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Original article

Prevalence and Antibiotic Resistance of Vaginal *Klebsiella pneumoniae* Isolates in Reproductive-Age Women in Zawia, Libya

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Abstract
Klebsiella pneumoniae is an opportunistic pathogen increasingly associated with vaginal infections and known for its multidrug resistance. Limited local data are available on its prevalence and resistance profiles among reproductive-age women in Libya. This study aimed to determine the prevalence of K. pneumoniae among vaginal isolates in reproductive-age women in Zawia, Libya, and to assess its antibiotic resistance patterns. Vaginal swabs were collected from women aged 20–49 years between October 2022 and March 2023. Isolates were identified using standard microbiological techniques, and antibiotic susceptibility testing was performed using the disk diffusion method. Data were analyzed using descriptive and inferential statistics. Out of 58 vaginal pathogen isolates, K. pneumoniae accounted for 23 cases (39.7%), with the highest prevalence observed in the 40–49 age group (65.3%). Antibiotic susceptibility testing revealed high resistance rates to multiple antibiotics, including 100% resistance to gentamicin and chloramphenicol, over 90% resistance to co-trimoxazole and cefixime, and notable resistance to cephalosporins and carbapenems. However, relatively higher susceptibility was noted for nitrofurantoin, ceftriaxone, meropenem, and colistin. The study demonstrates a notable prevalence of K. pneumoniae in vaginal infections among older reproductive-age women in Zawia, with alarming multidrug resistance patterns. These findings highlight the need for ongoing surveillance, appropriate antibiotic stewardship, and targeted preventive strategies to limit the spread of resistant strains and ensure effective treatment options remain available.
Keywords. *Klebsiella pneumoniae, Vaginal Isolates, Antibiotic Resistance, Reproductive-Age Women, Multidrug Resistance, Susceptibility Patterns.*

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Introduction

Bacterial vaginosis (BV) is one of the most prevalent vaginal infections among women of reproductive age. It represents a significant inflammatory condition that can lead to a range of adverse health outcomes. These include ectopic pregnancy, adverse pregnancy outcomes (like spontaneous abortions, preterm delivery, low birth weight), cervical dysplasia, increased risk of postoperative infection [1], amplified HIV and HSV-2 acquisition and transmission [1, 2], and pelvic inflammatory disease (PID) with subsequent tubal factor infertility. The condition known as bacterial vaginosis arises when the normal hydrogen peroxide (H2O2)-producing Lactobacillus species, which normally make up the balance of the vaginal flora, are dysregulated and replaced by high concentrations of harmful bacteria such as Gardnerella vaginalis, Prevotella, Mobiluncus, and Bacteroides species [3], as well as some aerobic bacteria, such as *E. coli, S. aureus, E. faecalis*, and group B streptococcus (GBS), which can occasionally cause aerobic vaginitis [3, 4] and vulvo-vaginal candidiasis [5].

A thin, gray/off-white, uniform, foul-smelling adherent vaginal discharge that is more obvious during sexual activity and menstruation is a clinical symptom of BV [6]. The devastating implications of bacterial vaginosis, such as premature birth, immaturity, uterine membrane rupture, spontaneous abortions, and the risk of sexually transmitted infections, make it a significant inflammatory condition [7].

The vaginal environment can be changed by a number of factors, such as the use of antibiotics, and it has been discovered that receiving treatment without doing a susceptibility test may contribute to the increase in resistance patterns [8]. The global report states that 44,000 deaths and over 2.6 million antibiotic-resistant bacterial illnesses are reported each year [9, 10]. Worldwide, and particularly in underdeveloped nations, reproductive tract infections (RTIs) are a major cause of sickness for women of reproductive age [11].

Current information on the prevalence of bacterial vaginosis, a disease that is sometimes disregarded, including bacterial vaginal infections, is provided by this study. The baseline data generated by this study will be useful for epidemiological research in the future. Therefore, the purpose of this study was to

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determine how common bacterial vaginosis, specifically (Vaginal *Klebsiella pneumoniae*) was among Libyan women residing in Zawia City.

Methods

Study Design and Sample Collection

From October 2022 to March 2023, this cross-sectional study was conducted in Zawia City, Libya. At the Gynecology Department at Zawia Medical Center, 79 vaginal swabs were taken from women of reproductive age (20–50 years) who had abnormal vaginal discharge. Of these, 21 samples (26.6%) had no bacterial growth, and 58 samples (73.4%) had it. Age and sex were among the pertinent patient data that was also documented.

Laboratory Procedures

As soon as possible, the obtained swabs were taken to the lab for cultivation. After being aseptically inoculated onto MacConkey agar, Mannitol agar, and blood agar, the samples were incubated at 37°C for the entire night. For vaginal infections, samples with a colony count more than 10,000 CFU/mL were deemed significant [12]. Following international norms, the isolated bacteria were then identified using standard biochemical assays, colony morphology, and Gram staining. The oxidase, urease, catalase, IMViC, and Simon's citrate tests were among them.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines [13]. The antibiotics tested included: Amikacin (AK), Amoxicillin-Clavulanic Acid (AMC), Trimethoprim-Sulfamethoxazole (SXT), Cefixime (CFM), Cefotaxime (CTX), Ceftriaxone (CRO), Ciprofloxacin (CIP), Ertapenem (ERT), Gentamicin (CN), Meropenem (MER), Nalidixic Acid (NA), Nitrofurantoin (NIT), Norfloxacin (NOR), Tetracycline (TE), Levofloxacin (LEV), Azithromycin (AZM), Colistin (COL), and Chlorpromazine (CPZ).

