Tropical Journal of Pharmaceutical Research May 2025; 24 (5): 687-701
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v24i5.7

Original Research Article

The pattern of antimicrobial susceptibility among urinary tract bacterial pathogens and the associated resistance profile

Lorina Ineta Badger-Emeka[1]*, Promise M Emeka[2]
[1]Department of Biomedical Sciences, Microbiology Division, College of Medicine, [2]Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, Saudi Arabia

For correspondence:* ***Email:* *lbadgeremeka@kfu.edu.sa*

Sent for review: 23 March 2025 *Revised accepted: 12 May 2025*

Abstract

Purpose: *The global trend in disease burden due to bacterial infections of urinary tract is on the rise, thus necessitating continual surveillance.*
Methods: *One hundred and sixty-seven (167) bacterial isolates of urinary tract infections collected between 2014 and 2020 were explored. Their identification (IDs) confirmation, antimicrobial susceptibility test (AST) and extended-spectrum beta-lactamases (ESBLs) were determined using Vitek Compact 2 (BioMerieux, Marcy L'Etoile, France. Results were computed and analyzed using GraphPad Prism version 10.4.1 (627).*
Results: *The pathogens were mainly Escherichia coli (52 %), Klebsiella pneumoniae (21.56 %) and Pseudomonas aeruginosa (10.77 %). Others include Proteus mirabilis (6 %), Enterobacter species (4.79 %), Providencia stuartii (2.39%) and Acinetobacter baumannii (2.39 %). Source of isolation was significantly ($p < 0.0001$) more from urine (82 %) than from catheter tip samples (18 %). Significantly more of the pathogens were multidrug-resistant (MDR; 59 %) than were susceptible strains (SS; 22.1 %) or extensively drug-resistant (XDR; 18.5%), with results varying among bacterial species. While E. coli strains were sensitive to three antimicrobials (imipenem, tigecycline and colistin), K. pneumoniae was highly resistant to the tested antibiotics including colistin (55.56 %), but with a reduction in resistance to ticarcillin (15 %), meropenem (19.44 %), gentamicin (22.22 %), and aztreonam (44.44 %).*
Conclusion: *The findings of this study show that E. coli, K. pneumoniae and P. aeruginosa continue to be troublesome UTI pathogens, progressing from MDR to XDR. Therefore, previously considered gold-standard antibiotics used in treating UTIs might remain the choice drugs for much longer as seen from the results of this study.*

Keywords: *Urinary tract infections, UTIs, Antimicrobial resistance, Antibiotics, ESBLs, Escherichia coli, Klebsiella pneumoniae*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Urinary tract infections (UTIs) are common amongst the immunocompetent as well as the immunocompromised. These infections are grouped as some of the most frequently encountered infections in clinical settings [1]. UTIs are prevalent across genders and age groups in communities and healthcare centers [2]. These infections have also been attributed to be more common among female patients than males because they are the gender group whose

urine specimens are most frequently collected in hospital microbiology laboratories [3,]. As a result of the aforementioned reasons, urinary tract infections remain a global public health burden.

An estimated 404.6 million individuals are reported to have had UTIs globally in 2019, and death resulting from such infections is placed at 236,786 in the same year [4]. The global disease burden was reported to be on the rise, differing across geographical regions [4]. In the USA, the prevalence of uncomplicated cases of UTIs is 8 – 11 % [2]. Although it has been reported that there are no national estimates available for Saudi Arabia [2], some researchers place the prevalence in the range of 9.8 – 24 % [5]. Besides, the disease burden of UTIs is placed second to those of the respiratory tract [6]. There is also the yearly worldwide financial burden of $6 billion attributed to direct healthcare expenses [7]. Furthermore, the number of 'ambulatory' hospital visits was placed at $10.5 million in 2007 in the USA, of which 0.9 % of the visits are to emergency departments [4]. Moreover, there is a significant impact of UTIs on health-related quality of life (QoL) comprising both economic and health burdens, while recurrent infections are accompanied by psychological problems associated with depression and anxiety symptoms [8].

Generally, UTIs are classified [1] to be a broad term that includes a wide variety of 'infection syndrome', of which the most common mechanism of infection acquisition is the colonization of the urethra or the periurethral space by bacterial pathogens.

Bacteria of the Enterobacteriaceae family are the major causative agents of UTIs with uropathogenic *Escherichia coli* (UPEC) accounting for about 80 % of uncomplicated cases while those of community- and hospital-acquired infections constitute 95 % of infections [9]. Other documented UTI bacterial pathogens include *Acinetobacter baumannii, Klebsiella pneumoniae, Proteus mirabilis*, and Group B *Streptococcus* amongst others [1]. While *E. coli* could be implicated in complicated and uncomplicated UTIs, species of *Enterococcus, P. aeruginosa, and P. mirabilis* cause complicated infections in hospitalized patients as well as in long-term stay care facilities [1]. Thus, the distribution of infection types differs with patients and comorbidity states.

Saudi Arabia, like other regions of the world, remains burdened by UTIs and they reportedly account for about 10 % of all infections in the Kingdom, second only to admissions to the emergency department [10]. Concerning though is the re-admission of about 10 % of the patients previously discharged from the hospital as a result of UTI, suggesting ineffective treatment.

Generally, broad-spectrum antibiotics are employed in the treatment of UTIs. However, the alarming rate of bacterial resistance to antimicrobials has also created difficult-to-treat strains associated with UTIs globally as well as here in the Kingdom of Saudi Arabia [10]. Besides, for the reason that there is an ever rapidly changing antimicrobial susceptibility by bacterial pathogens, there is a need for continual updates to track the emergence of bacteria strains in line with the recommendations for global and regional surveillance aimed at mitigating the spread of resistant strains.

The suggested treatment of uropathogens associated with UTIs varies for symptomatic and asymptomatic bacteriuria [11]. Some antibiotics such as ertapenem, imipenem, co-trimoxazole, and nitrofurantoin remain in the front line of treatment choice. However, bacterial susceptibility patterns continue to change rapidly so too, leading to the evolution of varying bacterial phenotypes with different phenotypic resistance traits.

For regional surveillance, this study seeks to examine the phenotypes of bacterial pathogens associated with UTIs in the Al-Ahsa subregion of Southeastern region of Saudi Arabia. The associated resistance factors are required to provide an insight into the antimicrobial susceptibility pattern of the isolates thereby providing a much-needed update to healthcare providers and professionals in the region. The information provided on resistance phenotypes could help in treatment modifications as well as in control measures aimed at mitigating the spread of resistant bacterial strains, in addition to bridging the knowledge gap for this region of investigation.

EXPERIMENTAL

Ethical consideration

Protocol for the research was approved by the Research Ethics Committee at King Faisal University with approval no. KFU-REC-2024-NOV-ETHICS2907. Subject to the provisions of the National Committee of Bioethics Saudi Arabia (kacst.gov.sa), subsection 11, the study is exempted from informed consent. Bacteria isolates stored in the microbank in the Microbiology Division of College of Medicine

were used for the investigation. Patients were not involved.

Bacteria isolation and confirmation identity (ID)

Samples were retrieved from the archives in the Microbank of the Microbiology laboratory at the College of Medicine, King Faisal University. They had been preserved with codes from 2014 – 2020.

The recovery of isolates was according to the recommended guidelines of the manufacturers, (https://www.pro-labdirect.com/v/vspfiles/microbank/microbank-wwp-portfolio.pdf). Each bacteria isolate was plated out on MacConkey or blood agar, and incubated at 37 °C for 24 h. The resultant overnight growth was sub-cultured to obtain pure colonies now used for the identification (IDs) of the isolates. Confirmation of IDs and antimicrobial susceptibility test (AST) of the isolates was conducted with Vitek Compact 2 (BioMerieux, Marcy L'Etoile, France) using GP, GN, ID and AST cards according to manufacturer's guidelines. Production of extended-spectrum beta-lactamases (ESBLs) was detected by a Vitek 2 Automated System and confirmed according to earlier recommended guidelines (CLSI) [12], on Muller–Hinton agar and incubated aerobically at 35 °C for 24 h.

