Archives of Razi Institute, Vol. 78, No. 1 (2023) 31-43

DOI: 10.22092/ARI.2022.358775.2307

Original Article

Antibiotic Susceptibility Profile of Bacteria Causing Aerobic Vaginitis in Women in Iraq

Kareem Raheem, Z[1 *], Abdulhamid Said, L[1]

1. Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq

Received 11 May 2022; Accepted 8 June 2022
Corresponding Author: zahraa91kr@gmail.com

Abstract

Aerobic vaginitis (AV) is a vaginal infectious condition characterized by abnormal vaginal discharge, high inflammatory response, signs of epithelial atrophy, an increase in aerobic bacteria of intestinal origin and a decrease in the normal flora, especially *Lactobacillus spp.*. It is one of the most common reproductive tract infections among women. This study aimed to analyze the antimicrobial susceptibility levels of the dominant bacterial species found in the vaginae of women infected with AV. A total of 89 high vaginal swabs (HVS) were collected from women aged (18-50) years old attending some hospitals and private gynaecology clinics in Baghdad City. All obtained swabs were cultured on different culture media, and the primary diagnosis was performed according to standard laboratory diagnosis protocols. To confirm the diagnosis and to determine the antibiotic susceptibility profile of bacterial isolates, VITEK 2 Compact Automated System GP and GN colourimetric identification cards and AST GN and AST GP cards were used according to Manufacturer Company constructions (BioMérieux / France). Out of 89swabs, ninety-five pathogenic strains were obtained, including 62 isolates (65.2%), Grampositive and 33 isolates (34.7%), Gram-negative bacteria. *Staphylococcus* spp. (46.3%) The most represented active strain was *Escherichia coli* (15.7%). All Gram-positive bacterial strains displayed the highest resistance rates (100%) toward penicillins and cephalosporins, while the highest sensitivity rates were toward daptomycin, followed by vancomycin and gentamicin (*P*=0.001). Gram-negative bacteria displayed the highest resistance rates toward penicillins, beta-lactam combination, monobactam and cephalosporins, while the highest sensitivity rates were toward amikacin followed by imipenem meropenem and gentamicin (*P*=0.001). It is worth mentioning that Gram-positive bacteria showed 100% sensitivity toward tigecycline. Thirty-eight (40 %) of all obtained bacterial strains were extensively drug-resistant XDR, 57 (60%) were multidrug resistance MDR and no pan-drug resistance PDR was reported. Gram-positive bacteria include 21% XDR and 44.2% MDR strains, while Gram-negative bacteria include 18.9% XDR and 15.7% MDR strains.

Keywords: Aerobic vaginitis, VITEK 2 system, GP and GN colourimetric cards, Aerobic bacteria, Antibiotic susceptibility, AST GP and AST GN cards, Iraq

1. Introduction

Human vaginal flora is a complex and protective environment which enables the maintenance of vaginal pH levels and microbial balance to withstand the invasion of pathogenic microorganisms. However, any imbalance in the naturally occurring bacterial flora may result in infections such as bacterial vaginitis (BV) and aerobic vaginitis (AV) (1). Donders first defined aerobic vaginitis in 2002 as a type of vaginal infection caused by aerobic bacteria, and AV was diagnosed according to Donders' score criteria (2). Aerobic vaginitis is not a new entity, but the second description of a type of bacterial vaginitis was first

reported by Scheffey in 1956 and named Desquamative inflammatory vaginitis by Gardner in 1968 (3). The clinical symptoms of AV include abnormal leucorrhea, increased discharge, vulval itching, burning pain, dyspareunia, erythema, and edema. Secondary infections may also occur, the outcomes of which can be severe, including miscarriage, infertility, and pelvic inflammatory disease (1, 4). Women infected with AV may suffer from many conditions, such as amniotic fluid infection, premature rupture of membranes, preterm labor, chorioamnionitis, cervical intraepithelial neoplasia disease and sexually transmitted infections (5). Previous studies have shown that AV is associated with an increased risk of amnionitis (6), placental histological inflammation, preterm delivery (6, 7), foetal funisitis (8) and major cervical cytological abnormalities (9). The main AV causative agents are bacteria of intestinal origin, including *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae* and some Gram-positive bacteria, including *Streptococcus agalactiae*, *Staphylococcus aureus* and Coagulase Negative staphylococci (5, 10). Diagnosis of AV is based on analyzing the patient's clinical signs and laboratory tests through microscopic analysis, cultural examination and antibiotic susceptibility tests. The global problem of AV is strongly linked to the emergence of antimicrobial resistance. Women with AV can develop an adverse outcome following antibiotic treatment (11). Aerobic vaginitis is often misdiagnosed and is not easy to treat due to the developing spread of multidrug-resistant bacterial strains. In Iraq, limited studies on AV have been conducted, so this study aimed to evaluate the aerobic bacterial types and antimicrobial susceptibility profile in AV patients, also to detect the incidence of multidrug-resistance (MDR), extensively drug-resistance (XDR) and pan drug-resistance (PDR) bacterial isolates.

2. Materials and Methods

2.1. Sample Collection

The present study samples were collected from women attending Al -Elwea Maternity Hospital and many private gynaecology clinics in Baghdad City. Eighty-nine high vaginal swabs were collected from women suffering from AV symptoms over 6 months from January 2021 to June 2021. Medical history was obtained from all patients after obtaining their verbal consent using a Questionnaire form; women who were treated with any antibiotics in the preceding 2 weeks were excluded from the study.

2.2. Cultivation and Identification of Bacterial Isolates

All obtained swabs were used for aerobic culturing aimed at the detection of aerobic bacterial growth by inoculating in MacConkey agar (Oxoid / England), blood agar (Oxoid / England), and mannitol salt agar (Oxoid / England) and incubating at 37 °C for 24-48 hours aerobically. After 24 hours of incubation, the plates were examined for the presence of growth (12). The colonies obtained were read out as standard microbiological methods based on morphological characteristics and biochemical tests (13). VITEK 2 Compact System and GN, GP colourimetric identification cards (BioMérieux/ France) were used to confirm the identification of bacterial isolates.

