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CHARACTERIZATION OF *ESCHERICHIA COLI* ISOLATED FROM GYNAECOLOGICAL AND UTI PATIENTS IN DISTRICT D.I. KHAN, KHYBER PAKHTUNKHWA

Nasreen Saif[1], Amina Abid[2*], Tauseef ul Hassan[3], Rahila Bano[4], Aleena Idrees[5], Muhammad Faisal[6]

[1]Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
[2*]Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
[3]Assistant Professor in Plastic Surgery Department, Mufti Mehmood Teaching Hospital, D.I. KHAN
[4]Associate professor in Pathology Department, Gomal Medical College, D.I. KHAN
[5]Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN
[6]Assistant professor in community Medicine Department, Gomal Medical College, D.I. KHAN

***Corresponding Author:** Amina Abid
*Demonstrator in Pathology Department, Gomal Medical College, D.I. KHAN Email: aminatauseef1@gmail.com

Abstract
Gynaecological and urinary tract infections, which can lead to severe outcomes, are major global health issues, particularly for women. Ensuring the maintenance of a strong vaginal microbiota in women throughout their reproductive years is extremely important. Infections caused by microbial pathogens, particularly *Escherichia coli*, have significant implications for the well-being of women and the welfare of newborns. The rising prevalence of *E. coli* and antibiotic resistance underscores the urgent worldwide need to achieve safe and efficient therapy. The objective of this study was to provide a detailed description of *E. coli* strains obtained from gynaecological patients and analytically examine their drug resistance profiles. This analysis included the identification of antibiotic resistance genes such as blaNDM-1, blaTEM, and OXA.Acquired gynaecological infections were detected in 55% of the female patients. In the present investigation, *the E. coli* strains collected had a prevalence rate of 47.5%. The age cohort ranging from 36 to 45 exhibits the highest level of pollution, reaching 80%, with those aged 16 to 25 closely trailing behind. 60%. The findings indicated that individuals with GNB infections in the region exhibited multidrug resistance (MDR). Approximately 60% of the *E. coli* (GNB) bacteria in the area have demonstrated resistance to amoxicillin, Nitrofurantoin, Ampicillin, Sulphamethox/trimethoprim, and Meropenem, suggesting the existence of multidrug-resistant (MDR) colonies. The present study aims to provide valuable assistance to clinical practitioners in the appropriate prescription of drugs for the treatment of gynaecological infections in pregnant women.

Key words: Ampicillin; E.coli; gynaecological; UTI

Introduction

Women's vaginal tract can host a significant amount of germs in the body. For women in their reproductive years, it is crucial to have the correct composition of microorganisms in the vagina in

order to maintain a healthy environment. Any disruptions in the normal balance of microorganisms in females can be attributed to a variety of factors, both external and internal. These factors include inadequate sanitation or unhygienic sexual activity, weakened immune system, hormonal fluctuations during pregnancy, pre-existing health conditions, and the use of antibiotics. These disruptions can result in infections such as urinary tract infection (UTI) and gynecological infections.

Gynecological infections can be caused by various types of pathogens, such as bacteria, viruses, fungi, and parasites (Dehkordi *et al.,* 2020). Moreover, this can result in diseases such as Pelvic Inflammation, Mycotic Vaginitis, and Bacterial Vaginosis, all of which pose a threat to the well-being of women, their unborn babies, and newborns (Peterson *et al.,* 2009). The primary bacteria responsible for gynaecological and urinary tract infections in women are *Escherichia coli*, *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* species, *Streptococcus* species, and *Enterococcu*s species. Among these, *Escherichia coli* is particularly contagious and commonly infects patients with urinary tract infections and gynaecological infections (Sarwar *et al.,* 2020).

The prevalence of gynaecological infections is remarkable. Each year, millions of women are affected, experiencing a range of different levels of severity. Gynaecological infections encompass a variety of disorders that impact the female reproductive system. These infections are a notable healthcare issue, as they have a substantial impact on women's physical, psychological, and reproductive health (Sobel *et al.,* 2000). Sexually transmitted infections (STIs), bacterial vaginosis, candidiasis (yeast infections), pelvic inflammatory disease (PID), and urinary tract infections (UTIs) are examples of diseases caused by different factors and characterized by different clinical signs. Certain infections, like yeast infections, are common and typically not severe, while others, such as pelvic inflammatory disease (PID) or certain sexually transmitted infections (STIs), can lead to significant health complications if left untreated.