Confirmation of ESBLs production and molecular assay of ESBLs genotypes

ESBLs were detected with Vitek 2 Automated System (BioMerieux, Marcy L'Etoile, France). Confirmation was by combined disc test (CDT) as recommended by CLSI [17]. Briefly, positive isolates were seeded together with 30 µg cefotaxime (CTX), and 30 µg ceftazidime (CAZ), combined with 10 µg clavulanic acid (CLA) on Muller Hinton agar. The setup was aerobically incubated overnight at 37 °C. Result interpretation centered on resistance to a single test against cefotaxime and ceftazidime individually and separately in combination with clavulanate as recommended by CLSI [13].

Qiagen DNA extraction kit was used for genomic DNA extraction according to the manufacturer's guidelines. A final PCR product volume of 50 µL composed of master mixer (25 µL), primers (2 µL each), and genomic DNA template (100 ng) was constituted and used for DNA amplification. Applied Biosystems (AB) thermal Cycler (Foster City, California 94404, USA) was used to perform the cycling program which required 30 cycles of 94 °C for 1 min, 62 °C for 45 s, and 72 °C for 1 min. Amplification by PCR for SHV, TEM, and CTX genes was ascertained using the primers in Table 1 [14,15].

Table 1: Primer sequence of genes used

Gene	Sequence
TEM-F	AGATCAGTTGGGTGCACGAG
TEM-R	CAGTGCTGCAATGATACCG
CTX-M-F	ATGTGCAGYACCAGTAARGTKATGGC
CTX-M-R	TGGGTRAARTARGTSACCAGAAYCAGCGG
SHV-F	GGGTTATTCTTATTTGTCGC
SHV-R	TTAGCGTTGCCAGTGCTC

The resultant PCR amplified products were stained using 0.5 µg/mL ethidium bromide, then resolved on 2 % agarose gel by gel electrophoresis, and DNA strands were analyzed by visualizing with an ultraviolet (UV) light illuminator and photo documentation system. The detailed method used is as previously described by Zaniani *et al* [17].

Antimicrobial susceptibility test and resistance profile groupings

The isolates where tested against the following antibiotics: amoxicillin (Amox), ampicillin (Amp), amikacin (Amk), augmentin (Aug), aztreonam Atm), cephalotin (Ch), cefuroxime (Cfx), ceftriaxone (Cro), cefotaxime (Cft), cefazolin (Cfz), ceftazidime (Caz), cefepime (Pime), cefoxitin (Ctt), ceftizoxime (Ctx), cefixime (Cef), cefodox (Cpd), imipenem (Imp), meropenem (Mer), gentamicin (Gm), ciprofloxacin (Cip), colistin (Cs), tigecycline (Tig), levofloxacin (Levo), trimethoprim/sulfamethoxazole (Sxt), piperacillin/tazobactam (Ptz), ticarcillin/clavulanic acid (TICA), nitrofurantoin (Nit), nalidic acid (Na), norfloxacin (Nxn), tetracycline (Te), tobramycin (Tob), doxycycline hydrochloride (Doxycycline HCl). The minimum inhibitory concentration values for the tested antibiotics were provided by Vitek Compact 2 (BioMerieux, Marcy L'Etoile, France). The pathogens were then classified as either susceptible strains (SS), multidrug-resistant if isolates were resistant (MDR) to at least one agent in three or more classes of antibiotics, including cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, piperacillin-tazobactam, ampicillin-sulbactam as defined by the Centers for Disease Control and Prevention (CDC) [18]. Also, other pathogens were classified as extensive drug-resistant (XDR) defined as non-susceptibility by any of the isolates to a minimum of one agent in all but two or fewer classes of antibiotics.

Statistical analysis

Data was collected on Microsoft Excel sheet and used for the analysis of antimicrobial assay results and presented as percentages (%). GraphPad Prism version 10.4.1 (627) was used to compare periodic data of mean ± SEM percentage resistant/sensitive of the bacterial pathogens against the tested antibiotics. Two-way ANOVA and Tukey's multiple comparison were used to compare the difference in susceptibility between *E. coli* and *K. pneumoniae* isolates with significance taken at $p < 0.05$. To compare data for other isolates (*P. aeruginosa, A. baumannii, P. mirabilis* and other pathogens), ordinary one-way ANOVA was used to compare the percentage (%) mean ± SEM, i.e., percentage resistant/sensitive, and significance taken at $p < 0.05$. In addition to these, MedCalc Software Ltd's Comparison of proportions calculator (https://www.medcalc.org/calc/comparison_of_proportions.php; version 23.1.5; accessed January 25, 2025) was used to compare significance between percentages, and significance taken at $p < 0.05$.

RESULTS

Bacterial isolates and source of samples

The isolates included in this investigation were *Escherichia coli* (52 %), *Klebsiella pneumoniae* (21.56%), *Pseudomonas aeruginosa* (10.77 %), *Proteus mirabilis* (6 %), *Enterobacter* species (4.79 %), *Providencia stuartii* (2.39 %) and *Acinetobacter baumannii* (2.39 %) as shown in Table 2. More significantly ($p < 0.0001$) isolated from urine (82 %) than catheter tips samples (18 %) were *E. coli* and *K. pneumoniae* isolates. Also, *E. coli* isolates were significantly more ($p < 0.05$) than those of *K. pneumoniae* and other bacteria pathogens. All of *A. baumannii* were from catheter tips while the remaining bacteria pathogens (*Proteus mirabilis, Providencia stuartii*, *Pseudomonas aeruginosa*, and species of *Enterobacter*), were from urine samples (Table 2). The origin of the samples was more from outpatient department (OPD; 35.32 %), followed by those from intensive care unit (ICU) and hospital wards (26.9 % each). The least (10.78 %) number of samples were those from the emergency room (ER). Additionally, *Klebsiella pneumoniae* isolates were significantly *(*$p < 0.05$) associated more with ICU (41.67 %) infections followed by those from the wards for this bacterial pathogen while *E. coli* isolates were more from OPD samples.

General antimicrobial profile of the urine pathogens

The results of the minimum inhibitory concentrations (MICs) of tested antimicrobials varied as shown in Table 3 a and b. The pathogens consisted significantly more of MDR (59.8 %) bacterial strains, than those that were either SS (22.1 %) or XDR (18.5 %; Table 4).

The results of susceptibility to the tested drugs are displayed in Figure 1. Overall percentage resistance against the penicillins (100 – 97.7 %) and cephalosporins (100 – 86.3 %) was high. For the quinolones, resistance ranged from 100 % for nalidixic acid to 79.6 and 75.57 % respectively for ciprofloxacin and levofloxacin, respectively (Figure 1). Also high were the overall resistance against meropenem (88.0 %), trimethoprim/sulfamethoxazole (Sxt; 80.8 %), norfloxacin (86.2 %), aztreonam (83.8 %) and augmentin (76.6 %). Between the aminoglycosides, the isolates were less susceptible to tobramycin (74 %) compared to amikacin (36.9 %) and gentamicin (44.63 %). There was generally a high sensitivity to imipenem (85.25 %) and colistin (82.35 %), while more than half of the isolates were sensitive to tigecycline (69.6 %), amikacin (63.1 %), and gentamicin (55.57 %), as shown in Figure 1.

