Journal of Taibah University Medical Sciences (2021) 16(1), 102–111

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Rising prevalence of multidrug-resistant uropathogenic bacteria from urinary tract infections in pregnant women

Umema Asmat, M.Phil, Muhammad Z. Mumtaz, Ph.D. * and Arif Malik, Ph.D.

Department of Biotechnology, Institute of Molecular Biology and Biotechnology, The University of Lahore, Main Campus, Lahore, Pakistan

Received 16 July 2020; revised 10 October 2020; accepted 11 October 2020; Available online 11 November 2020

الملخص

أهداف البحث: تهدف هذه الدراسة لتحديد مدى انتشار التهابات المسالك البولية عند الحوامل وتوصيف السلالات البكتيرية المسببة للأمراض البولية المرتبطة مع البيلة الجرثومية المصحوبة وغير المصحوبة بأعراض في لاهور، الباكستان.

طرق البحث: بين ديسمبر ٢٠١٨ ويونيو ٢٠١٩، قمنا بتحليل السلالات البكتيرية المسببة للأمراض البولية من عينات البول الوسطية لعدد ٨٠ سيدة حامل. وكانت الفئة العمرية للسيدات الحوامل من ١٩-٤٥ عاما وينتمون إلى المناطق الحضرية والريفية. كما قمنا أيضا بتسجيل العوامل الاجتماعية والاقتصادية لهذه المجموعة. وتم التعرف على السلالات المعزولة ظاهريا وتقييمها لنمط مقاومة الأدوية المتعددة ضد الأدوية المضادة للميكروبات الموصى بها.

النتائج: من بين ٨٠ سيدة حامل، كان ٦٥ لديهن التهابات المسالك البولية، ما يعكس انتشار (٨١٪) التهابات المسالك البولية عند السيدات أثناء الحمل. وكانت أعمار أغلب المشاركات ٢٤-٣٥ عاما، ومتعددات الحمل، وكن في الثلث الثالث من الحمل. كما أظهرت النتائج أن ٦٧ من السلالات البكتيرية المسببة للأمراض البولية تنتمي إلى الإشريكية القولونية (٣١٪)، والكلبسيلة الرئوية (٢٣٪)، والبكتيريا الزائفة (١٦٪)، والبكتيريا العقدية (٤٪) والمكورات المعوية (٤٪)، والمكورات العنقودية (٤٪)، والبكتيريا المتقلبة (٣٪). كما تم تحديدهم من خلال التوصيف الكيميائي الحيوي. ولوحظ أن أعلى أنماط المقاومة الإجمالية للأموكسيسيلين، وحمض البيميديك، والأمبيسلين ضد الإشريكية القولونية، وحمض البيميديك، والأمبيسلين، وسيفوتاكسيم ضد الكلبسيلة الرئوية، وسيبروفلوكساسين وسيفوتاكسيم ضد الزائفة. وتم تحديد ثلاثة أعلى سلالات لمقاومة الأدوية المتعددة التي كانت من سلالة الزائفة الزنجارية، وسلالة الإشريكية القولونية، وسلالة الكلبسيلة الرئوية.

الاستنتاجات: في هذه الدراسة، أظهرت السلالات المسببة لأمراض الجهاز البولي أعلى نمط لمقاومة الأدوية المتعددة. العلامات المقلقة لأمراض الجهاز البولي المقاومة للأدوية المتعددة نادرا ما يتم تناولها والاهتمام العاجل بهذه المسألة أمر ضروري.

الكلمات المفتاحية: مقاومة المضادات الحيوية؛ الإشريكية القولونية؛ الكلبسيلة الرئوية؛ الحمل؛ التهاب المسالك البولية

Abstract

Objectives: This study aimed to determine the prevalence of urinary tract infections (UTI) in pregnant women and characterise the uropathogenic bacterial strains associated with symptomatic and asymptomatic bacteriuria in Lahore, Pakistan.

Methods: Between December 2018 and June 2019, we analysed the uropathogenic bacterial strains from midstream urine samples in 80 pregnant women. The age of the pregnant women ranged from 19 to 45 years, and they resided in urban and rural areas. We also recorded socioeconomic factors in this cohort. The isolated strains were phenotypically identified and evaluated for multiple drug resistance (MDR) patterns against recommended antimicrobial drugs.

Results: Of the 80 pregnant women, 65 had UTI, reflecting an 81% prevalence of UTI in women during pregnancy. The majority of participants aged 24–35 years, were multipara, and were in their third trimester. Results showed that 67 uropathogenic bacterial strains belonged to *Escherichia* (31%), *Klebsiella* (23%), *Pseudomonas* (16%), *Streptococcus* (4%), *Enterococcus* (4%), *Staphylococcus* (4%), and *Proteus* (3%) genera, as identified using biochemical characterisation. The highest overall resistance of *Escherichia* was seen against

* Corresponding author. Institute of Molecular Biology and Biotechnology, The University of Lahore, Main Campus, Lahore, 54000, Pakistan.

E-mail: zahidses@gmail.com (M.Z. Mumtaz)

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2020.10.010

amoxicillin, pipemidic acid, and ampicillin; for *Klebsiella* against pipemidic acid, ampicillin, and cefotaxime; and for *Pseudomonas* against ciprofloxacin and cefotaxime. The three strains with the highest MDR were identified using 16S rRNA as *Pseudomonas aeruginosa* strain UA17, *Escherichia coli* strain UA32, and *Klebsiella pneumoniae* strain UA47.

Conclusion: In this study, the MDR uropathogenic strains showed the highest resistance pattern. The alarming signs of MDR uropathogenic infections are infrequently addressed and thus, urgent attention to this matter is essential.

