregnant women (A) and non-pregnant women (B), in both pregnant and non-pregnant (C)

Antimicrobial susceptibility profile of GP bacteria isolated from a non-pregnant woman

A cluster tree was designed using UPGMA in order to evaluate the similarity of the composition of bacterial communities across all samples. The results are presented in Figures 3, 4, 5 and 6.

Regarding the GP bacteria that were isolated from non-pregnant women, UPGMA cluterisation revealed four clusters were identified at a similarity level of 40%. C1 and C2 Clusters comprise a few numbers of isolates with a sensitivity range from intermediate to sensitive. All of the C3 cluster isolates were resistant and had a range of bacterial numbers (5-12), whereas, most of the C4 cluster isolates were sensitive and exhibited a variety of bacterial counts (8-12) (Figure 3A).

GP isolates were tested for susceptibility to 15 antibacterial drugs (Figure 3B). 53 gram-positive isolates were tested, and complete resistance (100%) to several antibiotics was found as follows: OX1 was (37 [69.8%]) followed by CM was (21[39.6%]), FA was (12 [22.6%]), TE was (7 [13.2%]), FT was (6 [11.3%]), VA, LZN, SXT, RA, and R were (5 [9.4%]), GM was (2[3.8%]), TGC, MXF, LEV, and TM were (1[1.9%]).

The GP isolates demonstrated a sensitivity level of 100% to TGC was (49[92.5%]), MXF was (22[41.5%]), VA was (21[39.6%]), FT was (20[37.7%]), LZN was (14[26.4%]), LEV was (13[24.5%]), TM was (12[22.6%]), GM was (10[18.9%]), RA was (7[13.2%]), SXT was (5[9.4%]), TE was (4 [7.5%]), OX1, CM, FA and R were (1[1.9%]).

The results of our study indicate that the S. haemolyticus was found to be the most prevalent GP bacteria in the non-pregnant and exhibited a notable sensitivity to OX1, CM, TE, FA, SXT, FT, RA, R, LNZ, VA, TGC, MXF, LEV, TM and GM. While demonstrating resistance to MXF, LEV, VA, LNZ, FT, SXT, RA, TE, R, CM, FA, TM, GM and OX1.

Staphylococcus sciuri and *Leuconostoc sp.* exhibited resistance to 15 antibiotic drugs, followed by *S. haemolyticus,* which was resistant to 14 antibiotic drugs. On the other hand, *Staphylococcus warneri* demonstrated resistance to one drug.