2.3. Antibiotic Susceptibility

Antibiotic susceptibility testing of all the bacterial isolates was performed using (AST-GP) and (AST-GN) cards of the VITEK 2 System according to the manufacturer's company (BioMérieux/ France). Antibiotics used in this study belong to 12 classes for Gram-positive bacteria and 9 classes for Gram-negative bacteria.

2.4. Data Analysis

Data were entered and analyzed using Microsoft Excel (2013) and the Unweighted Pair Group Method with Arithmetic mean (UPGMA) Clustering method and the similarity index (Dicecoefficient).

3. Results

3.1. Prevalence of Bacterial Types according to GP and GN Identification Cards

VITEK 2 System GP, GN identification cards revealed that ninety-five pathogenic bacterial isolates were obtained, including 62 isolate Gram-positive and 33 isolate Gram-negative bacteria, as shown in (Figures 1 and 2). Gram-positive bacteria belonging to 5 genera and 13 species. As a result, 44 isolates of *Staphylococcus* spp. were distributed among 11 isolates of *S. hominis*, 11 isolates of *S. haemolyticus*, 10 isolates of *S. aureus*, 7 isolates *of S. epidermidis*, 2 isolates of *S. saprophyticus*, 2 isolates *S. auricularis* and 1 isolate *S. warneri*. Six isolates of *Enterococcus* spp. were distributed between 5 isolates *of E. faecalis* and 1 isolate *of E. faecium*. Also, *Kocuria* spp. It Included 4 isolates of *K. kristinae* and 2 isolates of *K. rosea*. While *Micrococcus* and *Streptococcus* were represented in only one species for each when 5 isolates of *Micrococcus luteus* and 1 isolate *of Streptococcus agalactiae* were obtained. Gram-negative bacteria belong to 5 genera and 5 species; they included 15 isolates *of Escherichia coli*, 11 isolates of *Klebsiella pneumoniae*, 3 isolates *of Pseudomonas aeruginosa*, 2 isolates of *Acinetobacter baumannii* and 2 isolates *Proteus mirabilis*. Figures 1 and 2 show the percentages of Gram-positive and Gram-negative bacteria obtained in the present study.

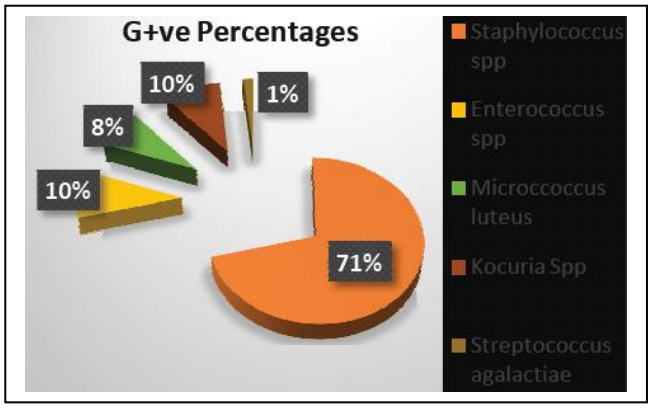

Figure 1. Prevalence of Gram-positive bacteria

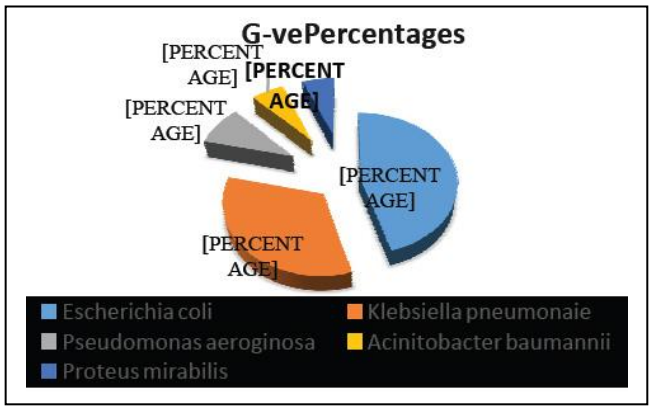

Figure 2. Prevalence of Gram-negative bacteria

3.2. Antibiotic Susceptibility Test of Gram-Positive Bacterial Isolates

According to GP AST cards, Gram-positive bacterial species showed susceptibility toward 30 antibiotics belonging to 12 groups. As shown in table 1, all Gram-positive bacterial types showed a high resistance rate (100%) toward penicillins and cephalosporins, while 100% sensitivity was reported toward glycylcyclines.

3.2.1. Susceptibility Profile of *Staphylococcus* spp.

Antibiotic susceptibility cards (AST GP) cards showed that the highest resistance of *Staphylococcus* spp. was against penicillins, cephalosporins (100%) and the highest sensitivity were observed toward tigecycline (100%), daptomycin (77.2%) followed by trimethoprim/sulfamethoxazole (70.4%), vancomycin (54.5%) and gentamicin (52.2%).

3.2.2. Susceptibility Profile of *Enterococcus* spp.

The resistance and sensitivity of *Enterococcus spp.* were tested toward many antibiotics, and results showed that the highest resistance rate (100%) was found against penicillins, cephalosporins, macrolides, fusidane groups, sulfonamides and pipemidic acid antibiotic from fluoroquinolones group, while the highest sensitivity rate was found toward tigecycline (100%), gentamicin (50%) and levofloxacin (50%).

3.2.3. Susceptibility Profile of *Kocuria* spp.

The results showed that the highest resistance of *Kocuria* spp. (100%) were found against penicillins, cephalosporins, macrolides, lincomycin, tetracyclines, pipemidic acid and norfloxacin antibiotics from fluoroquinolones, while the highest sensitivity was found toward tigecycline (100%), vancomycin and teicoplanin (50%).

3.2.4. Susceptibility Profile of *Micrococcus luteus*

Antibiotic susceptibility test of *Micrococcus luteus* revealed that the highest resistance (100%) was found against penicillins, cephalosporins and fluoroquinolones group, while the highest sensitivity was found toward tigecycline (100%), daptomycin, vancomycin (80%) followed by gentamicin, fusidic acid and erythromycin (60%).