There is an expectation that women in the majority of high-income countries (HICs) will have access to high-quality gynaecological therapy for a variety of disorders, such as uterine bleeding that is irregular and serious gynaecological cancer diagnosis. However, when it comes to the prevalence of sickness and death, gynaecological disorders have the most significant affect on women in low-resource countries (LRCs). This is due to the fact that women in LRCs frequently do not have access to specialist medical care (Wijeratne *et al.,* 2018). Throughout the course of the last three decades, there has been a substantial amount of attention and financial resources directed towards improving maternal health on a global scale.

According to the fact that Kassebaum *et al*. published their findings in 2014, the United Nations has formally designated the lowering of maternal death rates as one of its goals. In the context of gynaecological diseases, it is evident that the rates of death and illness, as well as the main barriers to reducing the occurrence of these diseases in low- resource countries (LRCs), are primarily related to high costs, a shortage of qualified healthcare professionals, and sociocultural factors related to gender bias and inequality. This is the case when comparing gynaecological diseases to other major global health issues such as maternal health, tuberculosis, malaria, and cardiovascular disease. In situations where a few local resource centers (LRCs) are still unable to provide universal basic healthcare to the entire community, Jakovljev *et al*. (2018) state that targeted external funding will likely be required in order to improve the delivery of gynaecological therapy. Within LRCs, there is a serious scarcity of trained healthcare professionals capable of offering gynaecological therapy that is both safe and compassionate, while also being backed by the most up-to-date research.

Allied health professionals, such as clinical officers, physician assistants, nurses, and midwives, as well as general practitioners, are examples of non-specialists who provide accessible therapy. Whether it is in the preservice curriculum, as part of continuing professional development (CPD), or through in-service training, non-specialist cadres have limited opportunity to get formal teaching in women's health. This is the case regardless of the setting. According to Wijeratne *et al*. (2018), many nations fail to recognize the significance of women's health due to the embedded gender bias and inequality that exists in their societies.

Statement of Problem

Gynaecological infections, which include bacterial vaginosis, pelvic inflammatory disease, and vulvovaginal candidiasis, are among the most frequent health disorders that affect women. The inappropriate and excessive use of antibiotics has resulted in the development of bacteria that are resistant to antibiotics, which can lead to the development of severe diseases. Gynaecological and urinary tract infections are becoming increasingly common, and antibiotic-resistant bacteria are becoming more prevalent in these diseases. Inadequate infection control procedures, broad antibiotic use without selectivity, and the ever-present existence of bacterial reservoirs that are resistant to treatment in hospital environments are some of the factors that are responsible for this state of affairs.

The prevalence of diseases that are resistant to treatment varies not just across geographic location but also between various healthcare settings. Women who are pregnant and women who are not pregnant are both affected by the problem of antibiotic resistance in gynaecological and urinary tract infections. This is a significant worry that affects both groups of women. Women who are pregnant and suffer gynaecological or urinary tract infections that are resistant to antibiotics are associated with an increased risk. It is possible for these illnesses to result in difficulties such as premature delivery, underweight babies, and infections in the mother. These complications can have a severe impact not only on the mother's well-being but also on the health of the baby who is experiencing development. Because there are fewer treatment options available for diseases that are resistant, the outcomes of pregnancies may be more problematic.

It is possible that non-pregnant women who have gynaecological and urinary tract infections that are resistant to antibiotics can develop recurrent or chronic illnesses that are difficult to control. The consequences of these events can include a decrease in the standard of life, a rise in the costs of medical care, and long-term consequences such as infertility or persistent pelvic pain.

Study Objectives:

The main objectives of this study are:

1. To isolate and characterize *E. coli* from UTI and gynaecological patients.
2. To estimate the prevalence of *E. coli* among UTI and gynaecological patients
3. To determine the antibiotic resistance profile of *E. coli* isolates.

Significance of the study

This was the first thorough study ever conducted with the purpose of isolating and characterization of *E. coli* from gynaecological patients at District D.I Khan who come from a variety of different backgrounds. An investigation into the genetic diversity of *E. coli* among gynaecological patients will be of great assistance in the management of this infection.