Keywords: Antibiotics resistance; *Escherichia coli*; *Klebsiella pneumoniae*; Pregnancy; Urinary tract infection

Introduction

Urinary tract infections (UTIs) are the most frequently occurring infections affecting the human population. They cause severe complications in millions of individuals annually. Estimates state that over seven million cases of UTI have been reported in emergencies, while around 100,000 cases of nosocomial infections have been reported in healthcare units annually.[1] Infections induced by bacteria that affect even a certain portion of the urinary tract are a major cause of UTI. These include increased urine frequency along with pain and the presence of cloudiness in the urine.[2] Other symptoms comprise dysuria, cramps in the lower abdominal region, back pain, chills, fever, and general weakness accompanied by nausea and vomiting.[3] Generally, urine contains not only bacteria, but also salts, wastes, and fluids. It has been seen that pathogenic bacteria entering and multiplying in the bladder or kidneys eventually become a cause of UTIs.[2,4] Asymptomatic bacteriuria, if left untreated, might cause acute cystitis and also pyelonephritis, which could eventually lead to serious consequences such as prematurity, low birth weight, and increased foetal mortality rates.[5–7]

Most commonly, UTI is caused by gram-negative aerobic bacilli that originate in the gastrointestinal tract. Frequent pathogens responsible for UTI include *Citrobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Serratia*, and *Staphylococcus*, which colonise the genito-urinary tract.[6,8–11] Ahmed et al.[8] revealed that *Klebsiella*, *Escherichia coli*, *Pseudomonas,* and *Proteus* spp. are the most influential gram-negative organisms responsible for UTIs. Among gram-positive bacterial pathogenic strains, *Staphylococcus* and *Streptococcus* spp. have been found to be the most common bacteria responsible for UTIs.[5] The main subject of concern to date has been the treatment of UTIs and whether it could decrease maternal or neonatal complications. Studies have revealed that antibiotic regimens have not shown any decrease in these complications and have imposed a huge financial burden on society.[12] Antibiotic resistance occurs when bacteria have or develop the ability to circumvent the mechanism, which drugs use against them.[13] The resistance of uropathogenic bacteria against antimicrobials is rising globally over time. It is directly dependent on the use and misuse of antimicrobial drugs, along with geographical location. Knowing the consequences of resistance is vital as the varying rates of resistance exert a massive influence on empirical therapies of UTIs.[7]

Microorganisms such as *Acinetobacter*, *Escherichia*, and *Pseudomonas aeruginosa* are notably distinguished due to their intrinsic capability of exposing multidrug resistance (MDR). Microbes that have been treated for several years have been showing resistance to drugs in recent times.[14] Colonisation with MDR bacteria can lead to infection that is more difficult to treat.[15] The conditions are even more challenging with the increasing prevalence of UTI as well as the reduced discovery of new antibiotics.[16] Dammeyer et al.[17] reported a higher risk of colonisation of methicillin-resistant *Staphylococcus aureus* and extended-spectrum beta-lactamase-producing *E. coli* transmitted from mother to child during pregnancy and after childbirth. The MDR in bacteria could be due to various mechanisms of action including antibiotic destruction, modification, alterations, and reduction in antibiotic accumulation due to inhibition of permeability and increased efflux.[18] Genes are transmitted within bacteria by various means, and the most vital means is conjugation. Reduced permeability prevents the antimicrobial agent from entering into the cell wall of bacteria, because of which the intracellular concentration of the antimicrobial agent is reduced.[19] Antibiotic pressure explains the fact that resistance towards antibiotics might arise with the emergence of resistant strains and the killing of susceptible strains. In patients infected with resistant strains, antimicrobial usage may inhibit susceptible strains and sanction resistant strains to multiply.[14] Therefore, the current study aimed to determine the prevalence of UTI and the frequency of MDR uropathogenic bacterial strains in pregnant women in Lahore, Pakistan. These uropathogenic bacterial strains were characterised biochemically and identified using 16S rRNA gene sequencing.

Materials and Methods

A cross-sectional study was conducted from December 2018 to June 2019. Urine samples (n = 80) were collected from secondary care, Hussain Memorial Hospitals, Lahore, Pakistan, and informed consent of participants was recorded on a form that included explicit consent for the collection, storage, and testing of the samples. In this study, pregnant women aged ≥18 years attending the antenatal clinics with symptomatic (dysuria, burning micturition, frequency, urgency, lower abdominal pain, and fever) and asymptomatic UTIs were included. Normally, patients with more than four to five pus cells, estimated through urine microscopy, which serves as a baseline antenatal visit, were investigated to

determine the prevalence of UTI and isolation of uropathogenic bacteria. Urine microscopy was performed by taking 10 mL of urine in a test tube that was then centrifuged at 3000 rpm for 10 min. The supernatant was decanted, and a drop of sediment was placed on microscopic slides. Microscopic slides were covered with a coverslip and observed under a light microscope. UTI patients who had taken antibiotics in the last two weeks were not included in the study. Socio-demographic characteristics, including medical and obstetrical history, clinical signs, and obstetric characteristics of the index pregnancy, were collected using a structured questionnaire.

Urine collection and isolation of uropathogenic bacteria

Midstream random voided urine samples (10−15 mL) were collected in sterile disposable containers and transferred to the Microbiology Laboratory, Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan, for bacterial isolation within two to 3 h. Bacterial strains were isolated through serially diluting (10^{-5}) urine samples and spreading on autoclaved cysteine-lactose-electrolyte-deficient (CLED) agar. Petri plates were incubated at 37 °C for 72 h in an incubator. The resulting colonies were manually counted to record the colony-forming units (CFUs) of bacteria in the urine sample. Distinctly different morphological colonies were purified using the multiple-streak method and preserved in glycerol stock at −20 °C for further culturing and characterisation experiments.

Characterisation of uropathogenic bacteria

For the identification of bacterial genera, strains were characterised through various morphological and biochemical tests as recommended by Hafeez and Aslanzadeh.[20] The strains were screened using Gram staining and differentiated into gram-positive and gram-negative by observing whether they were purple- or pink-stained, respectively.[21] Bacterial morphology was determined through a simple staining method as described by Cappuccino and Sherman.[22] The bacterial strains were cultured on mannitol salt agar (MSA); yellow colonies that fermented mannitol and exhibited coagulase and deoxyribonuclease (DNase) activity were considered to be *S. aureus*.[23] The strains were further cultured on MacConkey agar to differentiate lactose fermenting bacterial strains that appeared in the form of pink colonies.[24] The standard microbiological methods for triple sugar iron (TSI), hydrogen sulphide (H_2S), indole production, catalase, oxidase, urease, and citrate utilisation, bile-esculin, coagulase, and DNase tests were performed in triplicate by following the methods of Cappuccino and Sherman.[22]