Table 1. Antibiotic susceptibility of Gram-positive bacteria

Group Name	Antibiotics	Resistance of Gram-positive bacterial genera			
		***Staphylococcus spp* (44)**	***Enterococcus spp* (6)**	***Micrococcus luteus* (5)**	***Kocuria spp* (6)**
Penicillins	Ticarcillin ,Azlocillin, Mezlocillin, Piperacillin	44 (100%)	6 (100%)	5 (100%)	6 (100%)
Cephalosporins	Cefoxitin, Cefixime, Ceftazidime, Cefoperazone, Ceftriaxone, Cefepime, Cefpirome, Cefazolin	44 (100%)	6 (100%)	5 (100%)	6 (100%)
Aminoglycosides	Gentamycin	21 (47.7)	3 (50%)	2 (40%)	4 (66.6)
Fluoroquinolones	Pipemidic acid	42 (95.4.7%)	6 (100%)	5 (100%)	6 (100%)
	Ciprofloxacin	26 (59%)	5 (83.3%)	5 (100%)	5 (83.3%)
	Moxifloxacin	24 (54.5%)	5 (83.3%)	5 (100%)	4 (66.6%)
	Norfloxacin	28 (63.6%)	5 (83.3%)	5 (100%)	6 (100%)
	Ofloxacin	28 (63.6%)	5 (83.3%)	5 (100%)	5(83.3%)
	Levofloxacin	23 (52.2%)	3 (50%)	5 (100%)	5 (83.3%)
Macrolides	Azithromycin, Clarithromycin	40 (90.9%)	6 (100%)	3 (60%) 4 (80%)	6 (100%)
	Erythromycin	40 (90.9%)	6 (100%)	2 (40%)	6 (100%)
Lincomycin	Clindamycin	30 (68.1%)	5 (83.3%)	3 (60%)	6 (100%)
Cyclic lipopeptide	Daptomycin	10 (22.7)	5 (83.3%)	1 (20%)	2 (33.3%)
Glycopeptide	Vancomycin	20 (45.4%)	5 (83.3%)	1 (20%)	3(50%)
	Teicoplanin	27 (61.3%)	5 (83.3%)	3 (60%)	3 (50%)
Tetracycline	Tetracycline	32 (72.7%)	5 (83.3%)	3 (60%)	6 (100%)
Glycylcyclines	Tigecycline	0	0	0	0
Fusidane class	Fusidic Acid	34 (77.2%)	6 (100%)	2 (40%)	5 (83.3%)
Sulfonamides	Trimethoprim/Sulfamethoxazole	13 (29.5%)	6 (100%)	3 (60%)	4 (66.6)

P-value=0.001

3.3. Antimicrobial susceptibility profile and similarity rates of gram-positive isolates

The antimicrobial susceptibility types and the similarity rates of all Gram-positive bacterial isolates were investigated and reported in figure 3 and table 2. Tested isolates distributed into (MDR) and (XDR). Based on antibiotic test results in table 1, a Dendrogram of similarity between 62 Gram-positive isolates was provided by computing Dice Coefficient (DSC) (Figure 3); the isolates were separated into 2 main clusters, namely A and B formed at 0.88 (88%) similarity and both were divided to several sub-clusters. Cluster A consists of (40), isolates, including 4 genera and 11 species; it comprised between 41 *M. luteus* and 52 *S. auricularis* formed at 0.91 (91%) similarity rate and enclosed all isolates sharing the antibiotic resistance types, namely (A, B, C, and D, E, F and G), the second cluster named B consist of (22) isolate

included 5 genera and 10 species, it comprised between 39 *S. hominis* and 54 *S. saprophyticus* formed at 0.90.5 (90.5%) similarity and enclosed all isolates sharing the antibiotic resistance types namely (H, I, J, K, L, M, N and O) as shown in table 2. Cluster A showed the highest similarity were comprised between the isolates (28 *S. epidermidis*, 29 *S. hominis*, 42 *M. luteus*, and 50 *K. kristinae*, 51 *S. warneri*, 59 and 61 *E. faecalis*) at a similarity of 0.98 (98%) they shared the antibiotic group named (G) when they showed resistance against 28 antibiotics. Also, high similarity rates were shown between the isolates (14 *S. haemolyticus*, 30 and 31 *S. hominis*), (24 and 25 *S. epidermidis*), (3 and 4 *S.aureus*) at similarity 0.99 (99%), they shared the antibiotic types named (D and F) as shown in table 2. While according to cluster B, the highest similarity rate were shown between (37, 38 *S. hominis*), (34, 35 *S. hominis*), (5, 7, 9 *S. aureus*) at similarity 0.98 (98 %); they shared the antibiotic types named (M, O and J) as shown in table 2.

As shown in table 2, 20 isolates (32.2%) of Gram-positive bacteria showed (XDR) level of resistance when they showed resistance toward 10,11 antibiotic groups used in this study, and they share the types named (E, F, and G, L, M and O). The lowest number of groups of antibiotic resistance was reported in the type named (H) from cluster B when they showed resistance toward three antibiotic groups only. The multidrug resistance level of resistance was reported in 42 (67.7%) Gram-positive bacterial isolates when they showed resistance against less than 10 antibiotic groups—alarming the percentage of XDR *Staphylococcus* spp. Strains were (45%) from all Gram-positive isolates, representing the highest XDR rate among the XDR isolates in our study, and this is probably because of the total number of *Staphylococcus* spp. Strains isolated were (44) out of (62). It is worth mentioning that five out of six *Kocuria* spp. Showed XDR level of resistance as well as *Enterococcus* spp.

3.4. Antibiotic Susceptibility Test of Gram-Negative Bacterial Isolates

According to GN AST cards, Gram-negative bacterial species susceptibility results are shown in table 3, including susceptibility toward 30 antibiotics belonging to 9 groups and high rates of resistance against penicillins, beta-lactam combination and monobactam.