Limitations of the study:

This study is not representative of the entire province or country as it just focuses on gynaecological patients in District D.I Khan. Furthermore, the purpose of this study does not involve investigating the impact of individual sensitivity. Hence, it is crucial to investigate the specific impact that various factors have in the transmission of *E.COLI* among gynaecological patients

MATERIALS AND METHODS

Description of the Study Area

The current study was carried out during the months of June and July 2024 in the district of Dera Ismail Khan, which is situated in the province of Khyber Pakhtunkhwa, Pakistan. The district of D.I. Khan is the most southern district in Khyber Pakhtunkhwa (KP), and it is located approximately 300 kilometers away from Peshawar, the capital of the province to the south. The provinces of Punjab, Sindh, and Balochistan are all located on its border. According to the census completed in 2023, the total population of the district is greater than 1.8 million, with the number of

people who speak Saraiki being the majority of the population. This figure has been steadily growing over the course of the years.

Ethical Approval

This research was conducted after the approval from Ethical research Committee from both side Gomal University as well Gomal medical college Dera Ismail Khan.

Sample Collection and Processing

A total of 80 High vaginal swabs (HVS) samples were collected during the course of study. High vaginal swabs (HVS) were obtain from gynaecological patients admitted to Mufti Mahmood memorial hospital Dera Ismail Khan using swabs and pre-sterilized plastic containers. Collected samples were transported to the Microbiology Research laboratory of FVAS Gomal University for further investigation. Isolation and identified were done by growing on blood agar, MacConkey's agar, EMB agar, and nutrient agar incubated at 370C for 24-48 hours.

Isolation of bacteria

The growth media were prepare by following the protocol of manufactures and samples were inoculated in sterile condition into the growth mediums. The organism inoculated by streaking on Nutrient agar with the help of sterile wire loop. The culture medium then incubated at 370C for 24-48 hours.

All samples of high vaginal swab were inoculated into the blood agar and incubated at 370C for 24-48 hours. The growth of isolates were confirmed by Gram staining, Catalase test, Oxidase test, Indole test, Citrate test, Urease test, Motility test, TSI test and Eosin methylene blue fermentation test.

Identification of bacteria

Gram staining

The Gram staining procedure involves applying a specimen mixed with a drop of normal saline onto a clean slide. The Gram stain assessed using a bright field microscope equipped with a 100X oil immersion objective. The staining properties and microscopic morphological appearance of each individual bacterium were recorded.

Antibiotic Susceptibility Testing

Inoculation on Muller Hinton Agar (MHA)

The antimicrobial testing conducted using Muller Hinton Agar. The bacterial overnight culture compared to McFarland turbidity criteria. A 100µl aliquot of a bacterial broth culture used to inoculate the MHA medium using a sterile cotton swab. The antibiotic discs, including meropenem (10µg), imipenem (10µg), cefoperazone (75µg), sulphamethox/trimethoprim (25µg), amoxicillin (10µg), cefuroxime (30µg), nitrofurantoin (300µg), and ampicillin (10µg), will be applied to the plate. The MHA medium placed in an incubator set at a temperature of 37°C for a duration of 24 hours. Following the incubation of overnight cultures, the susceptibility assessed and documented.

Molecular Characterization

In the process of molecular characterization, DNA extracted and antibiotic resistance genes identified through the use of polymerase chain reaction (PCR). The presence of these genes further validated by gel electrophoresis. Finally, the PCR results were subjected to sequencing.

PCR product analysis

PCR products (5 µl) supplied from each tube. Afterwards, the samples were undergo vortexing and centrifugation. The samples, together with a 100 base pair ladder, placed into the wells of a gel electrophoresis device. Electrophoresis were conducted in 1XTAE buffer for a duration of 30

minutes at a voltage of 90V. The anticipated size of each TEM gene product will be 290 base pairs, the NDM gene product 234 base pairs, and the OXA gene product 250 base pairs.

RESULTS

The present work aimed to characterise *Escherichia coli* acquired from patients suffering from gynaecological infections in the D.I. Khan district of Khyber Pakhtunkhwa. 38 *E. coli* isolates were obtained from various clinical samples, including High vaginal swabs (HVS) and urine cultures. Within the patient population, the prevalence rate of *E.Coli* was 47.5% (38 out of 80).