Data Analysis

All collected data were organized, coded, and entered into an Excel file and then analyzed by using a statistical software package of SPSS, version (24.0). Descriptive statistics were used to summarize the demographic characteristics of the study participants, including age distribution and relevant clinical information. The prevalence of *Klebsiella pneumoniae* isolates among vaginal swabs was calculated and expressed as frequencies and percentages. Antibiotic susceptibility test results were analyzed to determine the resistance patterns of the *K. pneumoniae* isolates against the tested antimicrobial agents. Resistance rates were presented as percentages.

Results

The study enrolled 79 patients between October 2022 and March 2023. Of the total samples, 58 (73.4%) showed significant bacterial growth, whereas 21 (26.6%) showed no significant bacterial growth. The most affected age group was 40–49 years (46.5%), followed by the 30–39 age group (37.9%). The least affected group was those aged 20–29 years (15.6%).

Table 1. Distribution of vaginal pathogens according to age groups during October 2022 until March 2023.

Age group (Years)	October 2022 until March 2023 No (%)
20-29	9/ 58(15.6%)
30-39	22/58 (37.9%)
40-49	27/58 (46.5%)
Total	58

Among all patients, *Klebsiella pneumoniae* accounted for 39.7% (23/58) of the vaginal bacterial isolates. The highest percentage of *K. pneumoniae* isolates was found in patients aged 40–49 years (65.3%), followed by those aged 30–39 years (21.7%). While the lowest prevalence was observed in the 20–29 age group (13%) (Table 2).

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Table 2: Distribution of vaginal K. pneumoniae according to age groups during* October *2022 until* March *2023.

Age group (Years)	October 2022 until March 2023 No (%)
20-29	3/ 23(13%)
30-39	5/23 (21.7%)
40-49	15/23 (65.3%)
Total	23

In this study, all 23 *Klebsiella pneumoniae* isolates were evaluated for antimicrobial resistance against various antibiotics. The highest resistance rates were observed for Gentamicin (CN) and Chlorpromazine (CPZ), with 100% resistance. Similarly, Cefixime (CFM) and Sulfamethoxazole–Trimethoprim (SXT) exhibited very high resistance rates of 95.7% and 91.3%, respectively. Other antibiotics with high resistance levels included Nalidixic Acid (NA), Tetracycline (TE), and Azithromycin (AZM), each showing 87.0% resistance. These were followed by Ertapenem (ERT) and Norfloxacin (NOR), both with resistance rates of 60.9%.
In contrast, the highest sensitivity rates were recorded with Nitrofurantoin (NIT) at 73.9%, Meropenem (MER) and Ceftriaxone (CRO) both at 65.2%, and Colistin (COL) and Levofloxacin (LEV) at 60.9%. Moderate sensitivity was recorded for Ciprofloxacin (CIP) at 52.2% and Amikacin (AK) at 47.8%. Lower sensitivity rates were observed for Ampicillin–Clavulanic Acid (AMC) and Cefotaxime (CTX), at 43.5% and 34.8%, respectively.

Table 3: Susceptibility of the K. pneumoniae isolates against different antibiotics during October 2022 - March 2023.

Antibiotic agents (Disk content)	**Resistant No. (%)**	**Sensitive No. (%)**
(AK)	52.2%	47.8%
(AMC)	56.5%	43.5%
(SXT)	91.3%	8.7%
(CFM)	95.7%	4.3%
(CTX)	65.2%	34.8%
(CRO)	34.8%	65.2%
(CIP)	47.8%	52.2%
(ERT)	60.9%	39.1%
(CN)	100.0%	0.0%
(MER)	34.8%	65.2%
(NA)	87.0%	13.0%
(NIT)	26.1%	73.9%
(NOR	60.9%	39.1%
(TE)	87.0%	13.0%
(LEV)	39.1%	60.9%
(AZM)	87.0%	13.0%
(COL)	39.1%	60.9%
(CPZ)	100.0%	0.0%

Discussion

According to epidemiological research, *K. pneumoniae* strains that are hypervirulent and multidrug-resistant (MDR) are spreading throughout the world, especially in Southeast Asia, and they have significant clinical effects. These strains are linked to serious illnesses that are becoming more resistant to antibiotics used as a last resort, such as carbapenems, such as bloodstream infections (BSIs), pneumonia, and urinary tract infections (UTIs) [14].
As shown in Table 1, of the 58 positive isolates, the largest percentage (46.5% of all cases) occurred in women between the ages of 40 and 49. Following this, the age group of 30 to 39 years old accounted for 37.9% of the isolates, while the age group of 20 to 29 years old had the lowest prevalence, making up only 15.6% of all isolates. These results imply that older women in the reproductive age group were more likely to experience vaginal colonization or infection with bacterial pathogens, such as *Klebsiella pneumoniae*. Numerous causes, including hormonal changes, an increased risk of underlying gynecological disorders, or repeated antibiotic exposure with aging, could be responsible for this. These results are in contrast with other results obtained by other published studies [15].