Antimicrobial resistance of bacterial species

As per species type, susceptibility varied amongst the bacterial pathogens as displayed in Figure 2 A - F. Strains of *E. coli* were all sensitive to three of the tested antimicrobials (imipenem, tigecycline and colistin), while resistance was low for meropenem (1.3 %), nitrofurantoin (22.8 %), chloramphenicol (26.7 %) and tazocin (30 %). However, beyond than half of the isolates (52 %) were resistant to gentamicin as well as resistance ranging between 60 % for tetracycline to 100 % (piperacillin; Figure 2 A). Also, displayed is the antimicrobial susceptibility pattern for *K. pneumoniae* isolates (Figure 2 B).

For this pathogen, a high resistance is seen against most of the tested drugs inclusive of colistin (55.56 %). They were, however, less resistant to ticarcillin (15 %), meropenem (19.44 %), gentamicin (22.22 %), and aztreonam (44.44 %). Isolates of Enterobacter species (Figure 2 C) were sensitive to Amikacin and Tigecycline with a 33.33 % resistance to each for the tested carbapenems (imipenem and meropenem).

Table 2: Bacterial UTIs pathogens, sample source of isolation and their origin

Bacteria pathogen N (%)	**Sample source**				**Location**							
	Urine		***Catheter tips***		***OPD***		***ER***		***ICU***		***Wards***	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*(%)*	*N*	*(%)*
Klebsiella pneumoniae 36 (21.56)	22	61.11†	14	38.89*	9	25	2	5.56	15	41.67‡	10	27.78
Escherichia coli 87 (52.1)	72	82.76#	15	17.24	46	52.9	10	11.5	3	3.45	28	32.18
Acinetobacter baumannii 4 (2.39)	0	0	4	100	0	0	0	0	4	100	0	0
Providencia stuartii 4 (2.39)	4	100	0	0	0	0	0	0	3	75	1	25
Pseudomonas aeruginosa 18 (10.77)	18	100	0	0	0	0	2	11.1	12	66.7	4	22.2
Proteus mirabilis 10 (6.0)	10	100	0	0	0	0	0	0	8	80	2	20
Enterobacter species 8 (4.79)	8	100	0	0	4	50	4	50	0	0	0	0
Total N 167	134		33		59		18		45		45	
Percentage (%) (100)	**(82)¶**		**(18)**		**(35.32)**		**(10.78)**		**(26.9)**		**(26.9)**	

N = Number; % = percentage; OPD = Outpatient Department; ICU = Intensive Care Unit; ER = Emergency Room; * Comparison between % of catheter tips source of *E. coli* and *K. pneumoniae*, percentage differences are *significant p* = 0.0091. **†** Significant difference between urine and catheter tips for *K. pneumoniae* *(p* < 0.00017). # Significant difference between Urine and catheter tips sample for E. coli *(p* < 0.0001). ‡ represents significant difference between samples from ICU and hospital wards *(p* = 0.0396). ¶ Significant difference between urine and catheter tips samples used for the investigation *(p* < 0.0001)

Table 3a: Minimum inhibitory concentrations of tested antibiotics

Isolate	AMK	LEVO	AMP	TICA	AUG	CFX	CAZ	CTX	PIME	IMP
A. baumannii		8	32	128	32	Nt	64	Nt	64	8, 16
E. coli	2, 4, 64	Nt	16, 32	Nt	4, 8, 16, 32	4, 8, 16, 32, 64	1, 4, 64	1, 4	1, 4, 64	0.25, 1
K. pneumoniae	2, 64	Nt	32	Nt	2, 16, 32	4, 64	1, 2, 8, 64	1, 64	1, 64	0.25, 2, 16
P. aeruginosa	2, 4, 16	0.25, 1, 2, 4	Nt	128	Nt	Nt	16, 32, 64		8, 16, 64	2, 16
E. aerogenes	2	Nt	16	Nt	32	64	8	64	1	2
Enterobacter species	2	Nt	16	Nt	16	64	64	64	64	2
P. mirabilis	2	Nt	32	Nt	2, 8	4	1	1	1	2, 8
Providencia stuartii	2	Nt	32	Nt	Nt	4	1	1	1	1, 2

Table 3b: Minimum inhibitory concentrations of tested antibiotics *(contd)*

Isolate	MER	GM	CIP	TIG	PTZ	SXT	NIT	ATM	TOB
A. baumannii	16	16	4	4	Nt	320	Nt	Nt	16
E. coli	0.25, 16	1, 4, 16	0.025, 0.5, 4	0.025, 0.5	4, 8, 64, 128	20, 320	16, 32, 64, 256	Nt	Nt
K. pneumoniae	0.25, 4, 16	1, 16	0.25, 4	0.5, 1	4, 128	20, 320	32, 64, 128, 256	Nt	Nt
P. aeruginosa	1, 8, 16	1, 2, 4	0.25, 1, 4	8	Nt	Nt	Nt	16, 64	1, 16
E. aerogenes	0.5	1	0.25	0.5	128	20	128	Nt	Nt
Enterobacter species	0.25	2	8	0.12	128	Nt	32	Nt	Nt
P. mirabilis	0.25	1, 8, 16	2, 4	4	4	320	128	Nt	Nt
Providencia stuartii	0.25	4	4	8	4	320	256	Nt	Nt

Minimum inhibitory concentrations of the tested antibiotics; amikacin = AMK; levofloxacin= LEVO; ampicillin=AMP; augmentin = AUG; cefoxitin=CFX; ceftazidime= CAZ; ceftriaxone= CTX; cefepime=PIME; imipenem=IMP; meropenem=MER; gentamicin=GM; ciprofloxacin= CIP; tigecycline=TIG; piperacillin/tazobactam = PTZ; trimethoprim/sulfamethoxazole = SXT; nitrofurantoin=NIT; ticarcillin/clavulanic acid = TICA; aztreonam = ATM; tobramycin= TOB; not tested = NT. *A. baumannii = Acinetobacter baumannii, E. Coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae, P. aeruginosa = Pseudomonas aeruginosa, E. aerogenes = Enterobacter aerogenes, P. mirabilis = Proteus mirabilis, P. stuartii = Providencia stuartii*

Table 4: Dissemination of antimicrobial resistance pattern of UTI bacterial pathogens

Bacteria strain	All N (%)	SS N (%)	MDR N (%)	XDR N (%)
K. pneumonia	36 (100)	4 (11.1)	20 (55.5)	12 (33.3)
E. coli	87 (100)	25 (28.7)	52 (59.8)	10 (11.5)
A. baumannii	4 (100)	0 (0)	0 (0)	4 (100)
P. stuartii	4 (100)	0 (0)	4 (100)	0 (0)
P. aeruginosa	18 (100)	4 (22.2)	10 (55.5)	5 (27.7)
P. mirabilis	10 (100)	0 (0)	10 (100)	0 (0)
Enterobacter species	8 (100)	4 (50)	4 (50)	0 (0)
Total	***167 (100)***	***37 (22.1)***	***100 (59.8)****	***31 (18.5)***

*Significant difference between MDR, XDR and SS ($p < 0.0001$). SS = susceptible strain, MDR = multi-drug resistant, XDR = extensive drug-resistant. Percentages for bacterial strains were calculated as per total for each type of isolate in the row. While column percentages were as per their total. MDR was significantly more in number than SS and XDR isolates ($p < 0.0001$)