The present study demonstrated that *E. coli* produced sizable, spherical, grey, and moist colonies on blood agar Figure 1.

Figure 1 Proliferation of *Escherichia coli* on blood agar

E. coli colonies were examined under the microscope and that they were rod shaped and are typically arranged singly or in pairs and were gram negative as shown in fig Figure 2

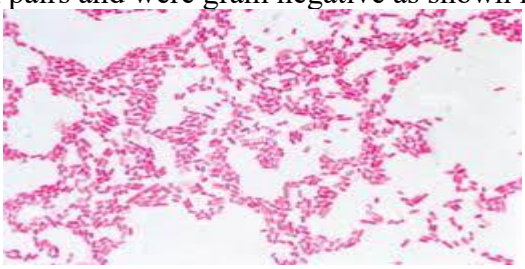

Figure 2 Gram staining of *E. coli*: gram negative rods

Antimicrobial Susceptibility Pattern of *E. coli*

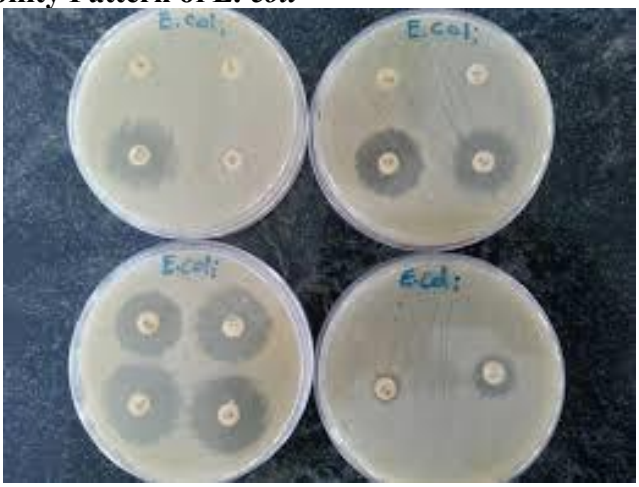

Figure 3. Antibiotic Disks were applied on MHA Media Antimicrobial Susceptibility Pattern of *E. coli*

Table 1: *E. coli* in UTI and Gynecological samples

Antibiotics	Resistant No (%)	Intermediate No (%)	Sensitive No (%)
Meropenem	18(47.3)	10 (26.32)	10(26.32)
Imipenem	12 (31.58)	14 (36.84)	12 (31.58)
Cefoperazone	10 (26.32)	12 (31.58	16 (42.11)
Sulphamethox/trimethoprim	20 (52.63)	0	18(47.3)
Amoxicillin	12 (31.58)	14 (36.84)	12 (31.58)
Cefuroxime	0	20 (52.63)	18(47.3)
Nitrofurantoin	10 (26.32)	16 (42.11)	12 (31.58)
Ampicillin	12 (31.58)	14 (36.84)	12 (31.58)

Reference table 1 presents the antimicrobial susceptibility pattern of *Escherichia coli*. With the exception of cefuroxime, all the antibiotics indicated exhibited resistance against *E. coli.* Sulphamethox/trimethoprim demonstrated a higher level of resistance compared to the other antibiotics.