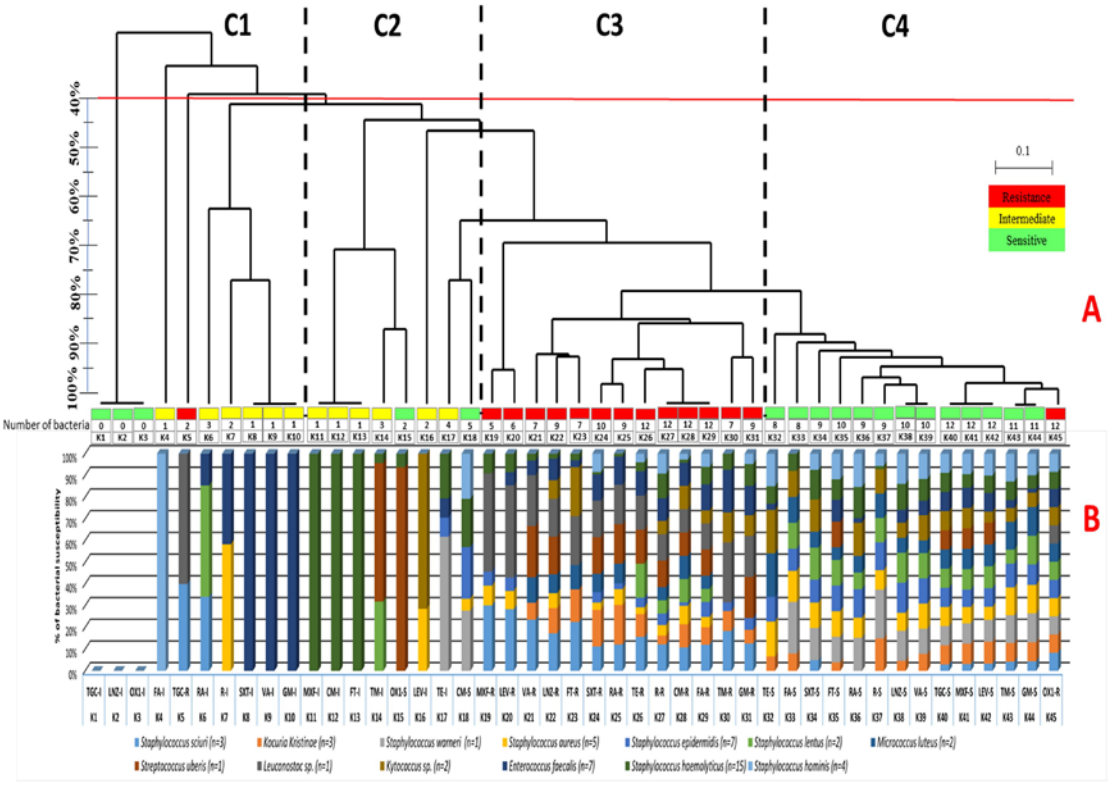

FIGURE 3. A) UPGM clusterisation of gram-positive bacteria isolated from a non-pregnant woman defined to the genus or species level and compared to percentage susceptibility to 15 antibacterial drugs. Clusters were defined at 40% similarity. B) non-pregnant woman bacterial diversity presented in percentage susceptibility (%) with the number of bacteria. Oxacillin (OX1), Gentamicin (GM), Tobramycin (TM), Nitrofurantoin (FT), Fusidic acid (FA), Vancomycin (VA), Clindamycin (CM), Erythromycin (R), Linezolid (LNZ), Levofloxacin (LEV), Moxifloxacin (MXF), Rifampicin (RA), Tetracycline (TE), Tigecycline (TGC) and Trimethoprim/Sulfamethoxazole (SXT)