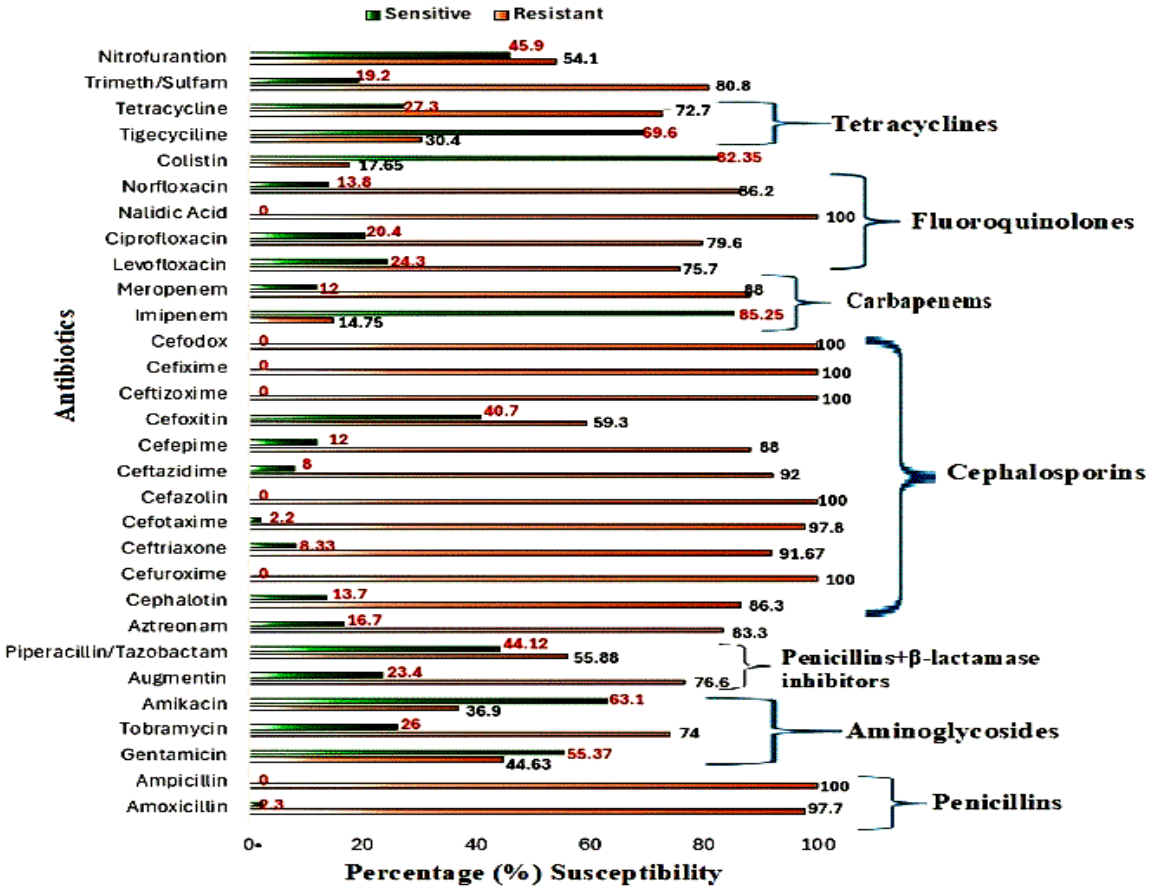

Figure 1: Distribution (%) of susceptibility of the UTIs pathogens to assessed antimicrobials. *Note*: Complete percentage resistance and sensitivity by the UTI pathogens against tested antibiotics and the antimicrobial groups

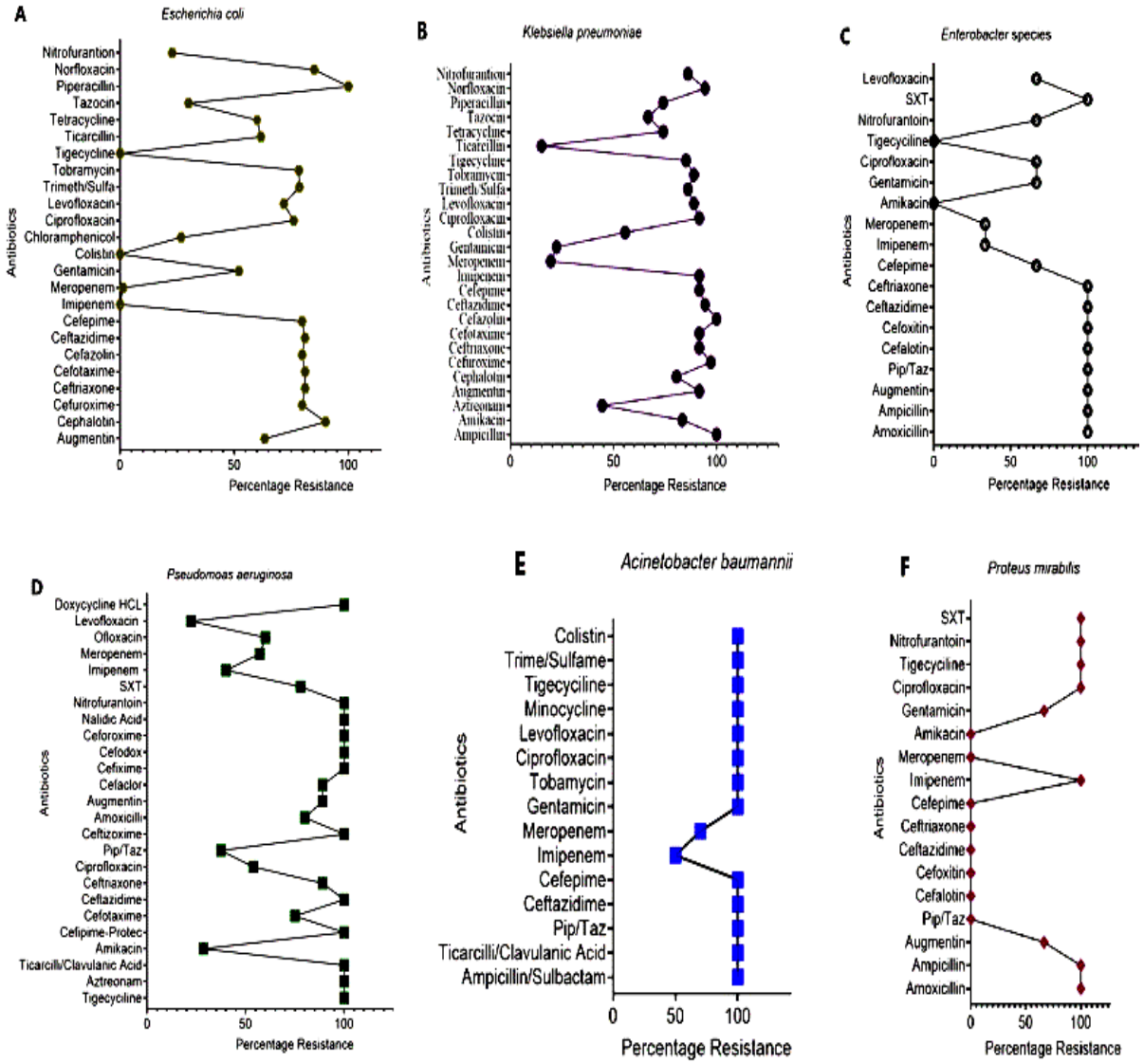

Figure 2: Distribution of antimicrobial resistance by UTI bacteria species to the tested antibiotics. **A-B:** Demonstrated here are *E. coli* (A) and *K. pneumoniae* (B) UTIs pathogens with their percentage resistance to the tested antibiotics. Pip/Taz = Piperacillin/Tazobactam, SXT = trimethoprim/sulfamethoxazole. **C-D:** Presentations of species of *Enterobacter* (C) and *P. aeruginosa* pathogens of UTIs and their percentage resistance to the tested antibiotics. Doxycycline HCl = Doxycycline Hydrochloride. **E-F:** Exhibition of the percentage resistance by species of *A. baumannii* (E) and *Proteus mirabilis* (F) pathogens of UTIs and their percentage resistance to the tested antibiotics

When compared to the aforementioned UTI bacterial pathogens, *P. aeruginosa* was more resistant to imipenem (50 %) and meropenem (57.14 %) while displaying a low resistance to amikacin (28.6 %), levofloxacin (22.22 %) and piperacillin/tazobactam (Ptz; 37.5 %) and the results are presented in Figure 2 D.