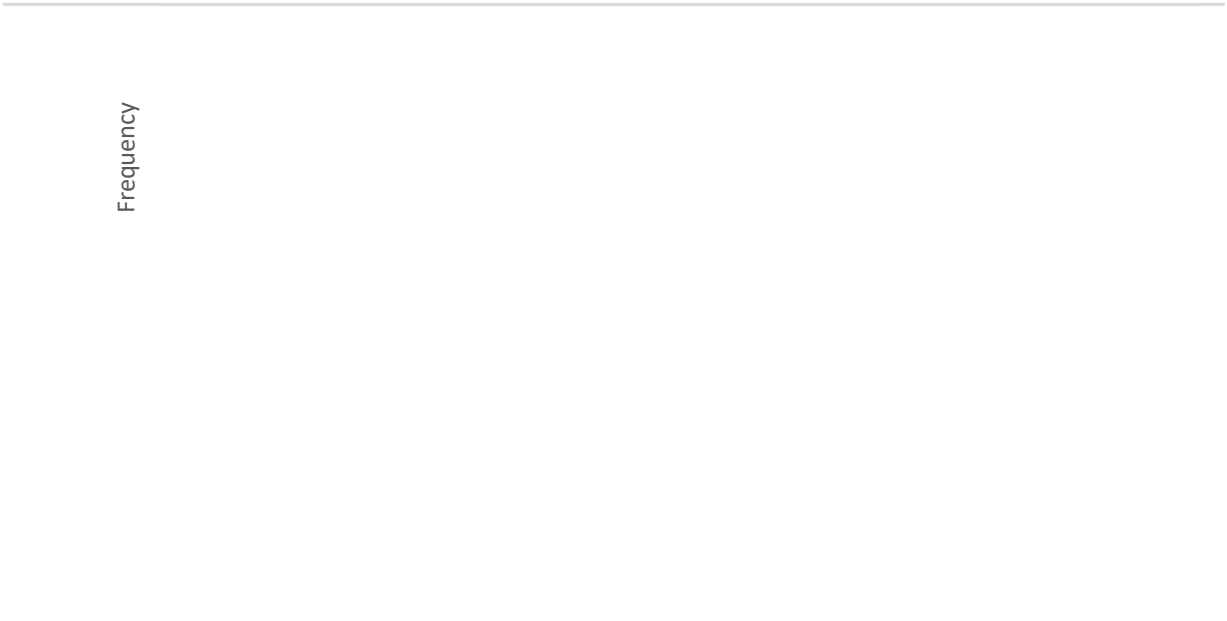

Figure 4 Graphical representation of antimicrobial susceptibility pattern of *E. coli* Prevalence of *Escherichia coli* by Age Groups

Table 2: Prevalence of *E. coli* isolated from Gynecological samples and UTI from different Age groups.

Age (years)	Total samples	*E. coli* +ve /%age	*E. coli* –ve /%age
16 – 25	20	12 (60)	8 (40)
26 – 35	30	10 (33.33)	20 (66.66)
36 – 45	15	12 (80)	3 (20)
46 – 55	15	4 (26.67)	11 (73.33)

Study table 2 presents the incidence of *Escherichia coli* categorized by age group. Every age group exhibited positive cases. The highest number of positive cases occurred in individuals aged 26 to 45 years old.

Figure 5 Prevalence of *Escherichia coli* by age groups

Table: 3 Presence of *E. coli* based on Marital Status of the respondents

Status	**Total samples**	***E.coli* % +ev**	***E.coli* % -ev**
Married	60	33 (55)	27(45)
Widows	20	5 (25)	15(75)

Table 3 illustrates the occurrence of *E. coli* dependent on marital status. Analysis revealed that married women had a higher prevalence of *E. coli* infection in comparison to widows.

Figure 6 Presence of E. coli based on Marital Status Prevalence of *Escherichia coli* infection in pregnant women

Table: 4 Prevalence of *E. coli* in pregnant and non-pregnant women

Variables	**Total Samples**	**E.coli (%) +ev**	**E.coli (%) +ev**
Pregnant	62	32(51.61)	28 (48.29)
Non-Pregnant	18	6(33.33)	16 (66.67)

Table 4 of the study shows the prevalence of E. coli infection in pregnant and non- pregnant women. Empirical evidence suggests that pregnant women are more susceptible to E. coli infection compared to those who are not pregnant.