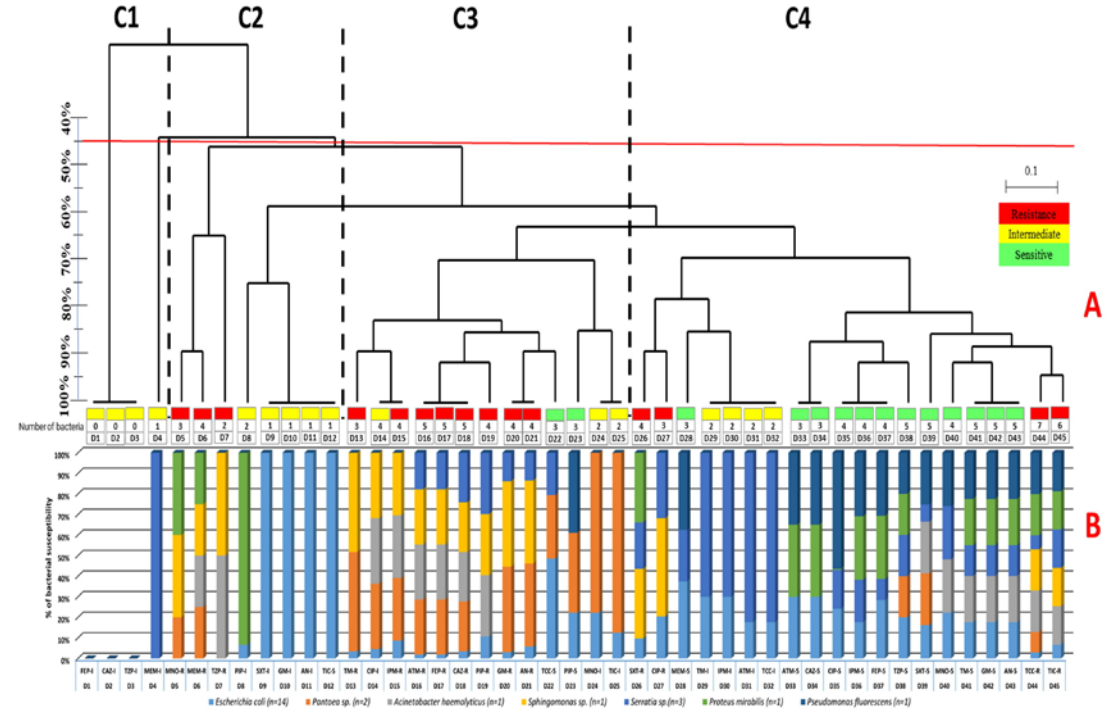

FIGURE 4. A) UPGM clusterisation of gram-negative bacteria isolated from a non-pregnant woman defined to the genus or species level and compared to percentage susceptibility to 15 antibacterial drugs. Clusters were defined at 45% similarity. B) non-pregnant woman bacterial diversity presented in percentage susceptibility (%) with the number of bacteria. Ticarcillin (TIC), Ticarcillin/clavulanic (TCC), Piperacillin (PIP), Piperacillin/Tazobactam (TZP), Ceftazidine (CAZ), Cefepime (FEP), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM), Amikacin (AN), Gentamicin (GM), Tobramycin (TM), Ciprofloxacin (CIP), Minocycline (MNO) and Trimethoprim/ Sulfamethoxazole (SXT)

Antimicrobial susceptibility profile of GN bacteria isolated from a non-pregnant woman

UPGMA cluterisation revealed four clusters with 45% similarity among the isolated GN bacteria from non-pregnant women. Most of the C1 and C2 cluster isolates were intermediate susceptibility, with few numbers of isolates. Most bacterial isolates in the C3 and C4 clusters were resistant and sensitive, respectively (Figure 4A).

GN isolates were tested for susceptibility to 15 antibacterial drugs (Figure 4B). 23 GN isolates were tested, and complete resistance (100%) to several antibiotics were found as follows: CAZ and TIC were (7 [30.4%]) followed by MEM and PIP were (5[21.7%]), IPM, ATM, FEP and TCC were (4 [17.4%]), TM, GM and AN were (3[13%]), MNO, TZP and SXT were (2[8.7%]), CIP was (1[4.3%]).

The GN isolates exhibited a sensitivity of 100% towards TZP was (21 [91.3%]), MEM was (15[65.2%]), MNO was (5 [21.7%]), SXT was (4 [17.4%]), PIP, TM, GM and AN were (3 [13%]), ATM, CAZ, CIP, IPM and FEP were (2 [8.7%]).

The most prevalent GN bacteria in samples of non-pregnant women namely, *E. coli* exhibited sensitivity to TIC, TCC, PIP, MEM, ATM, CAZ, CIP, IPM, FEP, TZP, SXT, MNO, TM, GM and AN, another serotype *E. coli,* demonstrated resistance to TM, IMP, ATM, FEP, CAZ, PIP, GM, AN, SXT, CIP, TCC and TIC.

The most resistant bacteria in our results was *Sphingomonas sp.* which was 100% resistant to 15 antibiotic drugs, followed by *Acinetobacter haemolyticus* and *Pantoea sp.* to 9 and 8 antibiotic drugs respectively. In general, the bacterial isolates exhibited multidrug resistance to at least two antibiotics, as observed in *Pseudomonas fluorescens.*

Antimicrobial susceptibility profile of GP bacteria isolated from a pregnant woman

In the isolated GP bacteria from pregnant, UPGMA cluterisation showed 2 main clusters at 55% similarity (Figure 5A). Most of the C1 cluster isolates were intermediate susceptibility, and they also had a few bacterial numbers. Whereas the larger (C2) cluster gathered sensitive and resistant high numbers of isolates.

The susceptibility of Gram-positive bacteria to 15 different antibacterial drugs was tested (Figure 5B).

In a total of 79 GP isolates, total resistance (100%) were detected against OX1 was (66 [83.5%]) followed by R was (41 [51.9%]), CM was (17[21.5%]), RA, VA, FT and FA were (8 [10.1%]), TE was (6 [7.6%]), SXT and LZN were(5[6.3%]), MXF, TGC, LEV, TM and GM were (3[3.8%]).