MDR assay

The MDR assay of uropathogenic bacterial strains was performed using the disk diffusion method.[25] All identified strains in the *Escherichia*, *Klebsiella*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Proteus* genera were tested for MDR. Autoclaved Mueller-Hinton (MH) agar plates (90-mm diameter) were prepared, and the bacterial inoculum of 10^8 CFU mL^{-1} was heavily streaked using a sterile cotton swab. For quality control, *E. coli* strain ATCC 25922 was also run parallel to the antibiotic assay. A total of 28 commercially-available paper antibiotic disks including amikacin (AK), amoxicillin (AML), amoxicillin clavulanate (AMC), ampicillin (AMP), aztreonam (ATM), ceftazidime (CAZ), cephradine (CE), ciprofloxacin (CIP), gentamycin (CN), ceftriaxone (CRO), cefotaxime (CTX), ceforaxime (CXM), doxycycline (DO), cefipime (FEP), fosfomycin (FOS), imipenem (IPM), levofloxacin (LEV), linezolid (LZD), meronem (MEM), moxifloxacin (MXF), nitrofurantoin (NF), novobiocin (NV), penicillin (P), pipemidic acid (PIP), tetracycline (TE), tobramycin (TOB), piperacillin tazobactam (TZP), and vancomycin (VA) of fixed concentration (Oxoid, UK) were placed on the inoculated agar surface and incubated for 24 h at 37 °C. The standard tested antibiotics were selected from the tables provided by the Clinical and Laboratory Standards Institute (CLSI) appropriate for testing *Enterobacteriaceae*, *Pseudomonas*, *Staphylococci*, *Enterococci*, and *Streptococci* spp.[26,27] After incubation, the diameter of zones of growth inhibition around the antibiotic disc were measured in millimetres. The diameter of the zone of growth inhibition was interpreted as sensitive, intermediate, or resistant as reported by CLSI.[26]

Molecular characterisation

The most MDR strains from the *Escherichia*, *Klebsiella*, and *Pseudomonas* genera were selected for identification using 16S rRNA partial gene sequencing. The selected strains were identified using genomic DNA isolation, amplification, sequencing of nucleotides, and phylogenetic analysis. The bacterial biomass was treated with proteinase-K treatment for the isolation of genomic DNA.[28] For PCR, 2.5 µL genomic DNA was used. The PCR reaction was performed using primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3′ and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'. The amplified PCR product was run on 1% agarose gel with GeneRuler 1 kb DNA (Fermentas) to confirm the size of the amplified 16S rRNA and further purified using a PCR purification kit (Favorgen, Taiwan). The amplified PCR products were sequenced using the commercial services of MACROGEN Seoul, Korea (http://macrogen.com/eng/). The resulting nucleotide sequences were blasted on NCBI servers by optimising highly similar sequences (Megablast). The sequences of closely related strain types were selected, and the phylogenetic tree was drawn using MEGA 7.0.14.[29]

Results

The demographic data of the pregnant women who underwent antenatal check-ups are summarised in Table 1. The study revealed a UTI prevalence of 65 out of 80 (81.25%) pregnant women. The prevalence of symptomatic bacteriuria in women was 62.5%, whereas the prevalence of asymptomatic bacteriuria was 37.5%. The assessment of

Table 1: Demographic data and prevalence of urinary tract infection in pregnant women.

Demographic characteristic		Number of patients (n = 80)	Percentage (%)
Age group	19–24	14	17.5%
	25–34	62	77.5%
	35–45	4	5.0%
Socio-economic status	Poor	40	50.0%
	Middle	31	38.8%
	Upper middle	9	11.2%
Employment	Housewives	50	62.5%
	Employed	30	37.5%
Residence	Rural	36	45.0%
	Urban	44	55.0%
Level of education	Illiterate	15	18.8%
	Primary to Intermediate	40	50.0%
	Secondary to Masters	25	31.3%
Parity	Multigravida	46	57.5%
	Primigravida	34	42.5%
UTI diagnosis	Symptomatic	50	62.5%
	Asymptomatic	30	37.5%
UTI Symptoms	Bacterial vaginosis	15	18.8%
	Intrauterine growth retardation	5	6.3%
	Preterm labour and PROM	5	6.3%
	Recurrent UTI	16	20.0%
Trimesters	First trimester	19	23.8%
	Second trimester	14	17.5%
	Third trimester	47	58.8%
Bacterial genera frequency (n = 67)	*Escherichia*	25	37.3%
	Klebsiella	18	26.9%
	Pseudomonas	13	19.4%
	Staphylococcus	3	4.5%
	Streptococcus	3	4.5%
	Enterococcus	3	4.5%
	Proteus	2	3.0%

UTI = urinary tract infection; PROM = premature rupture of membranes.

factors associated with UTI according to the previous and current history of patients was concluded as bacterial vaginosis (19%), preterm labour, premature rupture of membrane (PROM; 6%), intrauterine growth restriction (IUGR; 6%), and history of UTI in current pregnancy (20%). The majority of pregnant women (58.5%) were in the third trimester during the sample collection period (Table 1).

Characterisation of uropathogenic bacterial strains

Morphological and biochemical characterisation of 67 uropathogenic bacterial strains were performed. Based on the results, strains were classified into various bacterial genera including *Escherichia*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Proteus*. The characterisation results are listed in Table 2. The

Table 2: Characterisation of uropathogenic bacterial strains isolated from UTI in pregnant women.

	Escherichia	*Klebsiella*	*Pseudomonas*	*Staphylococcus*	*Streptococcus*	*Enterococcus*	*Proteus*
Gram-staining	−ve	−ve	−ve	+ve	+ve	−ve	−ve
Cell shape	Rods	Rods	Rods	Cocci in clusters	Diplococci	Diplococci	Rods
TSI	Y/Y, +ve gas	Y/Y, +ve gas	R/R, -ve gas	−ve	−ve	−ve	R/Y, +ve gas
H_2S production	−ve	−ve	−ve	−ve	−ve	−ve	+ve
Indole test	+ve	−ve	−ve	−ve	−ve	−ve	+ve
Catalase test	−ve	−ve	−ve	+ve	−ve	−ve	+ve
Oxidase test	−ve	−ve	+ve	−ve	−ve	−ve	−ve
Citrate utilisation	−ve	+ve	+ve	−ve	−ve	−ve	+ve
Urease test	−ve	−ve	+ve	−ve	−ve	−ve	+ve
Bile-esculin test	−ve	−ve	−ve	−ve	−ve	+ve	−ve
Coagulase test	−ve	−ve	−ve	+ve	−ve	−ve	−ve
DNase test	−ve	−ve	−ve	+ve	−ve	−ve	−ve
Growth on MSA	NG	NG	NG	Yellow growth	NG	NG	NG
Growth on MACA	Dark pink	Light pink	Pink to green	NG	NG	NG	NG

TSI = triple sugar iron; Y/Y = yellow slant-yellow butt; R/R = red slant-red butt; R/Y = red slant-yellow butt; DNase = deoxyribonuclease; MSA = mannitol salt agar; MACA = MacConkey agar; NG = no growth; +ve = presence of the traits; -ve = absence of the trait.