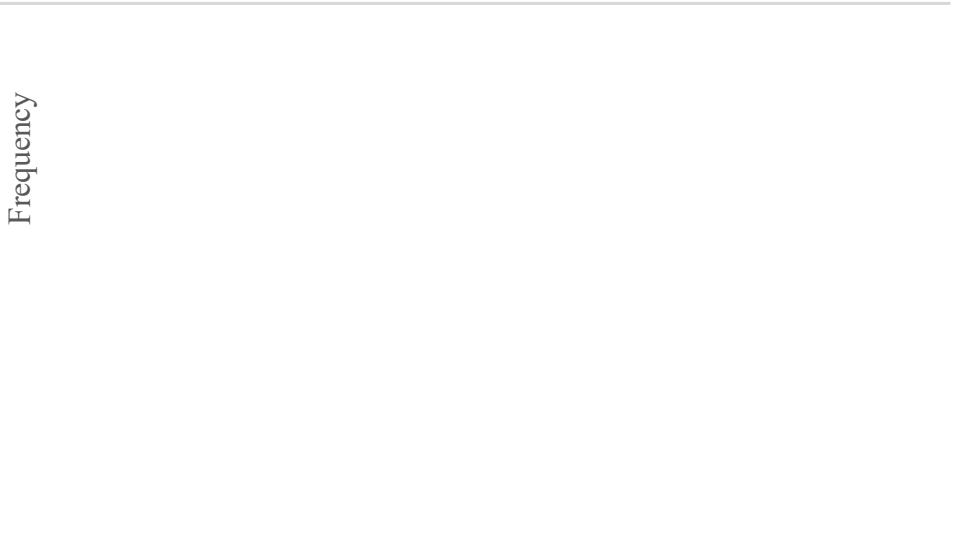

Figure 7 Graphical representation of *E.coli* among women

Table: 5 *E. coli* from the UTI and Gynecological infections

Type of Infections	Total Samples	E.coli (%) +ev	E.coli (%) -ev
UTI	30	20 (66.67)	10 (33.33)
Gynecological	28	9 (32.14)	19 (67.86)
Gynecological + UTI	22	9 (41)	13 (51)

Among the several types of infections caused by E. coli, UTI was the most prevalent, followed by gynecological infections.

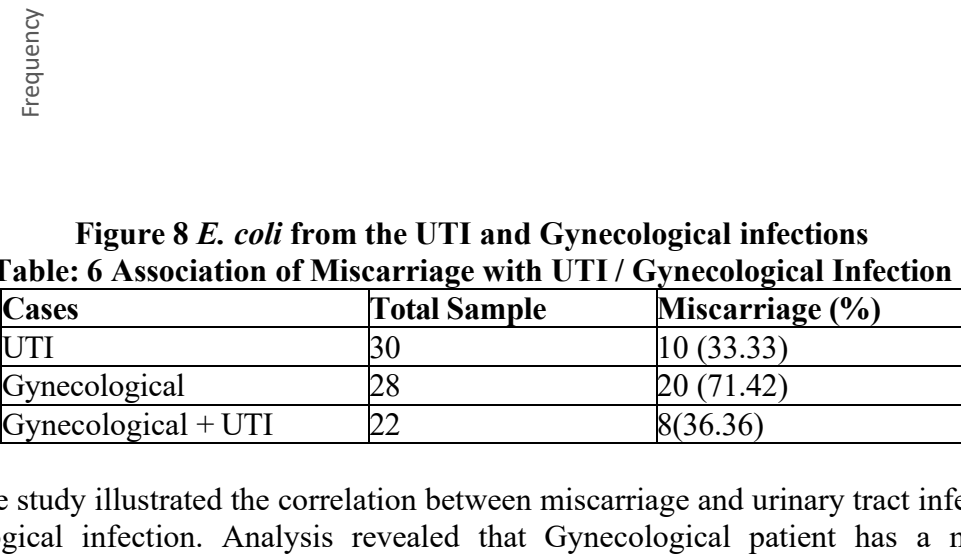

Figure 8 *E. coli* from the UTI and Gynecological infections

Table: 6 Association of Miscarriage with UTI / Gynecological Infection

Cases	Total Sample	Miscarriage (%)
UTI	30	10 (33.33)
Gynecological	28	20 (71.42)
Gynecological + UTI	22	8(36.36)

Table 6 of the study illustrated the correlation between miscarriage and urinary tract infection (UTI) or gynarcological infection. Analysis revealed that Gynecological patient has a more robust

correlation with Miscarriage than Gynaecological Infection.

MOLECULAR CHARACTERIZATION

Detection of PCR products by gel electrophoresis

PCR was amplified to detect antibiotic resistance genes, such as ^{bla}*NDM-1,* ^{bla}*TEM,* and *OXA* in *E. coli*. Gel electrophoresis was utilised in order to carry out a drug resistance gene analysis in *E. coli.* The sequencing of PCR products, which are illustrated in Figure 3.15, 3.16, and 4.20 respectively, then followed this.