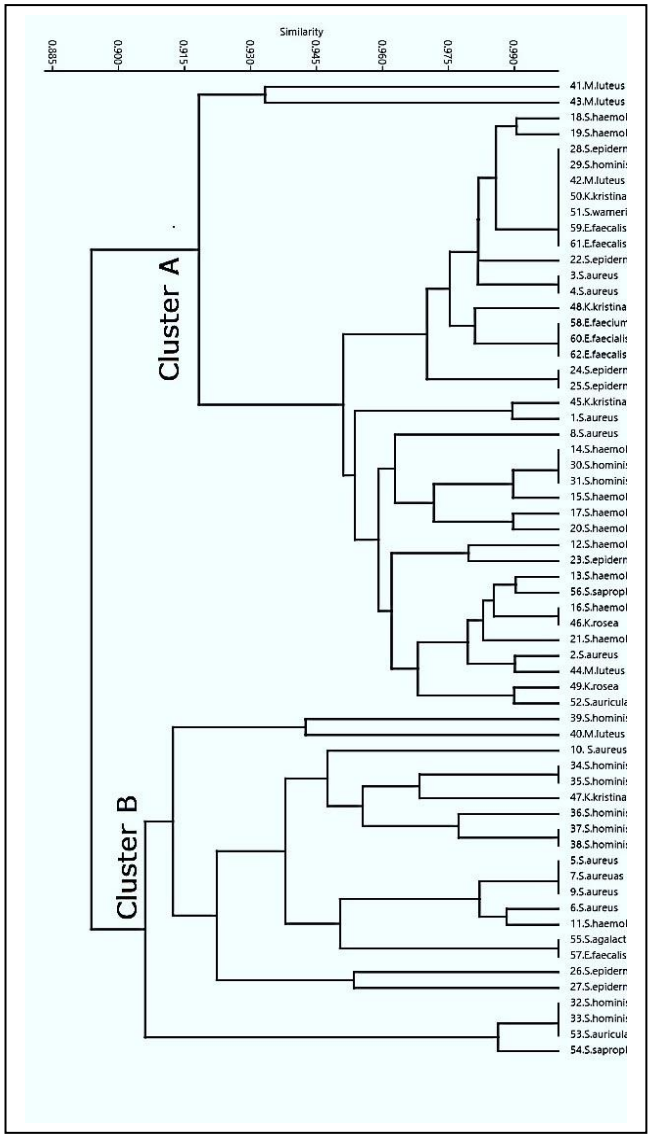

Figure 3. The hierarchy Dendrogram clarifies the relationship between Gram-positive bacterial isolates according to their susceptibility toward different antibiotics using UPGMA (Unweighted Pair Group Method with Arithmetic mean) Clustering method and similarity index (Dice coefficient)

Table 2. Distribution of Gram-positive bacteria according to the antimicrobial susceptibility types and resistance level

Type	Names of Bacterial isolates	The numbers of antibiotics the isolates resist	Number of groups	Resistance level
	Cluster A			
A	41 *M. luteus* , 43 *M. luteus*	20	5,6	MDR
B	1*S.aureus*, 45 *K. kristinae*	22	6	MDR
C	15 *and* 17 *S. haemolyticus*	24	7-8	MDR
D	12, 14, 20 *S. haemolyticus*, 23 S. epidermidis, 30 and 31 *S. hominis*24 and 25 *S. epidermidis*	25	7-9	MDR
E	8 *S. aureus*, , 44 *M. luteus*, 49 *K. rosea*, 52 *S. auricularis* 2,3,4 *S. aureus*, 21 *S. haemolyticus*, 22 *S. epidermidis*,	26	9-11	MDR, XDR
F	13, 16 *S. haemolyticus*, 56 *S. saprophyticus*, 46 *K. rosea*	27	9-11	MDR, XDR
G	18,19, 29 *S. haemolyticus*, 28 *S. epidermidis*, 42 *M. luteus*, 48 and 50 *K. kristinae*, 51 *S. warneri* 58 *E. faecium*, 59,60,61 *E. faecalis*, 62 *E. faecalis*	28	8-11	MDR, XDR
	Cluster B			
H	54 *S. saprophyticus*	12	3	MDR
I	32 and 33 *S. hominis*, 53 *S. auricularis*,	13	8, 3, 3	MDR
J	5, 7, 9 *S. aureus*	17	5, 8, 8	MDR
K	6 *S. aureus*	18	8	MDR
L	11 *S. haemolyticus*, 26 *S. epidermidis*, 36 *S. hominis*, 57 *E. faecalis*, 55 *S. agalactiae*	19	6-11	MDR, XDR
M	37 and 38 *S. hominis*, 10 *S. aureus*, 27 *S. epidermidis*, 39, *S. hominis*, 47 *K. kristinae*	21	7-10	MDR, XDR
N	40 *M. luteus*	22	7	MDR
O	34 and 35 *S. hominis*	24	10	X

Table 3. Antibiotic susceptibility of Gram-negative bacteria

Group Name	Antibiotics	Resistant of Gram-negative bacterial genera				
		E. coli **15**	***K. pneumoniae*** **11**	***P. aeruginosa*** **3**	***A. baumannii*** **2**	***P. mirabilis*** **2**
Penicillins	Ticarcillin	15 (100%)	11 (100%)	3 (100%)	2 (100%)	2 (100%)
	Azlocillin	14 (93.3%)				
	Piperacillin	14 (93.3%)	11 (100%)	3 (100%)	2 (100%)	2 (100%)
Beta-lactam combination	Piperacillin/tazobactam	14 (93.3%)	7 (63.6%)	3 (100%)	2 (100%)	2 (100%)
Monobactam	Aztreonam	15 (100%)	9 (81.8%)	3 (100%)	2 (100%)	2 (100%)
Cephalosporins	Cefazolin Ceftobiprole Cefixime Cefepime	14 (93.3%)	9(81.8%)	3 (100%)	2 (100%)	2 (100%)
	Cefditorin Ceftriaxone	14 (93.3%)	10 (90.0%)	2 (66.6%)	1 (100%)	1 (50%)
	Ceftazidime	14 (93.3%)	3 (27.2%)	3 (100%)	2 (100%)	2 (100%)
	Nitrofurantoin	7 (46.6%)	3 (27.2%)	2 (66.6%)	2 (100%)	2 (100%)
Fluoroquinolones	Nalidixic acid	15 (100%)	9 (81.8%)	3 (100%)	2 (100%)	2 (100%)
	Fleroxacin	14 (93.3%)	7 (63.6%)	0	1 (50%)	2 (100%)
	Levofloxacin	9 (60%)	1 (9.0%)	0	1 (50%)	2 (100%)
	Moxifloxacin	9 (60%)	2 (18.1%)	0	1 (50%)	2 (100%)
	Sparfloxacin	4 (26.6%)	2 (18.1%)	0	1(50%)	2 (100%)
	Ciprofloxacin	14 (93.3%)	7 (63.6%)	2 66.6%)	1 (50%)	2 (100%)
Carbapenems	Doripenem	3 (20%)	7 (63.6%)	3 (100%)	2 (100%)	2 (100%)
	Etrapenem	3 (20%)	7 (63.6%)	1 (33.3%)	2 (100%)	2 (100%)
	Imipenem	2 (13.3%)	1 (9.0%)	1 (33.3%)	0	0
	Meropenem	2 (13.3%)	1 (9.0%)	1 (33.3%)	0	1 (50%)
Aminoglycoside	Amikacin	4 (26.6%)	0	0	0	0
	Gentamicin	7 (46.6%)	1 (9.0%)	0	0	0
	Netlimicin	9 (60%)	1 (9.0%)	0	1 (50%)	2 (100%)
	Tobramycin	9 (60%)	1 (9.0%)	0	1 (50%)	1 (50%)
Tetracycline	Minocycline Tetracycline	9 (60%)	1 (9.0%)	2 (66.6%)	1 (50%)	2 (100%)
Sulfonamides	Trimethoprim/Sulfamethoxazole	14 (93.3%)	7 (63.6%)	3 (100%)	2 (100%)	2 (100%)