Between the UTI bacterial pathogens investigated, the highest resistance profile were those displayed by *A. baumannii* isolates that showed 100 % resistance to the tested antibiotics with the exception of imipenem (50 %) and meropenem (70 %; Figure 2 E). *Proteus mirabilis* isolates were sensitive to eight (Amk, Mer, Pime, Caz, Ctt, Cro, Ch, and Pip/Taz) of the seventeen tested antibiotics (Figure 2 F).

Antimicrobial resistance by year of isolation

Results on susceptibility by year of isolation were determined and presented as mean ± SEM of percentages resistance and sensitivity and displayed in Figure 3 A - F.

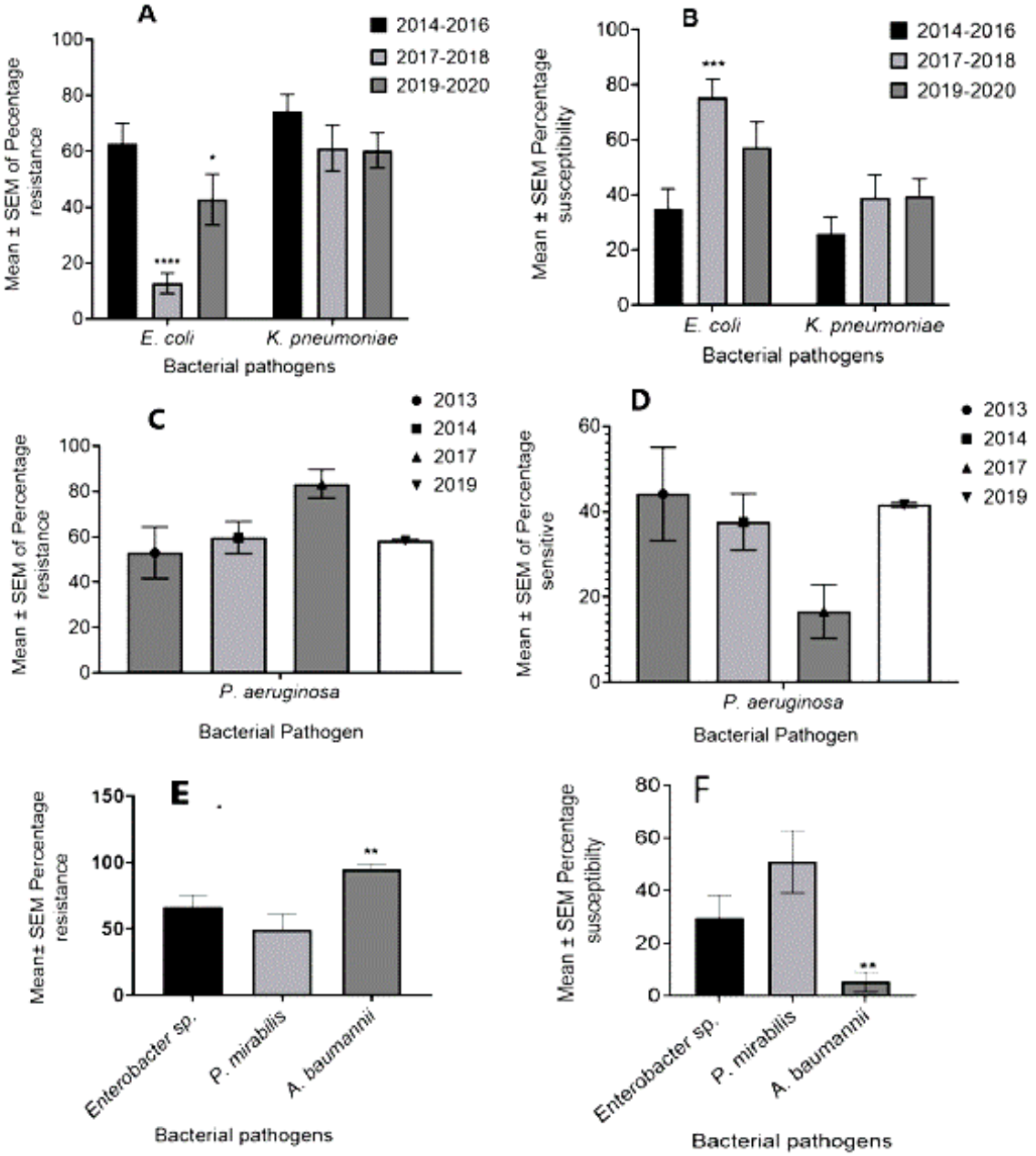

Figure 3 A-F: Presentation of the distribution of susceptibility pattern by year of isolation of UTIs pathogens. **A-B:** Mean ± SEM for resistance (A) and sensitivity (B) against the tested antibiotics by *E. coli* and *K. pneumoniae* bacterial UTIs pathogens for the period between 2014 and 2020. ****$P < 0.00001$, ***$p < 0.0005$ and *$p < 0.05$. ***C-D:*** Illustrations of mean ± SEM for resistance (C) and Sensitivity (D) exhibited by *P. aeruginosa* UTIs pathogens for 2013, 2014, 2017 and 2019. Differences in susceptibility for this pathogen based on the year of isolation were not significant. ***E-F:*** *Illustrations of Mean ± SEM for resistance (E) and Sensitivity (F) exhibited by other UTIs pathogens (Enterobacter species,* Proteus mirabilis *and* A. baumannii*); **p < 0.005*

Antimicrobial assay showed no specific pattern in regard to period of isolation. The figure shows *E. coli* isolates of 2017-2018 were significantly ($P < 0.0001$) the least resistant to the test antibiotics as compared with those collected before or after that period (Figure 3 A - B).

These differences in resistance are also reflected in the profile of sensitive strain period of isolation. A high resistance is displayed by *K. pneumoniae* isolates collected over the same period, highest for those of 2014 - 2016. Though percentage resistance reduced between the years 2017 – 2018, and remained slightly lower through 2019 - 2020, differences were not significant ($p < 0.75$, $p < 0.72$ and $p < 0.99$, respectively), as shown in Figure 3 A - B. Significant differences in resistance for *E. coli* and *K. pneumoniae* for the years of 2014 - 2016, 2017 - 2018 and 2019 - 2020 ($p < 0.0001$). Also, comparison of mean ±

SEM of percentages sensitivity for *E. coli* and *K. pneumoniae* showed significant differences between periods of isolation with *p*-values of 0.01, 0.016 and 0.036, respectively. For *P. aeruginosa* isolates, mean ± SEM of percentages resistance for the isolate was highest in 2017 (83.3 %) as compared to previously collected isolates or those of the latter years. Differences as regards the year of isolation were not found to be significant (p >0.05). Results did not follow any specific pattern of displayed resistance against antimicrobials (Figure 3 C - D). However, for other isolates such as *A. baumannii*, *P. mirabilis* and *Enterobacter* species (Figure 3 E - F), results of mean ± SEM of percentages resistance showed those of *A. baumannii* to be significantly higher (p < 0.002).