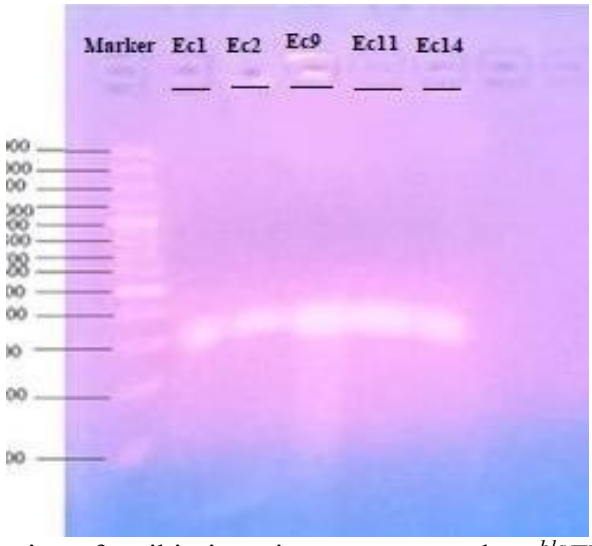

Figure 9 Molecular detection of antibiotic resistance gene such as ^{bla}*TEM* of *E. coli.* DNA from bacterial cells was extracted followed by PCR amplification of ^{bla}*TEM* gene in *E. coli* isolates (1, 2, 9, 11, 14) by using specific primers. The ^{bla}*TEM* gene product size was 300bp were successfully obtained.

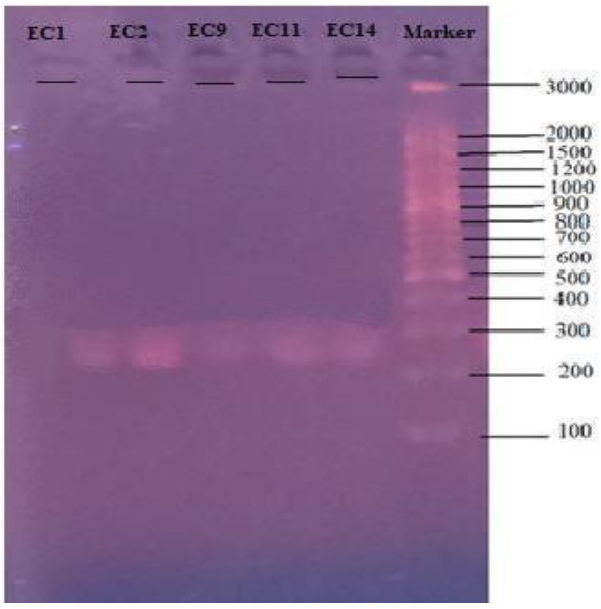

Figure 10: Molecular detection of antibiotic resistance genes such as ^{bla}*NDM* of *E. coli.*

DNA from bacterial cells was extracted followed by PCR amplification of ^{bla}*NDM* gene in *E. coli* isolates (1, 2, 9, 11, 14) by using specific primers. The ^{bla}*NDM* gene product size was 210bp and was successfully obtained.

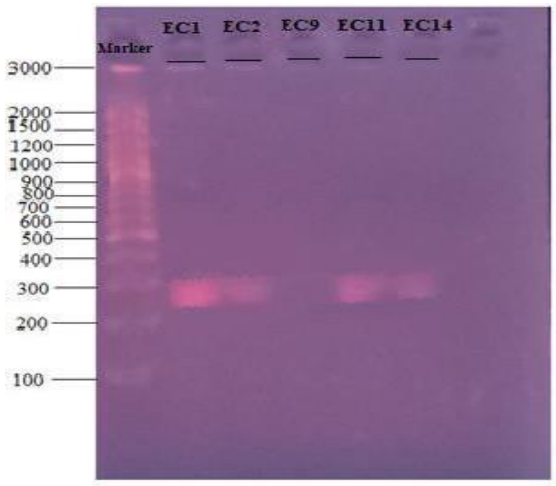

Figure 11: Molecular detection of antibiotic resistance genes such as *OXA* of *E. coli.*

DNA from bacterial cells was extracted followed by PCR amplification of *OXA* gene in *E. coli* isolates (1, 2, 11, and 14) by using specific primers. The *OXA* gene product size was 254 bp and was successful.