P-value=0.001

3.4.1. Susceptibility Profile of *Escherichia coli*

As a result, *E. coli* showed the highest resistance rate (100%) against ticarcillin from the penicillins group, monobactam and only nalidixic acid from the fluoroquinolones group, while the highest sensitivity rate was toward imipenem and meropenem (86.6%) followed by sensitivity (80%) towards doripenem, ertapenem and (73.3%) toward amikacin.

3.4.2. Susceptibility Profile of *Klebsiella pneumonia*

K. pneumoniae showed the highest resistance rate (100%) against antibiotics belonging to the penicillin group, while the highest sensitivity rate (100%) was found toward amikacin, followed by (90.9%) sensitivity toward all levofloxacin, imipenem, meropenem, gentamicin, netilmicin, tetracycline and minocycline, the sensitivity rates toward moxifloxacin and sparfloxacin were (81.8%).

3.4.3. Susceptibility Profile of *Pseudomonas aeruginosa*

Antibiotic susceptibility test of *pseudomonas aeruginosa* revealed that the highest resistance rate (100%) was found against penicillins, beta-lactam combination monobactam, sulfonamides, cefazolin and ceftazidime from cephalosporins group, nalidixic acid from fluoroquinolones class. While the highest sensitivity rate (100%) was found toward 8 used antibiotics, including fleroxacin, levofloxacin, moxifloxacin, sparfloxacin, amikacin, gentamicin, netilmicin, tobramycin.

3.4.4. Susceptibility Profile of *Acinetobacter baumannii*

A. baumannii showed the highest resistance rate (100%) against penicillins, beta-lactam combination, monobactam, and cephalosporins, nalidixic acid from fluoroquinolones group, sulfonamides, doripenem and ertapenem from carbapenem group, while the highest sensitivity (100%) were found toward imipenem, meropenem, amikacin and gentamicin.

3.4.5. Susceptibility Profile of *Proteus mirabilis*

As a result, *P. mirabilis* showed the highest resistance rate (100%) against penicillins, beta-lactam combination, monobactam, cephalosporins (except ceftriaxone 50%), fluoroquinolones, carbapenems (doripenem and ertapenem), tetracyclines, sulfonamides and netilmicin from aminoglycosides group. A high sensitivity rate (100%) was reported toward imipenem, amikacin and gentamicin.

3.5. Antimicrobial Susceptibility Profile and Similarity Rates of Gram-Negative Isolates

Based on antibiotic resistance numbers and percentages in table 3, a dendrogram of similarity between G-ve bacterial isolates was provided by computing Dice Coefficient (Figure 4); the isolates were included in two clusters, namely A and B formed at 0.24 (24%) similarity and cluster A divided into sub-clusters. Cluster A consists of (30), isolates included 5 genera and 5 species; it comprised between 4 *K. pneumoniae* and 5 *K. pneumoniaeformed* at 0.83 (83%) similarity and enclosed all isolates sharing the antibiotic types, namely (A, B, C, D, E, F, G, H, I, J, K, L, M) as in table 4. While cluster B consists of (3) isolates including 2 genera and 2 species, it formed between 3 *K. pneumoniae* and 20 *E. coli* at 0.31 (31%) similarity and enclosed the isolates sharing the types named (N and O) as shown in table 4.

As shown in table 4, 18 isolates (54.5%) of Gram-negative bacteria showed (XDR) level of resistance when they showed resistance toward (7-9) antibiotic groups, and they shared the types named (E, F, G, and H, I, J, K, L and M). The lowest number of groups of antibiotics resistance was reported in the types named (N and O) from cluster B when they showed resistance toward one or two antibiotic classes only, while the highest resistance was reported in the types named (L and M) when they showed resistance toward antibiotics from all of the nine classes used in the present study. The multidrug resistance level was reported in 15 (45.4%) Gram-negative isolates that shared the antibiotic resistance types named (A, B, C, D, N, and O). Alarming that the percentage of XDR *E. coli* strains was (50%) from all Gram-negative isolates, it represents the highest XDR rate between Gram-negative isolates, all of *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Acinetobacter baumannii* showed XDR level of resistance.