Table 3: Antibiotic resistance pattern in *Escherichia*, *Klebsiella*, and *Pseudomonas* spp. against various antimicrobial drugs.

	Escherichia (n = 25)			*Klebsiella* (n = 18)			*Pseudomonas* (n = 13)		
Drugs	S	IR	R	S	IR	R	S	IR	R
AK	5	4	16	5	2	11	4	3	6
AML	0	0	25	NT	NT	NT	NT	NT	NT
PIP	0	1	24	4	1	13	9	0	4
AMC	6	5	14	1	7	10	NT	NT	NT
AMP	0	0	25	2	0	16	NT	NT	NT
DO	6	8	11	12	0	6	NT	NT	NT
FEP	2	0	23	2	3	13	6	2	5
CTX	2	2	21	5	0	13	5	0	8
CAZ	3	4	18	5	6	7	8	2	3
CRO	4	0	21	6	2	10	NT	NT	NT
CXM	3	7	15	NT	NT	NT	NT	NT	NT
SCF	11	2	12	12	3	3	6	1	6
CIP	2	0	23	4	4	10	3	1	9
FOS	11	3	9	6	4	8	NT	NT	NT
CN	5	5	15	NT	NT	NT	8	3	2
IMP	13	2	10	10	1	7	9	4	0
MEM	16	0	9	12	0	6	12	0	1
TZP	NT	NT	NT	10	3	5	8	1	4
NF	NT	NT	NT	4	7	7	4	4	5
ATM	NT	NT	NT	NT	NT	NT	1	5	7

NT = not tested; S = sensitive, IR = intermediate resistant, R = resistant, AK = amikacin, AMC = amoxicillin clavulanate, AML = amoxicillin, AMP = ampicillin, ATM = aztreonam, CAZ = ceftazidime, CIP = ciprofloxacin, CN = gentamycin, CRO = ceftriaxone, CTX = cefotaxime, CXM = ceforaxime, DO = doxycycline, FEP = cefipime, FOS = fosfomycin, IPM = imipenem, MEM = meronem, NF = nitrofurantoin, PIP = pipemidic acid, TZP = piperacillin tazobactam.

frequency of these isolated uropathogenic bacterial strains is given in Table 1. The majority of strains were gram-negative, rod-shaped cells, with a yellow slant and butt with gas production, and were negative for H_2S production, indole test, catalase, oxidase, urease, coagulase, and bile-esculin test. The genus *Escherichia*, *Klebsiella*, and *Pseudomonas* cultures were able to grow on MacConkey agar demonstrating dark pink, light pink, and pink to green colour colonies, respectively.

Prevalence of multidrug resistance uropathogenic bacterial strains

Overall, 67 uropathogenic bacteria strains of the *Escherichia*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Proteus* genera were evaluated for MDR, and the results are reported in terms of susceptibility, intermediate resistance, and resistance to antimicrobial drugs (Tables 3 and 4). The highest MDR was observed in *Escherichia* and *Klebsiella* strains compared with in strains of other genera. A total of 25 uropathogenic *Escherichia* strains were tested for MDR, and their results are depicted in Table 3. The results revealed that 90–100% of *Escherichia* strains were resistant to AML, AMP, CIP, FEP, and PIP, 80–90% of *Escherichia* strains were resistant to CTX and CRO, and 55–80% of *Escherichia* strains were resistant to AK, AMC, CAZ, CN, and CXM. Out of the 25 *Escherichia* strains, 16 strains were sensitive to MEM, while 40–50% of *Escherichia* strains were sensitive to FOS, IMP, SCF, and TZP. A total of 18 *Klebsiella* strains were screened against antimicrobial drugs, and the results are shown in Table 3. The highest resistance in the 16 *Klebsiella* strains was found against AMP. More than 55% of *Klebsiella pneumoniae* strains were resistant to AK, AMC, CIP, CRO, CTX, FEP, and PIP. Additionally, 33% of *Klebsiella* strains had intermediate resistance against AMC, NF, and CAZ. The highest antimicrobial drug sensitivity was found in 67% of *Klebsiella* strains for DO, MEM, and SCF, while 55% of *K. pneumoniae* strains were sensitive to IMP and TZP.

A total of 13 uropathogenic *Pseudomonas* strains were screened against various antimicrobial drugs to determine their MDR pattern, and the results are depicted in Table 3.

Table 4: Antibiotic resistance pattern in *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Proteus* spp. against various antimicrobial drugs.

	Sensitive	Intermediate	Resistant
Staphylococcus (n = 3)	n = 3 [VA, LEV, MXF, CIP, NF, NV, CN] n = 1 [P]	n = 3 [PIP]	n = 3 [TE, DO, TOB] n = 2 [P]
Streptococcus (n = 3)	n = 2 [FOS, MXF, NF] n = 1 [CRO, VA, LZD]	n = 1 [CTX, MXF, NF]	n = 3 [CE, LEV, TE, P, DO, CIP] n = 2 [CRO, VA, LZD, CTX] n = 1 [FOS]
Enterococcus (n = 3)	n = 3 [LZD] n = 2 [TE] n = 1 [FOS, DO, MXF, NF, IPM]	n = 1 [FOS, CTX, MXF, NF]	n = 3 [CRO, VA, CE, LEV, P, CIP] n = 2 [CTX, DO, IPM] n = 1 [FOS, TE, MXF, NF]
Proteus (n = 2)	n = 2 [IMP, MEM, SCF] n = 1 [AK, CTX, CAZ, CIP, TZP, CRO, AMP, AMC]	n = 1 [TZP]	n = 2 [PIP, NF, FOS] n = 1 [AK, CTX, CAZ, CIP, CRO, AMP, AMC]

VA = vancomycin, LEV = levofloxacin, AMP = ampicillin, AMC = amoxicillin clavulanate, MXF = moxifloxacin, CIP = ciprofloxacin, NF = nitrofurantoin, NV = novobiocin, CN = gentamycin, PIP = pipemidic acid, TE = tetracycline, DO = doxycycline, TOB = tobramycin, P = penicillin, FOS = fosfomycin, CRO = ceftriaxone, LZD = linezolid, CTX = cefotaxime, CE = cephradine, IPM = imipenem, MEM = meronem, AK = amikacin, CAZ = ceftazidime.