DISCUSSION

The preservation of a robust vaginal microbiome is of paramount significance, particularly for women in their reproductive years. The female reproductive system harbours a diverse array of germs, making it imperative for women to engage in this practice. Inadequate hygiene, immunosuppression, hormonal imbalances, sickness, and antibiotic usage are all causative elements that can disturb this equilibrium, leading to infections such urinary tract infections (UTIs) and gynaecological diseases caused by various microorganisms. Chronic pelvic inflammation, bacterial vaginosis, and mycotic vaginitis are all medical disorders that can greatly endanger the well-being of women, as well as the health of unborn children. *Escherichia coli, Staphylococcus aureus*, and antibiotic-resistant Coagulase

The four predominant bacterial species responsible for these pathologies are Staphylococcus species, Streptococcus species, and Enterococcus species. Pathogens such as bacteria, viruses, fungi, and parasites are the primary causes of female mortality worldwide. Reproductive system infections, affecting the vagina, cervix, uterus, fallopian tubes, and ovaries, are the primary etiological factors of illness. The objective of this study was to analyse drug resistance profiles, detect antibiotic resistance genes including blaNDM-1, blaTEM, and OXA, and establish a thorough description of *E.coli* strains obtained from gynaecological patients.

The prevalence of gynaecological illnesses among patients in the District Dera Ismail Khan region was the primary focus of this study. One study found that female patients had an 71% frequency of gynaecological infections. Within the total number of bacteria that were cultivated for this investigation, *Escherichia coli* strains constituted the majority (47%%) of the results. In accordance with the antibacterial susceptibility pattern that Javed et al. had previously observed, the findings of this study are consistent.

The bulk of the bacterial isolates that were found in our inquiry were found in patients who were between the ages of 26 and 45, as indicated by the findings of our investigation. Those individuals who were between these ages were the ones that had the largest number of positive cases during the study. According to the findings of a study that was carried out in Kolkata, India, the female group that had the highest frequency of genital infections was constituted of women who were between the ages of 15 and 45.

There was a positive result for cefoperazone in every single bacterial isolate. It is possible to employ cefoperazone in situations where bacteria are resistant to or unresponsive to other antibiotics. This is due to the fact that cefoperazone has been successful in the Pakistani population. Susceptibility to

sulfamethoxazole and trimethoprim

According to the findings of our investigation, the overall level of antimicrobial resistance to aminopenicillins and nitrofurantoin in both GNB was significantly higher than what had been previously observed. Based on this information, it appears that antimicrobial resistance (AMR) is growing in the area, and it is imperative that healthcare practitioners pay immediate attention to this matter. In addition to this, it had a strong antibody response to both ampicillin (27.7%) and nitrofurantoin (27.7%).

The susceptibility rates of antibiotics that were available for oral administration were found to be 63.8% for imipenem and 47.2% for amoxycillin. These results were in close agreement with a prior study that was carried out in Pakistan. It is possible to compare the antibiotic resistance pattern of the current inquiry with that of a study that was carried out in Pakistan and another that was carried out in Uganda. Both of these studies indicated that the majority of Gram-negative bacteria (GNB) exhibited resistance to ampicillin, amoxicillin, and Nitrofurantoin.

Based on the findings of our research, it appears that the level of *E.coli* resistance in Pakistan is higher than what is stated in western literature. Furthermore, our findings suggested that individuals infected with GPB and GNB in the region exhibited multidrug resistance (MDR), which is a form of resistance to many drugs. Over sixty percent of *E.coli* (GNB) strains displayed resistance to antibiotics such as amoxicillin, nitrofurantoin, ampicillin, sulfamethox/trimethoprim, and other antibiotics. This finding provided evidence that multidrug-resistant bacteria (MDR) are prevalent in the region.

CONCLUSION

In conclusion, this study emphasises the high occurrence of gynaecological infections among female patients in the Dera Ismail Khan area, with *E. coli* being the predominant causative entity. Significantly, the heightened infection rates seem to be influenced by cultural hygiene concerns peculiar to certain castes and age groups. In addition to revealing concerning trends in resistance to crucial antibiotics such aminopenicillins and nitrofurantoin, the antimicrobial susceptibility profile identifies feasible options such as Cefoperazone and Sulfamethoxazole Trimethoprim. The proliferation of multidrug resistance (MDR) in *E. coli* isolates underscores the urgent requirement for strategic healthcare interventions to tackle the escalating problem of antimicrobial resistance (AMR) in the region.

To unravel the molecular mechanisms of drug resistance in *E. coli* and to have a better understanding of the virulence characteristics of *E. coli*, additional comprehensive research is required.

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