GP isolates demonstrated 100% sensitivity to TGC was (76[96.2%]), RA was (54[68.4%]), VA was (44[55.7%]), FT was(39[49.4%]), MXF was (37[46.8%]), LZN was(36[45.6%]), LEV was (26[32.9%]), TM was

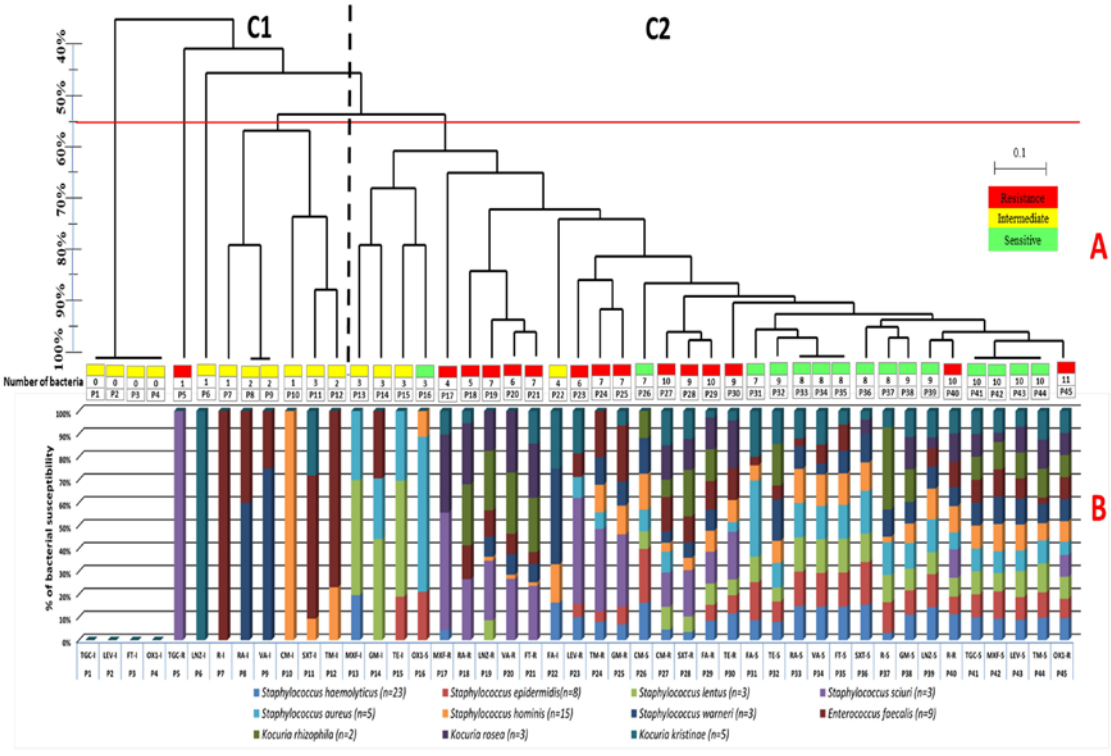

FIGURE 5. A) UPGM clusterisation of gram-positive bacteria isolated from a pregnant woman defined to the genus or species level and compared to percentage susceptibility to 15 antibacterial drugs. Clusters were defined at 55% similarity. B) pregnant woman bacterial diversity presented in percentage susceptibility (%) with the number of bacteria. Oxacillin (OX1), Gentamicin (GM), Tobramycin (TM), Nitrofurantoin (FT), Fusidic acid (FA), Vancomycin (VA), Clindamycin (CM), Erythromycin (R), Linezolid (LNZ), Levofloxacin (LEV), Moxifloxacin (MXF), Rifampicin (RA), Tetracycline (TE), Tigecycline (TGC) and Trimethoprim/Sulfamethoxazole (SXT)

(13[16.5%]), CM was (8[10.1%]), GM was (5[6.3%]) and R was (2[2.5%]).