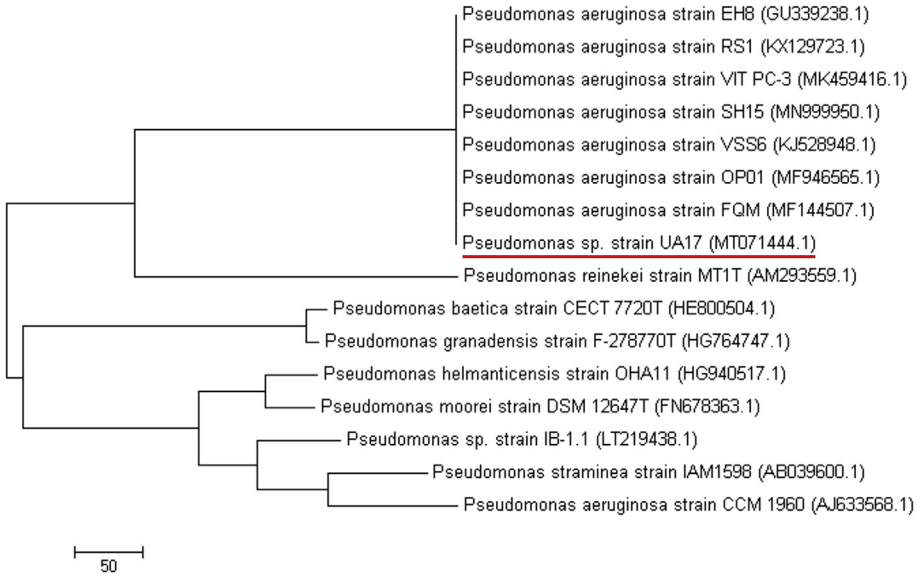

Figure 1: Phylogenetic tree of *Pseudomonas* strain UA17 (accession number: MT071444).

Approximately all tested *Pseudomonas* strains were sensitive to MEM, whereas more than 60% of *Pseudomonas* strains were sensitive to CAZ, CN, IMP, PIP, and TZP. More than 50% of *Pseudomonas* strains had MDR patterns against ATM, CIP, and CTX. Three strains each from the *Staphylococcus*, *Streptococcus*, and *Enterococcus* genera, and two from the *Proteus* genus were evaluated for sensitivity to and resistance against various antimicrobial drugs, and their results are given in Table 4. Among the three strains of *Staphylococcus*, all strains were resistant to DO, TE, and TOB, and they had intermediate resistance against PIP. All strains of *Staphylococcus* were sensitive to

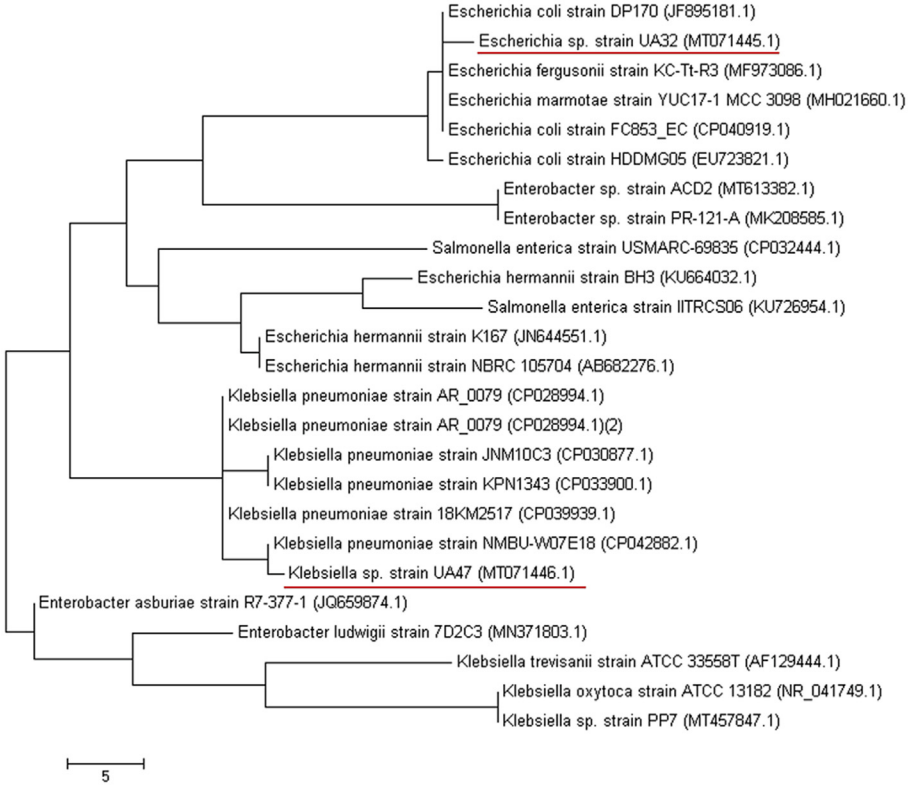

Figure 2: Phylogenetic tree of *Escherichia* strain UA32 and *Klebsiella* strain UA47 (accession numbers: MT071445 and MT071446, respectively).

CIP, CN, LEV, MXF, NF, NV, and VA. In case of *Streptococcus* (n = 3), all strains were resistant to CE, CIP, DO, LEV, P, and TE. Two strains of *Streptococcus* were sensitive to FOS, MXF, and NF. All strains of *Enterococcus* were resistant to CE, CIP, CRO, LEV, P, and VA and were sensitive to LZD. Two *Proteus* strains were resistant to FOS, NF, and PIP and were sensitive to IMP, MEM, and SCF.