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The reduced frequency in the youngest age group could be attributed to less comorbid diseases, less frequent past antibiotic usage, and usually better vaginal flora balance. But these trends may also be influenced by behavioral, social, and sexual health variables, which call for more research.

On the other hand, the largest proportion was detected among women aged 40–49 years, representing 65.3% of the cases. The 30–39 age group accounted for 21.7% of isolates, while only 13% were identified in the youngest age group (20–29 years). This age-specific distribution pattern indicates that *K. pneumoniae* vaginal colonization or infection is markedly more prevalent among women in the older reproductive age bracket. Compared to the general distribution of vaginal pathogens in Table 1, *K. pneumoniae* shows an even stronger concentration in the 40–49 age group. This may suggest that certain host-related factors — such as hormonal fluctuations approaching perimenopause, changes in vaginal microbiota, or cumulative exposure to healthcare interventions could increase susceptibility to *K. pneumoniae* colonization. Comparative studies across different regions and larger sample sizes would help validate whether this age trend is consistent and whether specific interventions are needed for high-risk age groups. This result is in line with the results of other studies, which obtained that infection rate was higher between the age group of 29-55 years, followed by 15 – 28 years [16]. Additionally, the data highlight a worrying trend of multidrug resistance among these isolates, which poses a significant public health challenge, especially for reproductive-age women who may be at risk of recurrent or complicated genital tract infections. The results show that the highest resistance rates were observed against a number of antibiotics such as CN and CPZ, with all isolates (100%) being resistant. This finding is particularly concerning as CN is commonly used for treating Gram-negative infections, and resistance indicates limited treatment options.

Additionally, 91.3% and 95.7% of isolates, respectively, showed high resistance to SXT and CFM. This is consistent with data from around the world showing that *K. pneumoniae* is becoming more resistant to sulfonamides and third-generation cephalosporins, most likely as a result of extensive abuse and overuse. Significantly strong resistance to TE, AZM, and NA was also observed (87% each). Because of their distinct structure, Gram-negative bacteria are now known to be more resistant than Gram-positive bacteria, which also helps to explain why they inflict such a high burden of morbidity and mortality worldwide [17]. Due to a variety of plasmid-mediated extended spectrum β-lactamases (ESBL) genes present in Enterobacteriaceae, particularly in E. coli and *K. pneumoniae*, Gram-negative bacteria have demonstrated a marked increase in resistance to β-lactam antibiotics in recent years [18].

On the other hand, resistance to CTX and ERT was detected in 65.2% and 60.9% of isolates, respectively, highlighting the emergence of extended-spectrum beta-lactamase (ESBL)-producing strains and possible carbapenemase producers. This is alarming as carbapenems are often used as last-resort antibiotics for multidrug-resistant Gram-negative bacteria. Conversely, relatively higher susceptibility rates were observed for NIT (73.9% sensitive), CRO and MER (65.2% sensitive each), COL and LEV (60.9% sensitive each). This suggests that these agents may still be effective therapeutic options for treating *K. pneumoniae* vaginal infections in this population. However, reliance on these few remaining options increases the risk of further resistance development if appropriate stewardship is not enforced.

It is worth mentioning here that virulence features and/or antibiotic resistance can be acquired by Klebsiella species through genetic elements and mutations, ultimately resulting in the creation of convergent clones known as multidrug-resistant and hyper-virulent (MDRhv) Klebsiella spp [19,20]. Moreover, a large variety of MDR-hv strains of Klebsiella species that have evolved through different methods have been reported in numerous publications from different continents throughout the world [21].

Results of the current study revealed that the moderate resistance rates to CIP, NOR, and AK indicate partial effectiveness, but continued surveillance is necessary as resistance to fluoroquinolones and aminoglycosides is known to increase rapidly due to misuse. Accordingly, the high prevalence of multidrug resistance observed in this study underlines the urgent need for rational antibiotic prescribing, strict infection control measures, and routine antimicrobial susceptibility testing to guide appropriate therapy. Although Klebsiella species are currently regarded as an urgent hazard to human health due to their multidrug resistance, the MDR profile differs from nation to nation, even when it comes to the extensive use and abuse of antimicrobial drugs [22].

Conclusion

This study highlights a concerning prevalence of *Klebsiella pneumoniae* among vaginal isolates in reproductive-age women in Zawia, Libya, with the highest occurrence in older age groups and a high level of multidrug resistance to commonly used antibiotics. These findings underscore the urgent need for regular screening, prudent antibiotic use, and continuous local surveillance to prevent further spread of resistant strains and to guide effective treatment strategies.

Conflict of Interest. Nil

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