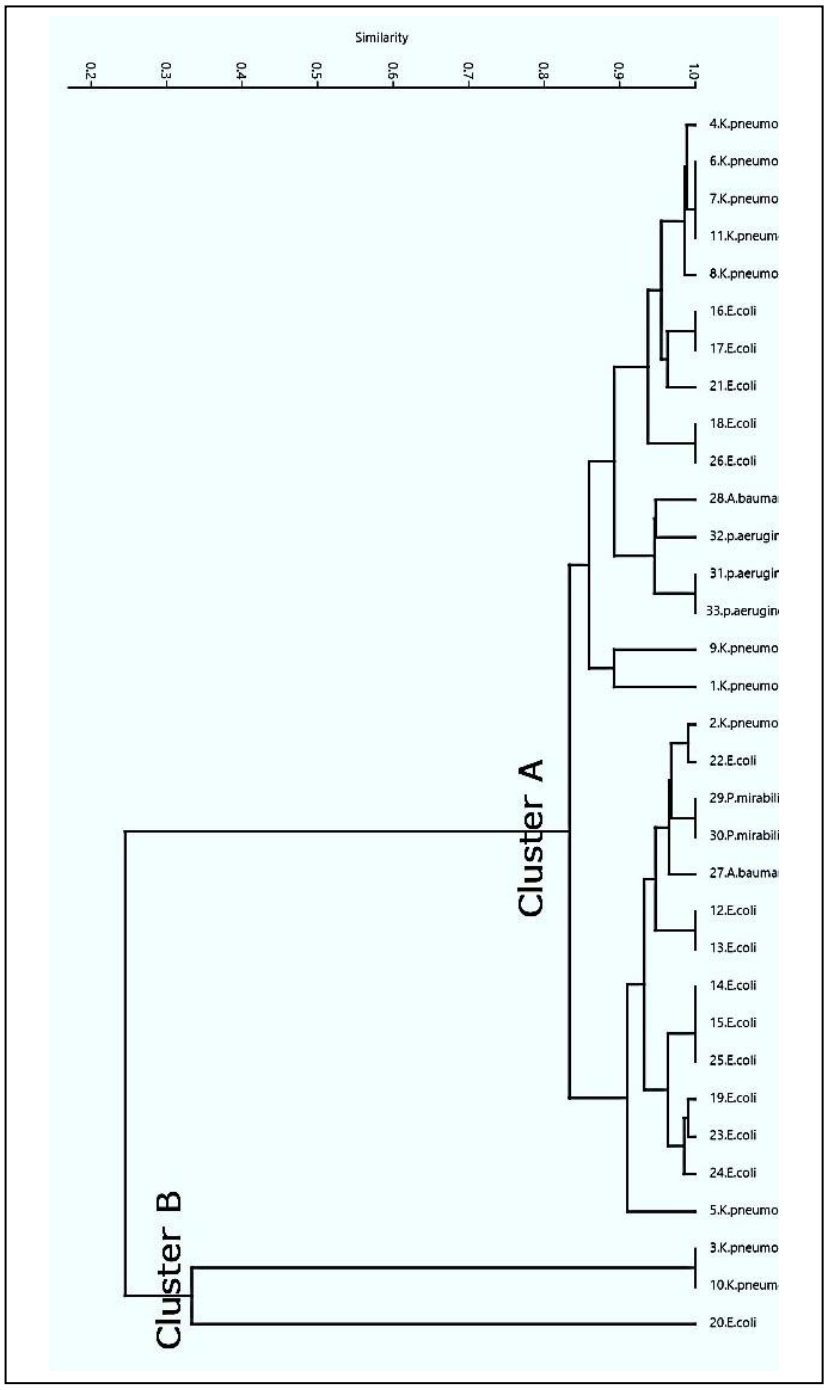

Figure 4. The hierarchy Dendrogram clarifies the relationship between Gram-negative bacterial isolates according to their susceptibility toward different antibiotics using the UPGMA clustering method and similarity index (Dice coefficient)

Table 4. Distribution of Gram-negative bacteria according to the antimicrobial susceptibility types and resistance level

Type	Name of bacterial isolates	The numbers of antibiotics the isolates resist	Numbers of groups	Resistance level
	Cluster A			
A	1, 9 *K. pneumoniae*	13	5, 4	MDR
B	21 *E. coli*	15	4	MDR
C	16,17,18, 26 *E. coli*	16	5	MDR
D	4,6,7,11,8 *K. pneumoniae*	17	6	MDR
E	28 *A. baumannii*, 32 *P. aeruginosa*	18	7	X
F	31, 33 *P. aeruginosa*	19	8	X
G	5 *K. pneumoniae*	22	7	X
H	14,15,24,25 *E. coli*	23	14,15 (7), 24,25 (8)	X
I	19 *E. coli*	24	8	X
J	23 *E. coli*	25	8	X
K	27*A.baumannii*	26	8	X
L	2 *K. pneumoniae*, 22 *E. coli* , 29, 30 *P. mirabilis*	27	9	X
M	12, 13 *E. coli*	28	9	X
	Cluster B			
N	3, 10 *K. pneumoniae*	3	1	MDR
O	20 *E. coli*	4	2	MDR

4. Discussion

Genital tract infections are causing a series of women's health challenges worldwide and can lead to medical complications such as substantial discomfort, frequent medical visits, significant morbidity, premature rupture of the fetal membranes and low birth weight (14). According to Donders, Bellen (15), AV in pregnant women was correlated to many risks, including preterm birth, premature rupture of membranes and fetal infections. Aerobic vaginitis complications are probably not diagnosed or treated in infected women, but the AV treatment is strongly suggested based on antibiotic susceptibility profiles. The present study reported the prevalence of Gram-positive and Gram-negative bacteria isolated from Iraqi women infected with aerobic vaginitis to determine the antibiotic susceptibility profile and the most effective drug against different isolated bacterial strains.

Many researchers around the world have studied the prevalence of AV pathogens. A study by Jabuk (16) found that the most prevalent aerobic bacteria isolated from infected women were *E. coli*, followed by *S. aureus* and *K. pneumoniae*. In another study conducted by Yasin, Ayalew (17), they found that 67.8% of obtained isolates from AV were Gram-positive and 32.2% were Gram-negative; this result is in agreement with the present study results. The most prevalent aerobic bacteria was *E. faecalis*, followed by *S. aureus*, *E. coli* and *K. pneumoniae* in a study conducted by Yasin, Ayalew (17); these results agree with the present study results and another study conducted by Sangeetha, Golia (18). A recent study by Serretiello, Santella (19) in Italy reported different results from the present study when 50.4% of the obtained bacterial strains were Gram-negative, and 49.6 % were Gram-positive. *Enterobacteriaceae* were isolated from patients with aerobic vaginitis symptoms in the reproductive age group in many studies reported by Hayat, Nagat (20) and Kumar and Singh (21); this might be due to the poor some women's hygiene and pass of gut flora into genital tract causing vaginal infections.