ESBL-associated genotypes in association with antimicrobial resistance profiles

Of the 87 (100 %) *E. coli* isolates, 80 (92 %) of them were ESBL producers while 32 (88.89 %) of the *K. pneumoniae* isolates were ESBLs with non-significant difference (p-value 0.99) in the percentages between both species of the bacterial pathogens. Also, a non-significant difference is seen with results of the CDT test (Table 5) which confirmed that 80.56 % of *K. pneumoniae* were positive for each of CTX/CLA and CAZ/CLA while for *E. coli*, 81.6 % were positive CTX/CLA and 80.46 % CAZ/CLA (Table 5). The results presented in Figure 4 A show the heatmap of *E. coli* isolates with different ESBL gene (CTX, TEM and SHV) carriage. Nine (9) of the isolates inhabited all the 3 ESBL genes with varying unrated percentage resistance profiles. Most of the isolates lacked the TEM and SHV genes but these characteristics did not correlate with the resistance outlook. The finding therefore indicates that there is no correlation between ESBL gene carriage and resistance profile. Also, sources of isolation did not show any differences between the numbers of ESBL gene carriage and resistance profile. Overall, the results showed a high resistance profile irrespective of whether the isolates had a full carriage of ESBL genes present or not. The Heatmap in Figure 4 B shows the distribution of the ESBL genes (CTX, TEM and SHV) carriage by the *Klebsiella pneumoniae* isolates and the sample sources. These isolates displayed different resistance profiles. Thirteen (13) isolates were found to possess all the ESBL genes with only *Kp*. 9, 196, 228 and 232 isolates exhibiting similar resistance profiles. The rest of the 9 isolates showed different resistance profiles that appeared to be lower than 80 %. Therefore, the presence of ESBL carriage by the isolates did not confer a higher or similar resistance profile. Thus, there is no correlation between ESBL gene carriage and resistance (%) in the present study. Additionally, the source of sample isolation did not have any correlation with either resistance profile or ESBL gene carriage. However, resistance remained high among the various isolates.

DISCUSSION

The UTI pathogens listed in this report are those that have commonly been associated with such infections [19,20]. The number of isolates here is, however, commensurate with what was available at -80°C microbank in the Microbiology Division of College of Medicine of our institution, with *E. coli, K. pneumoniae* and *P. aeruginosa* being the highest in number. Nonetheless, they are also the most common pathogens associated with complicated and uncomplicated UTIs reported in other studies in Saudi Arabia.

Table 5: Attributes of combined disc test

Bacterial pathogens	Number tested	Extended-spectrum beta-lactamases (ESBL) *Characteristics combined disc test (CDT)*			
		Number positive (%)	***CTX/CLA N (%)***	***CAZ/CLA N (%)***	***Negative for CTX/CAZ/CLA (N (%))***
Escherichia coli	87	80 (92)	71 (81.6)	70 (80.46)	9 (10.34)
Klebsiella pneumoniae	36	32 (88.89)	29 (80.56)	29 (80.56)	3 (8.33)

Number of isolates tested and those positive are as provided by Vitek Compact 2 (BioMerieux, Marcy L'Etoile, France). CTX = cefotaxime; CAZ = ceftazidime; CLA = clavulanic acid; N = number; % = percent

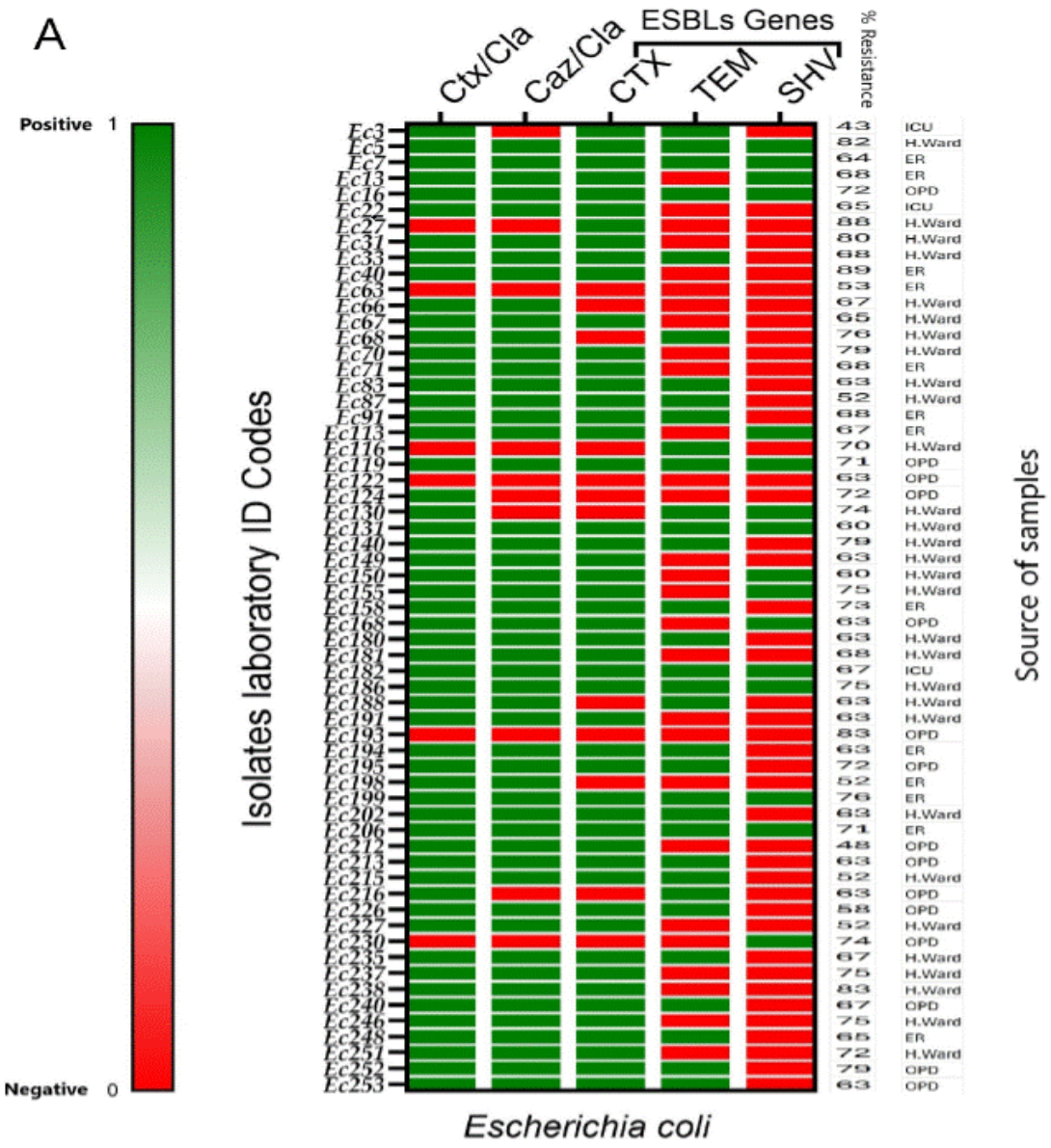

Figure 4 A: Heatmap showing *Escherichia coli* (A) isolates from various sources with ESBL gene carriage and resistance profile. ***Key:*** CTX = cefotaxime; CAZ = ceftazidime; CLA = clavulanic acid; CTX = C-terminal telopeptide; TEM = Temoniera; SHV = sulfhydryl variable; ICU = Intensive care unit; OPD = Out-patient department; ER = Emergency room; H.Ward = hospital ward

Thus, the numbers could simply be due to their association with UTI infections in the region of study. Also, the other listed UTIs pathogens (*Enterobacter* species, *Proteus mirabilis, Providencia stuartii* and *Acinetobacter baumannii*), align with reports that associated them with complicated, catheter-associated or recurrent cases of UTIs [19]. The *A. baumannii* isolates included in this study are catheter-associated UTIs, found among ICU patients suggesting the possibility of comorbid conditions. Generally, species of *Acinetobacter* are ubiquitous in nature with an enormous capability of colonizing to cause nosocomial infections in immunocompromised patients, particularly those with urinary catheters [21]. That they were from immunocompromised patients further highlights this bacterium as an opportunistic pathogen. Again, the isolates were extensively resistant to tested drugs with low sensitivity to the carbapenems, pointing to narrowing therapeutic options with differing recommendations for therapeutic regimens for XDR *A. baumannii* of which colistin is one of such [22]. Those in this investigation were resistant to colistin; thus, while therapeutic options are limited, they will differ across regions based on resistance assay.