The most prevalent GP bacteria in samples of pregnant women namely, *S. haemolyticus* were totally Sensitive 100% to VA, FT and LZN and totally resistant to OX1. However, the most resistant GP bacteria in our results were *Staphylococcus sciuri* which was 100% resistant to 15 antibiotic drugs, followed by *Enterococcus faecalis* and *Kocuria kristinae* to 12 and 11 antibiotic drugs respectively. In general, all the bacterial isolates were multidrug resistant to at least 6 antibiotics *(Staphylococcus aureus)*.

Antimicrobial susceptibility profile of GN bacteria isolated from a pregnant woman

Concerning isolated Gram-negative bacteria from pregnant, 6 UPGMA clusters were defined at 45% similarity. The Gram-negative bacteria structures' diversity was highly linked to antibiotic susceptibility with type bacterial species. Most of C1-C4, C5 and C6 cluster isolates were intermediate, sensitive and resistant respectively (Figure 6A).

Fifteen antibacterial drugs were utilized to test the susceptibility of GN isolates (Figure 6B). Among the 26 GN isolates tested, a complete resistance rate of 100% was observed against TIC was (6[23.1%]) followed by TM and PIP were (3[11.5%]), while AN, TZP, MNO, CAZ, GM), FEP and TCC) were (2[7.7%]), IPM, SXT and ATM were (1[3.8%]).

The GN isolates exhibited a sensitivity of 100% towards MEM was (16[61.5%]), AN was (14[53.8%]), TZP and MNO were (13[50%]), IPM was (12[46.2%]), ATM was (10[38.5%]), CIP and CAZ were (9[34.6%]), TM, SXT were (8[30.8%]), GM, FEP, TCC and PIP were (7[26.9%]), TIC was (4[15.4%]).

The most prevalent gram-negative bacteria in samples of pregnant women namely, *E. coli* exhibited sensitivity to ATM, TM, AN, TZP, MNO, IPM, SXT, CIP, GM, FEP, CAZ, TCC, PIP, and TIC. At the same time, another isolated serotype of *E. coli* demonstrated resistance to AN, TZP, MNO, IPM, SXT, ATM, TM, CIP, CAZ, GM, FEP, TCC, PIP, and TIC.

The results of our study indicate that *Burkholderia pseudomallei* exhibited the highest level of resistance among the tested bacterial strains, displaying 100% resistant to a total of 15 antibiotic drugs, followed by *E. coli* was resistant to 14 antibiotic drugs. In general, the bacterial isolates exhibited multidrug resistance to at least two antibiotics, as observed in *Klebsilla pneumoniae* and *Aeromonas sobria.*

DISCUSSION

Genital tract infections are a global concern that poses critical difficulties to women's health. These infections can result in many medical consequences, including considerable discomfort, frequent medi-