The sensitivity toward different classes of antibiotics was testified, analyzed and reported for all bacterial types obtained in the present study; the most effective antibiotics against Gram-positive bacteria were tigecycline, gentamicin, vancomycin, daptomycin, and

clindamycin (Table 1). While the most effective antibiotics against Gram-negative bacteria were doripenem, ertapenem, imipenem, meropenem, amikacin and gentamicin (Table 2). The present study results are partially in agreement with Yasin, Ayalew (17) when they found that the most effective antibiotics against Gram-positive bacteria were vancomycin, clindamycin and gentamicin, while for Gram-negative bacteria the most effective antibiotics were gentamicin, meropenem, and this result disagree with the present study results when they found that ciprofloxacin was very effective against the most of isolated bacterial species. A previous study by Serretiello, Santella (19) found that amikacin was very effective against *E. coli* isolated from AV-infected women; this result agrees with the present study results when 80% of *E. coli* was sensitive to amikacin. A new study by Tang, Yu (22) agrees with the present study results when they found that *E. coli* showed high sensitivity toward amikacin, imipenem, meropenem and ertapenem, and Gram-positive bacteria were susceptible to vancomycin.

In a study conducted by Krishnasamy, Saikumar (23), they reported that the most effective antibiotic against *K. pneumoniae* was meropenem when 100% of obtained *K. pneumoniae* showed sensitivity toward meropenem followed by imipenem 85.7% and amikacin, gentamicin (57.1%), identical results were obtained in the present study results. On the other hand, Krishnasamy, Saikumar (23) results disagree with the present study results when *P. aeruginosa* showed 100% resistance against ceftazidime and *P. aeruginosa* showed 100% sensitivity toward ceftazidime. In the present study, *Staphylococcus* spp. revealed resistance rates (72.7 %, 63.6%, 47.7%) toward tetracycline, norfloxacin and gentamicin, respectively and *Proteus* spp. revealed 0% resistance to gentamicin; these results were in agreement with the results obtained by Mulu, Yimer (24) when they found the resistance rates of *S. aureus* were (67%, 67% and 33%) for the same three mentioned antibiotics, respectively and also *Proteus* spp. showed 0% resistance to gentamicin. While Mulu, Yimer (24) results differed from the present study result when *Proteus* spp. showed 100% resistance against ciprofloxacin and, in contrast, their *Proteus* spp. showed 0% resistance to ciprofloxacin. *Escherichia coli* showed high susceptibility toward aminoglycosides and less susceptibility toward sulfonamides (trimethoprim/ sulfamethoxazole) and fluoroquinolones (25); this researcher's results were partially in agreement with the present study results when *E. coli* showed high sensitivity toward aminoglycosides and carbapenems.

Appropriate detection of vaginal infections is important for better management of the cases. However, the detection of vaginitis in reproductive age, especially during pregnancy, is significant due to the high risk of transmission of bacterial infections to the neonates during their delivery period by Group B streptococci, *Escherichia coli* and other coliforms leading to many risks (26). Treating bacterial vaginosis with different aetiology during pregnancy can reduce the outcomes, such as preterm birth, so WHO recommends not only treating symptomatic infections but also screening for vaginosis in pregnant women and women with a history of spontaneous abortion or preterm delivery (27, 28).

MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial groups, while XDR was defined as non-susceptibility toward one agent in all except two antimicrobial groups, PDR was defined as nom-susceptibility to all agents in all antimicrobial groups (29). In the present study, a total of 38 isolates (40%) out of 95 obtained isolates were XDR level of resistance, including 20 (21.0%) Gram-positive strains and 18 (18.9%) Gram-negative strains. The rapid spread of multidrug-resistant bacteria is considered a health challenge worldwide (30). Many reasons might contribute to the high MDR and XDR rates, such as poor antibiotics quality, non-proper drug usage, poor sanitation and lack of control for antibiotic use; there is no clear antibiotic usage controlling policy in Iraq, and it is common to buy any antibiotic from any private pharmacies without any prescription. Finally, the p-value was robust compared to different

antibiotic resistance percentages in this study and reported (P=0.001) as shown in tables 1 and 3, $P \leq 0.05$ considered statistically significant.

As concluded, it would be very important to find, characterize and report the aerobic bacterial pathogens that cause AV infection and their antibiotic susceptibility profiles through many epidemiological studies to direct physicians about the best treatment selections. It is worth mentioning that, for the first time in Iraq in the present study, six *Kocuria* spp. including four species *of Kocuria kristinaie* and two species *of Kocuria rosae*, were isolated from AV-infected women and showed very high resistance levels (1isolate MDR and 5 isolates XDR) against most of the antibiotic classes used during this study. All microbiology laboratories must mention early detection and examination of MDR and XDR strains to reduce antimicrobial resistance, a global problem.

The present study recommended using tigecycline, daptomycin, vancomycin, trimethoprim/sulfamethoxazole and gentamycin as a drug of choice for treating AV infected by *Staphylococcus* spp. women. While the drugs of choice against *Enterococcus* spp. were found to be tigecycline, gentamycin and levofloxacin. For women infected with *Kocuria* spp. drugs of choice were found to be tigecycline, vancomycin and teicoplanin. Tigecycline, daptomycin, vancomycin, gentamicin and fusidic acid were the drugs of choice against *Micrococcus luteus* AV infection. For women infected with Gram-negative bacteria, this study recommended using imipenem, meropenem, amikacin, gentamicin and tobramycin as a drug of choice for AV treatment.

Authors' Contribution

Study concept and design: Z. K. R.
Acquisition of data: Z. K. R
Analysis and interpretation of data: L. A. S.
Drafting of the manuscript: L. A. S.
Critical revision of the manuscript for important intellectual content: Z. K. R
Statistical analysis: Z. K. R
Administrative, technical, and material support: L. A. S.

Ethics

The Research Ethics Committee approved the study of the College of Science, Biology department at Mustansiriyah University.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors thank all patients who agreed to be study participants. Special thanks to the Al-Elwea Maternity Hospital medical staff in many private gynaecology clinics and microbiology laboratories in Baghdad City for their corporation, as well as many thanks to Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq (https://uomustansiriyah.edu.iq).