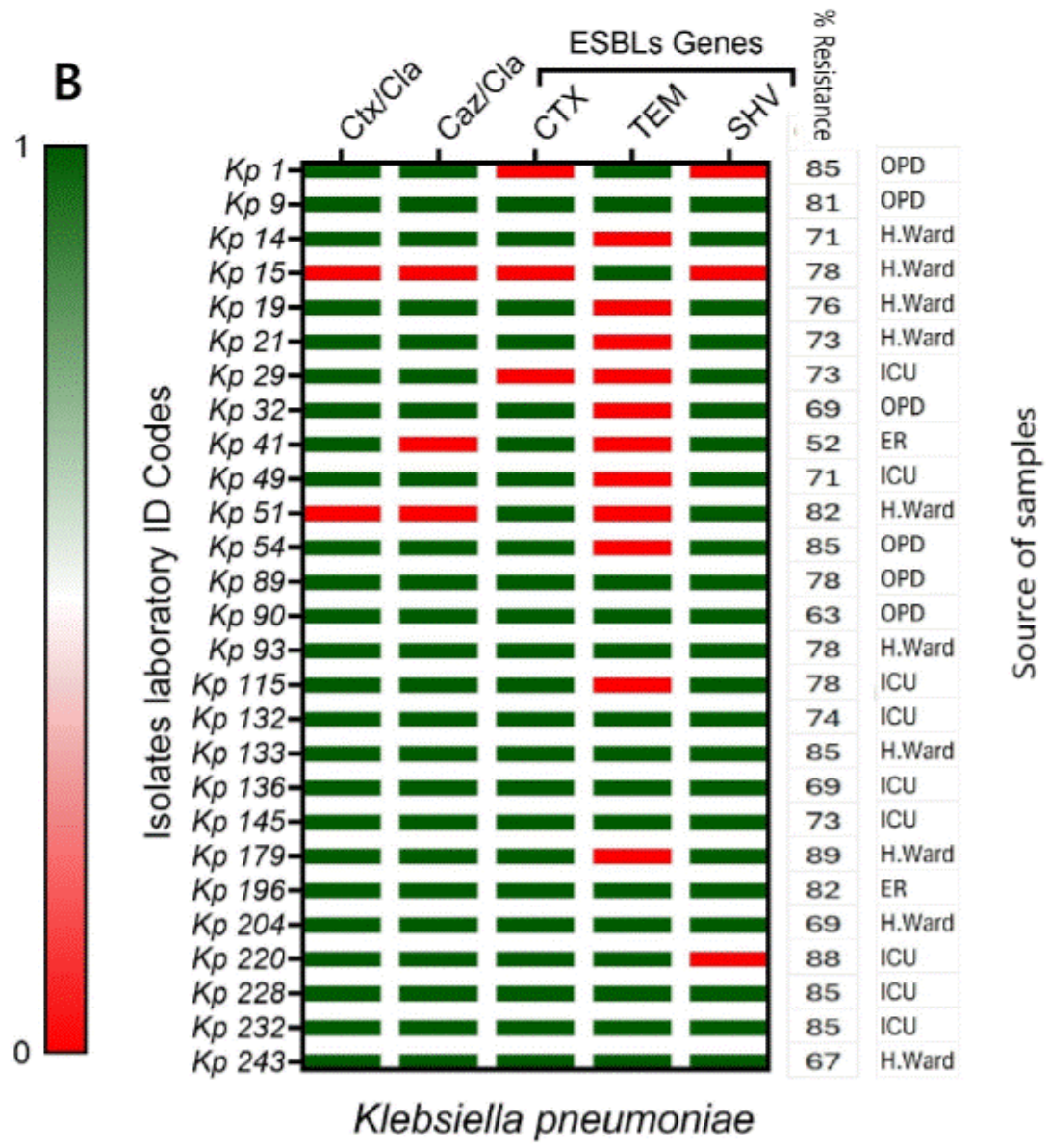

Figure 4 B: Heatmap showing *Klebsiella pneumoniae* (B) isolates from different sources with ESBL gene carriage and resistance profile. ***Key:*** CTX = cefotaxime; CAZ = ceftazidime; CLA = clavulanic acid; CTX = C-terminal telopeptide; TEM = Temoniera; SHV = sulfhydryl variable; ICU = Intensive care unit; OPD = Out-patient department; ER = Emergency room; H.Ward = hospital ward

In terms of the origin of the samples, most of them were from OPD, ICU and hospital wards. Generally, reports on UTIs distribution in healthcare settings differ, with some reporting higher prevalence from ER departments [10]. Again, the fact that the majority of the *E. coli* isolates were from OPD, may point to the possibility of uncomplicated cases of UTIs might not be consistent with other reports. However, the results which showed an overall significantly high drug resistance are consistent with those reporting a global rise of difficult-to-treat bacterial infections [23]. Of particular note was the high proportion of ESBL-producing *E. coli* and *K. pneumoniae* among the pathogens in this report. Principally, the ESBLs, *K. pneumoniae* and *E. coli* have been on the increase globally since 2000, particularly with regard to their resistance to the drug of choice [23].

The penicillins (ampicillin and amoxicillin) are shown in this report, not to be suitable therapeutic choices, findings that are in accord with those of earlier reports [24], thus suggesting the possibility of the circulating strains in the Southeastern region of Saudi Arabia. However, there are contrary reports from other regions of the world that indicate lower resistance against the penicillins, thereby suggesting the probability of geographical differences. Furthermore, the level of high resistance to the fluoroquinolones (norfloxacin, nalidic acid, ciprofloxacin, and

levofloxacin) seen in this report though higher than those of previous reports, are in harmony with earlier reports [24] from Saudi Arabia and other regions of the world. The fact that a similar pattern of resistance is seen with the cephalosporin group of antibiotics is a confirmation that they are unsuitable for the management of UTI bacteria strains in Al-Ahsa region of Southeastern region of Saudi Arabia. as had previously been suggested [24]. Among the carbapenems, only *E. coli* could still be treated with either imipenem or meropenem as they were highly susceptible to them.

Largely, the *E. coli* pathogens in this research responded well to imipenem with an overall resistance of 14.75 % as against meropenem (88 %). Carbapenems were once the gold standard in the management of UTIs, the need to conduct regular surveillance of the trends of antimicrobial susceptibility by UTI pathogens. Nevertheless, the findings reported here differ from those of a recent one [24] in which the carbapenems group was considered the choice of antibiotics. Thus, reports vary on the suitability of carbapenems in the management of UTIs although there has been a global rise in the resistance of bacterial pathogens to β-lactams, especially the carbapenems.

It is obvious that the isolates used in this investigation were sensitive only to colistin (except *A. baumannii*), tigecycline and imipenem but with differences amongst the pathogens, again highlighting limited choice of available antibiotics in the management of resistant bacterial pathogens. While the search for new antibiotics continues and the problem of antimicrobial resistance is not abating, the need for global surveillance cannot be over-emphasized.

CONCLUSION

The findings of this study show that *E. coli*, *K. pneumoniae* and *P. aeruginosa* are the top three bacterial pathogens associated with urinary tract infections over the period of 2014 - 2020 in Al-Ahsa, Saudi Arabia. High percentage of ESBL-producers are *E. coli* and *K. pneumoniae* while resistance to the tested antibiotics indicates that most of the pathogens are MDR. Although the majority of the *E. coli* pathogens coming from OPD are suggestive of uncomplicated UTIs, those of other pathogens (*Enterobacter* species, *Proteus mirabilis, Providencia stuartii*) are likely complicated UTIs. The presence of *A. baumannii* UTIs further highlights the opportunistic characteristics associated with catheterized patients. The carbapenems and nitrofurantoin, usually considered drugs of choice in the management of UTIs might not retain this status for too long due to the high resistance seen among the pathogens used in this research. Based on the foregoing, therefore, regional surveillance of these frequently encountered pathogens should be considered imperative. The surveillance should identify areas of increased infection within the regions of study, and hence frequently updating healthcare facilities 'antibiogram as a guided treatment protocol. This is to forestall the increasing pathogen resistance and optimize the use of appropriate antibiotics. Continuous sharing of antibiogram data within the region of this study should be promoted.