Identification of selected strains using 16S rRNA gene sequencing

Three uropathogenic bacterial strains, UA17, UA32, and UA47, were selected on the basis of their high MDR and identified using 16S rRNA partial gene sequencing. The selected strains were identified as *Pseudomonas* strain UA17, *Escherichia* strain UA32, and *Klebsiella* strain UA47. The accession numbers of these strains are MT071444, MT071445, and MT071446, respectively, obtained after submission in the NCBI browser. *Pseudomonas* strain UA17 showed a close resemblance to strains of *P. aeruginosa* with a similarity index of 100%, and its phylogenetic tree is presented in Figure 1. A combined phylogenetic tree of *Escherichia* strain UA32 and *Klebsiella* strain UA47 was drawn and is shown in Figure 2. *Escherichia* strain UA32 showed a close resemblance to *E. coli* with a 99.86% similarity index. *Klebsiella* strain UA47 was found to be similar to *K. pneumoniae* with a similarity index of 99.80%.

Discussion

MDR bacteria can resist the action of antimicrobial drugs, which causes treatment failure of diseases and spread of infections. MDR in bacteria is a natural phenomenon that is extensively promoted in immunosuppressed conditions. It is enhanced due to prolonged drug exposure and the persistence of infections despite therapy. Among infections, UTI is a severe threat in our community, especially to pregnant women.[1] Asymptomatic bacteriuria is a frequently occurring bacterial infection of the urinary tract that requires medical treatment even during pregnancy. Gram-negative and gram-positive bacteria are involved in the occurrence of UTI; however, gram-negative bacteria more commonly cause UTIs.

In the present study, uropathogenic bacterial strains were isolated from 80 pregnant women aged 19–45 years with 78% of participants aged 25–34 years (Table 1). The participants were multigravida (57.5%) and primigravida (42.5%) with UTI in pregnancy as it has repeatedly been reported to be an element for a substantial rise in UTI.[30] The association between UTIs and parity is because of the physiological variations that emerge in pregnancy due to UTIs.[31] Maternal age, parity, and morbid obesity have been formerly witnessed as threat elements for UTI in pregnant women.[32] The prevalence of UTIs in our study population was comparable to that in other studies. In our study, we sampled pregnant women, including both urban and rural residents during all trimesters with the majority in the third trimester in the study catchment zone, presenting at antenatal check-ups in Lahore, Pakistan. Sarwar et al.[33] isolated 370 pathogenic bacterial isolates from 520 pregnant female genitalia residing in various areas of Punjab, Pakistan. They reported that 71% of gynaecological infections were caused by *E. coli* (41.6%), *S. aureus* (15.4%), and coagulase-negative staphylococci (12.2%). Similarly, Kumarasamy et al.[34] isolated *Enterobacteriaceae* isolates from UTI, pneumonia, and bloodstream infection and reported 239 metallo-β-lactamase 1 producing isolates from India, 37 isolates from the United Kingdom, and 25 isolates from Pakistan, which were found to be carbapenem-resistant. Recently, a study conducted in Bangladesh on UTI in pregnant women reported high-burden bacteriuria (5.7%) in both symptomatic and asymptomatic cases.[35] Similarly, Dashtizade et al.[36] also reported the prevalence of UTI in Iranian pregnant women. They reported that the population most commonly affected by UTIs were those aged between 21 and 30 years (48.3%), with high school education (38.8%), and who were housewives (90.8%). In the current study, both symptomatic and asymptomatic UTI cases were investigated. Previously, both symptomatic and asymptomatic bacteriuria studies have also been conducted in Bangladesh,[37] Ethiopia,[38] and Iran.[36] Symptomatic bacteriuria, similar to the current study, was also reported in Pennsylvania,[39] Iran,[40] and India.[41]

In the present study, uropathogenic bacterial strains were isolated from 80 pregnant women with bacteriuria. A total of 67 uropathogenic isolates underwent a series of biochemical characterisations (Table 2) based on the recommendations of Lennox and Ackerman.[42] Based on these biochemical characteristics, the uropathogenic bacterial strains were identified at the genus level and grouped into *Escherichia*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Proteus*. Similarly, Rave et al.[43] also adopted biochemical identification techniques before profiling the antibiotic susceptibility pattern. In addition, the molecular identification of selected uropathogenic bacterial strains was also conducted in this study by performing 16S rRNA partial gene sequencing to verify the correct identification of strains of the bacterial genus. The UTI aetiology in the current study revealed that 81.3% of participants were affected by UTI caused by uropathogenic gram-negative and gram-positive bacterial strains (Tables 1 and 2). In this population, 90% of the isolated uropathogenic strains belonged to gram-negative bacteria. There was a high rate of bacterial isolation from the *Escherichia*, *Klebsiella*, and *Pseudomonas* genera. Similarly, other UTI aetiology studies have reported the predominance of gram-negative bacteria, especially strains of *E. coli* and *Klebsiella* spp. Recently, Lee et al.[35] reported that 50% of bacteriuria was caused by *E. coli* and *Klebsiella* spp. in pregnant women in Bangladesh. They also reported bacteriuria due to staphylococcal (23% of isolates) and Group B streptococcus species (5.3% of isolates).[35] Similarly, Majumder et al.[44] and Haque et al.[45] reported a predominance of *E. coli* comprising up to 75% of isolates and *Klebsiella* species accounting for up to 11% of isolates. Untreated recurrent UTI could be a major cause of foetal and maternal adverse effects such as low birth weight, preterm delivery, cystitis, and pyelonephritis.[46] Uropathogenic bacteria might enter the urothelium, thus avoiding normal clearance during emptying of the bladder, which later causes recurrent UTI.[47]

Rising antibiotic resistance is a global concern, particularly in low-income countries, including Pakistan. The current study demonstrates the higher rates of antibiotic resistance to common antimicrobial agents for the treatment of UTI in pregnancy. The general pattern was that *Escherichia* spp. were resistant against AML, PIP, and AMP; *Klebsiella* spp. were resistant against PIP, AMP, and CTX, and *Pseudomonas* spp. were resistant against CIP and CTX (Table 3). Other strains from the *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Proteus* genera also showed a variable MDR pattern (Table 4). The current study concluded that gram-negative uropathogenic bacterial strains were highly resistant to third-generation cephalosporin antibiotics. Similarly, the World Health Organization (WHO) also reported the resistance of *E. coli* (68%) and *Klebsiella* (81%) isolates to third-generation cephalosporin antibiotics.[48,49] Similarly, other relevant studies on MDR in strains of *E. coli*, *Klebsiella*, *Pseudomonas,* and other uropathogenic bacteria have also been reported.[5,35,36,41,43,50–52] The current study revealed that for uncomplicated and complicated bacteriuria, carbapenems should be preferred over fluoroquinolones and cephalosporins. The data from the current study highlights the need for antibiotic stewardship and the development of new effective antimicrobials that are safe for use in pregnancy.