References

1. Li T, Liu Z-H, Li K, Bai H-H. Evaluation of the vaginal microbiome in clinical diagnosis and management of vaginal infectious diseases. Chin Med J. 2019;132(09):1100-3.
2. Donders GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG. 2002;109(1):34-43.
3. Gardner HL. Desquamative inflammatory vaginitis: a newly defined entity. Am J Obstet Gynecol. 1968;102(8):1102-5.
4. Petricevic L, Domig KJ, Nierscher FJ, Sandhofer MJ, Fidesser M, Krondorfer I, et al. Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. Sci Rep. 2014;4(1):1-6.
5. Donders GG, Bellen G, Grinceviciene S, Ruban K, Vieira-Baptista P. Aerobic vaginitis: no longer a stranger. Res Microbiol. 2017;168(9-10):845-58.

6. Čuržik D, Dražančić A, Hrgović Z. Nonspecific aerobic vaginitis and pregnancy. Fetal Diagn The. 2001;16(3):187-92.
7. Lamont R, Taylor-Robinson D. The role of bacterial vaginosis, aerobic vaginitis, abnormal vaginal flora and the risk of preterm birth. BJOG. 2010;117(1):119-20.
8. Rezeberga D, Lazdane G, Kroica J, Sokolova L, Donders GG. Placental histological inflammation and reproductive tract infections in a low risk pregnant population in Latvia. Acta Obstet Gynecol Scand. 2008;87(3):360-5.
9. Vieira-Baptista P, Lima-Silva J, Pinto C, Saldanha C, Beires J, Martinez-de-Oliveira J, et al. Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. Eur J Clin Microbiol Infect Dis. 2016;35(4):657-64.
10. Martora F, Pinto F, Folliero V, Cammarota M, Dell'Annunziata F, Squillaci G, et al. Isolation, characterization and analysis of pro-inflammatory potential of Klebsiella pneumoniae outer membrane vesicles. Microb Pathog. 2019;136:103719.
11. Kaambo E, Africa C, Chambuso R, Passmore J-AS. Vaginal microbiomes associated with aerobic vaginitis and bacterial vaginosis. Front Public health. 2018;6:78.
12. Baron EJ. Specimen collection, transport, and processing: bacteriology. Manual Clin Microbiol. 2015:270-315.
13. Tille P. Bailey & Scott's diagnostic microbiology-E-Book: Elsevier Health Sciences; 2015.
14. Nu PAT, Nguyen VQH, Cao NT, Dessì D, Rappelli P, Fiori PL. Prevalence of Trichomonas vaginalis infection in symptomatic and asymptomatic women in Central Vietnam. J Infect Dev Ctries. 2015;9(06):655-60.
15. Donders G, Bellen G, Rezeberga D. Aerobic vaginitis in pregnancy. BJOG. 2011;118(10):1163-70.
16. Jabuk SIA. Prevalence of aerobic bacterial vaginosis among Intrauterine Contraceptive Device users women in Hilla city. J Babylon Univ. 2014:2424-31.
17. Yasin J, Ayalew G, Dagnaw M, Shiferaw G, Mekonnen F. Vulvovaginitis Prevalence Among Women in Gondar, Northwest Ethiopia: Special Emphasis on Aerobic Vaginitis Causing Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors. Infect Drug Resist. 2021;14:4567.
18. Sangeetha K, Golia S, Vasudha C. A study of aerobic bacterial pathogens associated with vaginitis in reproductive age group women (15-45 years) and their sensitivity pattern. IJRMS. 2015.
19. Serretiello E, Santella B, Folliero V, Iervolino D, Santoro E, Manente R, et al. Prevalence and Antibiotic Resistance Profile of Bacterial Pathogens in Aerobic Vaginitis: A Retrospective Study in Italy. Antibiotics. 2021;10(9):1133.
20. Hayat IM, Nagat SS, Nermine N, Zeinab AB. Prevalence of vaginal infection and associated risk health behaviors among married women in Ismailia city. Int J Curr Microbiol Appl Sci. 2015;4(5):555-67.
21. Kumar G, Singh K. Microbial Profile of High Vaginal Swab From Symptomatic Women of Reproductive Age Group: Data from Tertiary Care Hospital. Int J Sci Res. 2015;4(7):2672-73.
22. Tang Y, Yu F, Hu Z, Peng L, Jiang Y. Characterization of aerobic vaginitis in late pregnancy in a Chinese population: A STROBE-compliant study. Medicine. 2020;99(25).
23. Krishnasamy L, Saikumar C, Kumaramanickavel G. Aerobic bacterial pathogens causing vaginitis in patients attending a tertiary care hospital and their antibiotic susceptibility pattern. J Pure Appl Microbiol. 2019;13(2):1169-74.
24. Mulu W, Yimer M, Zenebe Y, Abera B. Common causes of vaginal infections and antibiotic susceptibility of aerobic bacterial isolates in women of reproductive age attending at Felegehiwot referral Hospital, Ethiopia: a cross sectional study. BMC Women's Health. 2015;15(1):1-9.
25. Wójkowska-Mach J, Pomorska-Wesołowska M, Romanik M, Romaniszyn D. Prevalence and antimicrobial susceptibility profiles of microorganisms associated with lower reproductive tract infections in women from southern poland—Retrospective laboratory-based study. Int J Environ Res Public Health. 2021;18(1):335.
26. Shaikh S, Waghmare P, Sharma A, Ingole K, Bawane R. A Retrospective Evaluation of Vaginitis in Women of Reproductive Age Group in a Tertiary Care Hospital in Solapur, India. Int J Curr Microbiol App Sci. 2018;7(02):762-8.
27. Organization WH. Sexually transmitted and other reproductive tract infections: a guide to essential practice: World Health Organization; 2005.
28. Strus M, Pilarczyk-Żurek M, Adamski P, Janeczko J, Heczko PB, Tomusiak A. Bacterial infections of the lower genital tract in fertile and infertile women from the southeastern Poland. Ginekol Pol. 2013;84(5).
29. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant,

extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

30. Chakraborty A, Pal NK, Sarkar S, Gupta MS. Antibiotic resistance pattern of Enterococci isolates from nosocomial infections in a tertiary care hospital in Eastern India. J Nat Sci Biol Med. 2015;6(2):394.