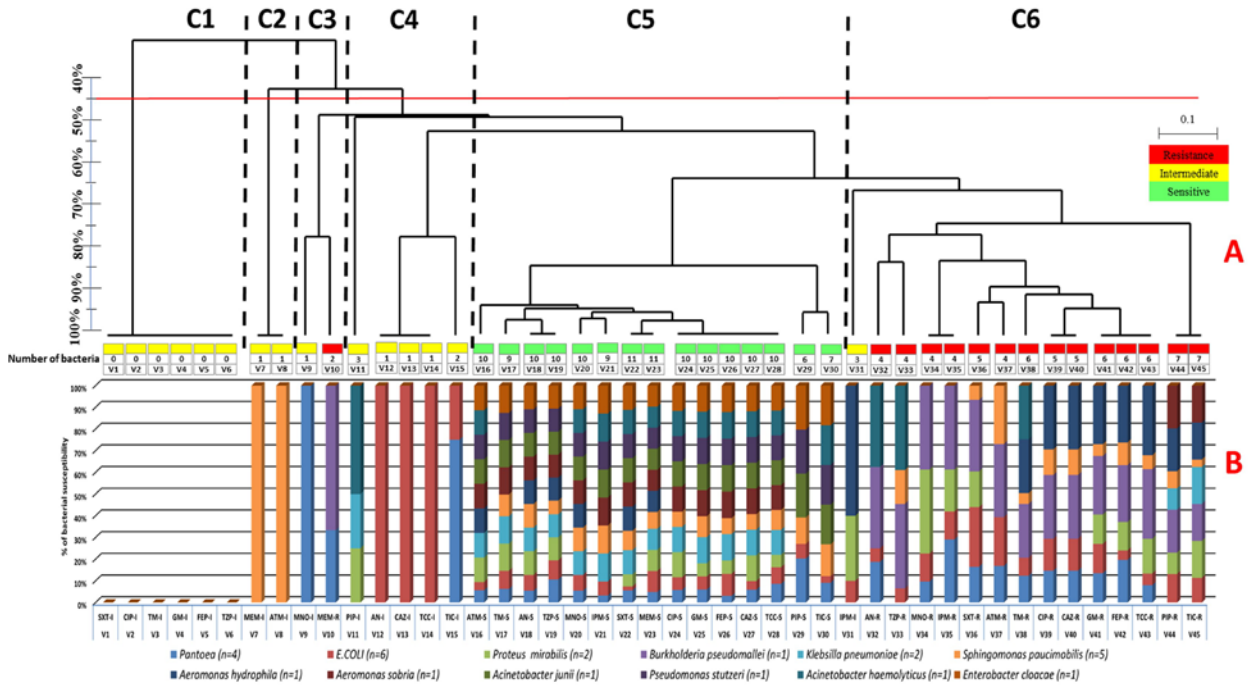

FIGURE 6. A) UPGM clusterisation of gram-negative bacteria isolated from a pregnant woman defined to the genus or species level and compared to percentage susceptibility to 15 antibacterial drugs. Clusters were defined at 45% similarity. B) pregnant woman bacterial diversity presented in percentage susceptibility (%) with the number of bacteria. Ticarcillin (TIC), Ticarcillin/clavulanic (TCC), Piperacillin (PIP), Piperacillin/Tazobactam (TZP), Ceftazidine (CAZ), Cefepime (FEP), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM), Amikacin (AN), Gentamicin (GM), Tobramycin (TM), Ciprofloxacin (CIP), Minocycline (MNO) and Trimethoprim/Sulfamethoxazole (SXT)

cal consultations, notable morbidity, premature rupture of pregnant membranes, and low birth weight [17]. A study by Donders and Bellen, 2011 [18] found that preterm delivery, premature membrane rupture, and fetal infections were all linked to aerobic vaginitis in pregnant women. Aerobic vaginitis consequences are probably not detected or treated in infected women; nonetheless, the Aerobic vaginitis treatment is strongly indicated based on susceptibility to antibiotics profiles.

The vaginal pH was measured in our study as it is considered a diagnostic tool for vaginal infection [19]. It is possible to measure the vaginal pH with either pH indicator strips or pH electrodes. Despite the accuracy of the latter, it is much more complicated and expensive. The strips are simple to use and provide a reasonably accurate result. However, the results may be affected by moisture and other factors. It is therefore recommended that women use pH electrodes if they want more reliable results [19].

The optimal vaginal pH ranges from 3.8 to 4.5 as a result form vaginal microflora and acid-base transporters balance in vaginal epithelium like Na+/H+ – exchangers, HCO3 – cotransporters, epithelial proton pumps and Na+ [20-21].

In our study, the acidic pH detected in non-pregnant women was approximately in the normal range of 3.5-5 and reflect a balanced growth of microbial flora. The measured vaginal pH of pregnant women ranged from normal acidic (4) to alkaline (8) indicating a disturbance of the protective effect of estrogen in maintaining acidic pH by providing a metabolic substrate (glycogen) for lactobacilli and the possibility of pathogen overgrowth. A similar observation of combined alkaline pH and overgrowth of abnormal vaginal bacteria was detected by Lykke et al. 2021 [20].

According to our study, pregnant women had significantly higher bacterial abundance than non-pregnant women (Figure 1), a finding that can be attributed to pregnancy-related hormonal changes, and physiologically modulated immune response [22].

Vitek system demonstrated a high accuracy rate of 86% in the identification of bacteria and a low rate of unidentified bacteria 14.2% which is in agreement with other studies [23-24]. We found a higher prevalence rate of GP bacteria than GN bacteria in both groups of our study; such an expected result can be explained by the low pH that favors an abundance of GP vaginal flora (Lactobacilli) and causes suppression of growth of facultative & obligatory anaerobic pathogens this agreement with previous studies by Onderdonk et al.2016 [25].