Conclusion

In the present study, the uropathogenic strains of the *Escherichia*, *Klebsiella*, and *Pseudomonas* genera showed significant resistance to PIP, AMP, AML, FEP, and CTX, and less resistance to MEM and TZP. Because of uropathogenic strains with emerging MDR, our conclusion highlights the limited use of antibiotics and recommends physicians to perform culture examination and identify some genetic mechanisms for UTI treatment during pregnancy.

Recommendations

The increase in antibiotic-resistant UTI in pregnant women from Lahore, Pakistan has been observed due to the increased use of antibiotics. National efforts are needed to control MDR UTI in pregnant women in the country by implementing evidence-based actions monitoring UTI treatment. Our study suggests that raising awareness of the judicious use of antibiotics is essential to limit the increase in resistance levels.

Source of funding

The current research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Ethical approval for the protocol of the study was granted by the Institute of Molecular Biology and Biotechnology at The University of Lahore, Pakistan, with reference number IMBB-734 and dated 04-10-2018.

Authors' contributions

UA-Conducted research, provided research materials, and collected and organised data. **MZM** conceptualised and supervised the study, analysed and interpreted the data, and wrote the initial and final draft of the article**. AM** provided research materials and logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Acknowledgments

The authors would like to acknowledge the research facility provided by the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL), Main campus, Lahore, Pakistan. The authors acknowledge the help of Dr. Ahmad Zaheer, IMBB, UOL, Main campus, Lahore, in analysing the sequencing of 16S rRNA. The authors also acknowledge English language proofreading of the manuscript by Dr. Munir Ahmad (College of Food and Agricultural Sciences, King Saud University, KSA) and Dr. Bisma Rauff (Institute of Molecular Biology and Biotechnology, The University of Lahore).

References

1. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. **Am J Med 2002**; 113: 5–13.
2. Rajivgandhi G, Vijayarani J, Kannan M, Murugan A, Vijayan R, Manoharan N. Isolation and identification of biofilm forming uropathogens from urinary tract infection and its antimicrobial susceptibility pattern. **Int J Adv Life Sci 2014**; 7: 352–363.
3. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. **Urol Clin North Am 2008**; 35: 1–12.
4. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. **Nat Rev Microbiol 2015**; 13: 269–284.
5. Gessese YA, Damessa DL, Amare MM, Bahta YH, Shifera AD, Tasew FS, et al. Urinary pathogenic bacterial profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo town, Central Ethiopia: a cross-sectional study. **Antimicrob Resist Infect Contr 2017**; 6: 132–141.
6. Nguefack CT, Ebongue CO, Chokotheu CN, Ewougo CE, Njamen TN, Mboudou E. Clinical presentation, risk factors and pathogens involved in bacteriuria of pregnant women attending antenatal clinic of 3 hospitals in a developing country: a cross sectional analytic study. **BMC Pregnancy Childbirth 2019**; 19: 143–148.
7. Moghadas AJ, Irajian G. Asymptomatic urinary tract infection in pregnant women. **Iranian J Pathol 2009**; 4: 105–108.

8. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: relationship with urinary tract infections. **Int J Health Sci 2019**; 3(2): 48–55.
9. Almalki MA, Varghese R. Prevalence of catheter associated biofilm producing bacteria and their antibiotic sensitivity pattern. **J King Saud Univ Sci 2020**; 32(2): 1427–1433.
10. Wojno KJ, Baunoch D, Luke N, Opel M, Korman H, Kelly C, et al. Multiplex PCR based urinary tract infection (UTI) analysis compared to traditional urine culture in identifying significant pathogens in symptomatic patients. **Urology 2020**; 136: 119–126.
11. Wiley Z, Jacob JT, Burd EM. Targeting asymptomatic bacteriuria in antimicrobial stewardship: the role of the microbiology laboratory. **J Clin Microbiol 2020**; 58(5). https://doi.org/10.1128/JCM.00518-18.
12. Rejali M, Ahmadi SS. Prevalence of urinary tract infection and associated effective factors during pregnancy in Shahrekord, Iran. **Int J Epidemiol 2018**; 5: 55–59.
13. World Health Organization. *Antibiotic resistance*; 2020 https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.
14. Gallagher JC, MacDougall C. *Antibiotics simplified*. Jones & Bartlett Learning; 2016.
15. Geffers C, Gastmeier P. Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). **Deutsch Arztebl Int 2011**; 108(6): 87–93.
16. Lowy FD. Staphylococcus aureus infections. **N Engl J Med 1998**; 339(8): 520–532.
17. Dammeyer AH, Heinze S, Adler AC, Nasri L, Schomacher L, Zamfir M, et al. Clinical relevance of colonization with antimicrobial-resistant bacteria (AMRB) and methicillin susceptible Staphylococcus aureus (MSSA) for mothers during pregnancy. **Arch Gynecol Obstet 2019**; 300(5): 1303–1316.
18. Christaki E, Marcou M, Tofarides A. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. **J Mol Evol 2020**; 88(1): 26–40.
19. Pumps E, Cost F, Ram B. Efflux pumps of gram-negative bacteria: genetic responses to stress and the modulation of their activity by pH, inhibitors, and phenothiazines. **Adv Enzymol Relat Area Mol Biol 2011**; 238: 61–167.
20. Hafeez S, Aslanzadeh J. Biochemical profile-based microbial identification systems. In: Tang YW, Stratton CW, editors. *Advanced techniques in diagnostic microbiology*. Cham: Springer; 2018. pp. 33–67.
21. Smith AC, Hussey MA. *Gram stain protocols*. American Society for Microbiology; 2005.
22. Cappuccino JG, Sherman N. *Microbiology: a laboratory manual*. 11th ed. Harlow, England: Pearson Education; 2018.
23. Davis JA, Farrah SR, Wilkie AC. Selective growth of Staphylococcus aureus from flushed dairy manure wastewater using acriflavine-supplemented mannitol salt agar. **Lett Appl Microbiol 2006**; 42(6): 606–611.
24. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of Escherichia coli O157: H7 associated with hemorrhagic colitis. **J Clin Microbiol 1986**; 23: 869–872.
25. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. **Am J Clin Pathol 1966**; 45(4): 493–496.
26. CLSI. *Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A10*. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2009. https://clsi.org/media/1631/m02a12_sample.pdf.
27. CLSI. *Performance standard for antimicrobial susceptibility test: approved standard Twelfth Edition. M02-A13*. Wayne, PA: Clinical and Laboratory Standard Institute (CLSI); 2019. https://clsi.org/standards/products/microbiology/documents/m02/.
28. Cheneby D, Perrez S, Devroe C, Hallet S, Couton Y, Bizouard F, et al. Denitrifying bacteria in bulk and maize-rhizospheric soil: diversity and N_2O reducing abilities. **Can J Microbiol 2004**; 50: 469–474.
29. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. **Mol Biol Evol 2016**; 33: 1870–1874.
30. Sescon NIC, Garingalao-Molina FD, Ycasiano CEJ, Saniel M, Manalastas R. Prevalence of asymptomatic bacteriuria and associated risk factors in pregnant women. **Phil J Microbiol Infect Dis 2003**; 32: 63–69.
31. Nandy P, Thakur A, Ray C. Characterization of bacterial strains isolated through microbial profiling of urine samples. **Online J Biol Sci 2007**; 7: 44–51.
32. Haider G, Zehra N, Munir AA, Haider A. Risk factors of urinary tract infection in pregnancy. **JPMA. J Pak Med Assoc 2010**; 60: 213–216.
33. Sarwar A, Butt MA, Hafeez S, Danish MZ. Rapid emergence of antibacterial resistance by bacterial isolates from patients of gynecological infections in Punjab, Pakistan. **J Infect Public Health 2020**. https://doi.org/10.1016/j.jiph.2020.06.011.
34. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt FA, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. **Lancet Infect Dis 2010**; 10: 597–602.
35. Lee AC, Mullany LC, Koffi AK, Rafiqullah I, Khanam R, Folger LV, et al. Urinary tract infections in pregnancy in a rural population of Bangladesh: population-based prevalence, risk factors, etiology, and antibiotic resistance. **BMC Pregnancy Childbirth 2020**. https://doi.org/10.1186/s12884-019-2665-0.
36. Dashtizade M, Zolfaghari MR, Yousefi M, Nazari-Alam A. Antibiotic susceptibility patterns and prevalence of streptococcus agalactiae rectovaginal colonization among pregnant women in Iran. **RBGO Gynecol Obstet 2020**. https://doi.org/10.1055/s-0040-1710299.
37. Ullah MA, Barman A, Siddique MA, Haque AK. Prevalence of asymptomatic bacteriuria and its consequences in pregnancy in a rural community of Bangladesh. **Bangladesh Med Res Counc Bull 2007**; 33(2): 60–64.
38. Gizachew Z, Kassa T, Beyene G, Howe R, Yeshitela B. Multidrug resistant bacteria and associated factors among urinary tract infection suspectedreproductive aged women in jimma university specialized hospital, Southwest Ethiopia. **Ethiop Med J 2019**; S1: 31–43.
39. Hillebrand L, Harmanli OH, Whiteman V, Khandelwal M. Urinary tract infections in pregnant women with bacterial vaginosis. **Am J Obstet Gynecol 2002**; 186: 916–917.
40. Sharami S, Afrakhteh M, Shakiba M. Urinary tract infections in pregnant women with bacterial vaginosis. **J Obstet Gynaecol Res 2007**; 27: 252–254.
41. Nasiri RM, Muhi FA. The proportion of pregnancy-associated asymptomatic bacteriuria in al-karkh hospital. **World J Pharmaceut Res 2019**; 5: 312–323.
42. Lennox VA, Ackerman VP. Biochemical identification of bacteria by replicator methods on agar plates. **Pathology 1984**; 16(4): 434–440.
43. Rave AFG, Kuss AV, Peil GH, Ladeira SR, Villarreal JP, Nascente PS. Biochemical identification techniques and antibiotic susceptibility profile of lipolytic Ambiental bacteria from effluents. **Braz J Biol 2019**; 79(4): 555–565.
44. Majumder MI, Ahmed T, Hossain D, Begum SA. Bacteriology and antibiotic sensitivity patterns of urinary tract infections in a tertiary hospital in Bangladesh. **Mymensingh Med J 2014**; 23(1): 99–104.

45. Haque R, Akter ML, Salam MA. Prevalence and susceptibility of uropathogens: a recent report from a teaching hospital in Bangladesh. **BMC Res Notes 2015**; 8: 1–5.
46. Delzell Jr JE, Lefevre M. Urinary tract infections during pregnancy. **Am Fam Physician 2000**; 61(3): 713–720.
47. Dwyer PL, O'Reilly M. Recurrent urinary tract infection in the female. **Curr Opin Obstet Gynecol 2002**; 14: 537–543.
48. WHO. *Antimicrobial resistance global report on surveillance*. Geneva, Switzerland: World Health Organization (WHO); 2014. https://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/.
49. WHO. *The world is running out of antibiotics*. Geneva, Switzerland: World Health Organization (WHO); 2017. https://www.who.int/medicines/news/2017/world-running-out-antibiotics-WHO-report/en/.
50. Mokube MN, Atashili J, Halle-Ekane GE, Ikomey GM, Ndumbe PM. Bacteriuria amongst pregnant women in the Buea Health District, Cameroon: prevalence, predictors, antibiotic susceptibility patterns and diagnosis. **PloS One 2013**; 8: 1–6.
51. Onuoha SC, Fatokun K. Prevalence and antimicrobial susceptibility pattern of urinary tract infection (UTI) among pregnant women in Afikpo, Ebonyi, Nigeria. **Am J Life Sci 2014**; 2: 46–52.
52. Tellapragada C, Eshwara VK, Bhat P, Acharya S, Kamath A, Bhat S, et al. Risk factors for preterm birth and low birth weight among pregnant Indian women: a hospital-based prospective study. **J Prev Med Public Health 2016**; 49: 165–175.

How to cite this article: Asmat U, Mumtaz MZ, Malik A. Rising prevalence of multidrug-resistant uropathogenic bacteria from urinary tract infections in pregnant women. J Taibah Univ Med Sc 2021;16(1):102–111.