The most commonly isolated GP bacteria in our study was *S. haemolyticus* (Table 1 & 2) suggested to be ascending to the vagina from the urethra where it is a commensal flora. This microorganism is a well-known multidrug-resistant opportunistic pathogen of immune-compromised host and has biofilm formation characteristics. *S. haemolyticus* was purely isolated from urethral discharge by VITEK® by Naha et al., 2015 [26]. On the other hand, in (Table 1 & 2) the most commonly isolated GN *E. coli* is a major commensal of the gastrointestinal tract and is a common cause of several infections including urinary and genital tracts as a result of poor hygiene [27-29].

We observed a high level of antimicrobial resistance to OX1 for GP bacteria isolated from a non-pregnant (69.8%) and pregnant (83.5%) (Figure 3B & 5B). GP bacteria may develop resistance to β-lactam antibiotics because they produce the enzyme β-lactamase, which hydrolyzes the antibiotic's amide bond [30].

According to this study, GN bacteria showed the highest percentage of resistant (100%) to CAZ and TIC (30.4% each) in non-pregnant whereas the highest resistance in pregnant samples was against TIC (23.1%) (Figure 4B & 6B). This β-lactam antibiotic resistance is again attributed to the production of β-lactamase enzymes by Enterobacteriaceae *(E. coli)* [31]. Weak activity of CAZ against GN bacteria has been detected in previous studies [32].

In this study, all types of bacteria were tested, analyzed, and found to be 100% sensitive to different classes of antibiotics. Antibiotics that were most effective against GP bacteria obtained from non-pregnant and pregnant women were TGC, RA, VA, FT, MXF, LZN, LEV, TM, CM, GM and R (Figure 3B & 5B). While the Antibiotics that were most effective against GN bacteria obtained from non-pregnant and pregnant women were TZP, MEM, MNO, SXT, PIP, TM, GM, ATM, CAZ, CIP, IPM and FEP (Figure 4B & 6B). The current study's findings partially agree with the research conducted by Yasin et al. (2021) [33], which demonstrated that VA, CM, and GM were the most effective antibiotics against GP bacteria. Similarly, their study indicated that GM, MEM, and CIP exhibited the most effective against GN bacteria from the vagina.

Alwaily et al. (2022) [34] found a high number of *S. haemolyticus* had a sensitivity to SXT, FT, VA, LEV and GM, and these results agree with the current study. Indeed, in the same study, *S. haemolyticus* was resistant to six antibiotics that have been tested in our study (LEV, VA, FT, SXT, TE and GM) but with a different percentage of resistance. Antibiotic resistance may be attributed to challenges in the penetration of antibiotics via biofilms, the bacteria's slow growth rate, and mechanisms that degrade antibiotics. Antibiotic resistance occurs due to the increased formation of biofilms, mainly resulting in the emergence of persistent infections [35-36].

In the present study, some of the tested GP and GN bacteria showed multidrug resistance ranging

from 2-15 antibiotics in both groups. In another study conducted by Kareem and Abdulhamid 2023 [37], they found multidrug resistance range from 3-17 antibiotics in GN bacteria isolated from the vagina. Several factors potentially contribute to the elevated rates of multidrug-resistant infections. These include substandard quality of antibiotics, inappropriate drug utilization, insufficient hygiene practices, and a lack of regulation regarding antibiotic usage. Notably, Iraq needs a comprehensive policy for controlling antibiotic usage, and it is commonplace for individuals to purchase antibiotics without a prescription from private pharmacies [29].

CONCLUSION

Antibiotics play a critical role in managing microbial infections, and culture sensitivity reports assist in enhancing their reasonable, empiric usage in clinical settings. The findings of this study showed that TZP and MEM were the most effective antibiotics against GN isolates in both groups. In contrast, GP bacteria were highly susceptible to TGC. Clinicians should use modern antibiograms to assess susceptibility patterns and justify antibiotic empiric therapy to minimize antibiotic resistance.

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