DECLARATIONS

Acknowledgment/Funding

The authors would like to thank Mr Hani Al-Farhan for his technical assistance. This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant no. KFU250163).

Conflict of interest

No conflict of interest is associated with this work.

Institutional Review Board statement

The procedure for the research was approved by the Research Ethics Committee at King Faisal University with approval no. KFU-REC-2024-NOV-ETHICS2907. No humans were included as test subjects in the research.

Data availability statement

Data can be made available from the corresponding author upon reasonable request.

Contribution of authors

Lorina Badger-Emeka conceived, designed and collected the data, did the statistical analysis of the data, as well as wrote and edited manuscript. She was also responsible for the grant acquisition and the integrity of the research. Promise Madu Emeka participated in the data collection process and analysis as well as writing of the manuscript. Both authors reviewed and approved the final draft of the manuscript for publication.

REFERENCES

1. Al Lawati H, Blair BM, Larnard J. Urinary Tract Infections: Core Curriculum 2024. Am J Kidney Dis 2024; 83(1): 90 – 100. https://doi.org/10.1053/j.ajkd.2023.08.009
2. Alghoraibi H, Asidan A, Aljawaied R, Almukhayzim R, Alsaydan A, Alamer E, Baharoon W, Masuadi E, Al Shukairi A, Layqah L, Baharoon S. Recurrent Urinary Tract Infection in Adult Patients, Risk Factors, and Efficacy of Low Dose Prophylactic Antibiotics Therapy. J Epidemiol Glob Health 2023; 13(2): 200-211. https://doi.org/10.1007/s44197-023-00105-4
3. Jung C, Brubaker L. The etiology and management of recurrent urinary tract infections in postmenopausal women. Climacteric 2019; 22(3): 242-249. doi: 10.1080/13697137.2018.1551871.
4. Zeng Z, Zhan J, Zhang K, Chen H, Cheng S. Global, regional, and national burden of urinary tract infections from 1990 to 2019: an analysis of the global burden of disease study 2019. World J Urol 2022; 40(3): 755-763. https://doi.org/10.1007/s00345-021-03913-0.
5. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol 2019; 11: 3-7. https://doi.org/10.1177/1756287219832172.
6. Klumpp DJ, Rycyk MT, Chen MC, Thumbikat P, Sengupta S, Schaeffer AJ. Uropathogenic Escherichia coli induces extrinsic and intrinsic cascades to initiate urothelial apoptosis. Infect Immun 2006; 74(9): 5106-5113. https://doi.org/10.1128/IAI.00376-06
7. Mann R, Mediati DG, Duggin IG, Harry EJ, Bottomley AL. Metabolic Adaptations of Uropathogenic E. coli in the Urinary Tract. Front Cell Infect Microbiol 2017; 7: 241. https://doi.org/10.3389/fcimb.2017.00241
8. Renard J, Ballarini S, Mascarenhas T, Zahran M, Quimper E, Choucair J, Iselin CE. Recurrent Lower Urinary Tract Infections Have a Detrimental Effect on Patient Quality of Life: a Prospective, Observational Study. Infect Dis Ther 2015; 4: 125–135. https://doi.org/10.1007/s40121-014-0054-6
9. Mlugu EM, Mohamedi JA, Sangeda RZ, Mwambete KD. Prevalence of urinary tract infection and antimicrobial resistance patterns of uropathogens with biofilm-forming capacity among outpatients in Morogoro, Tanzania: a cross-sectional study. BMC Infect Dis 2023; 23(1): 660. https://doi.org/10.1186/s12879-023-08641-x
10. Sula I, Alreshidi MA, Alnasr N, Hassaneen AM, Saquib N. Urinary Tract Infections in the Kingdom of Saudi Arabia, a Review. Microorganisms 2023; 11: 952. https://doi.org/10.3390/microorganisms11040952
11. Lee SJ. Clinical Guideline for Childhood Urinary Tract Infection (Second Revision). Child Kidney Dis 2015; 19(2): 56-64. http://dx.doi.org/10.3339/chikd.2015.19.2.56
12. CLSI 2020. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed.; CLSI supplement M100; Clinical and Laboratory Standards Institute: Pittsburgh, PA, USA, https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI2020.pdf.
13. Clinical Laboratory Standards Institute 2012. Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grows Aerobically, 9th ed.; (M07-A9); Clinical Laboratory Standards Institute: Wayne, PA, USA.
14. Yazdi MKS, Mohammad MSD, Hedroosha MAM, Ailar S, Jalil FM, Lari AR, Eshraghian MR, Saneiee AF, Abdar MH, Bakhtiari R. Molecular detection of TEM broad spectrum βlactamase in clinical isolates of Escherichia coli. Afr J Biotechnol 2011; 10(46): 9454-9458
15. Mulvey MR, Bryce E, Boyd D, Ofner-gostini M, Christianson S, Simor AE, Paton S. Ambler Class A Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and Klebsiella spp. in Canadian Hospitals. Antimicrob Agents Chemother 2004; 48(4): 1204-1214.
16. Sima SS, Mehdi G, Fattaneh, S. "Relation between bla TEM, bla SHV and bla CTX-M genes and acute urinary tract infections". J Acute Dis 2016; 5(1): 71-76.
17. Zaniani FR, Meshkat Z, Naderi NM, Khaje-Karamadini M, Ghazvini K, Rezaee A, Esmaily H, Nabavinia MS, Darban HM. The Prevalence of TEM and SHV Genes among Extended-Spectrum Beta-Lactamases Producing Escherichia Coli and Klebsiella Pneumoniae. Iran J Basic Med Sci 2012; 15(1): 654-660.
18. Centers for Disease Control and Prevention (CDC). Antimicrobial-resistant phenotype definitions, last updated 1/2022. https:// www.cdc.gov/nhsn/pdfs/ps-analysis-resources/ phenotype_definitions.pdf (accessed on January 26, 2024).
19. Alzahrani MA, Ali MS, Anwar S. Bacteria causing urinary tract infections and its antibiotic susceptibility pattern at tertiary hospital in Al-Baha region, Saudi Arabia: A retrospective study. J Pharm Bioall Sci 2020; 12: 449-456.
20. Abalkhail A, AlYami AS, Alrashedi SF, Almushayqih KM, Alslamah T, Alsalamah YA, Elbehiry A. The Prevalence of Multidrug-Resistant Escherichia coli Producing ESBL among Male and Female Patients with Urinary Tract Infections in Riyadh Region, Saudi Arabia. Healthcare (Basel) 2022; 10(9): 1778. https://doi.org/10.3390/healthcare10091778
21. Bagińska N, Cieślik M, Górski A, Jończyk-Matysiak E. The Role of Antibiotic-Resistant A. baumannii in the Pathogenesis of Urinary Tract Infection and the Potential of Its Treatment with the Use of Bacteriophage Therapy. Antibiotics (Basel) 2021; 10(3): 281. https://doi.org/10.3390/antibiotics10030281
22. Jiménez-Guerra G, Heras-Cañas V, Gutiérrez-Soto M, Aznarte-Padial MDP, Expósito-Ruiz M, Navarro-Marí JM, et al. Urinary tract infection by Acinetobacter baumannii and Pseudomonas aeruginosa: Evolution of antibiotic resistance and therapeutic alternatives. J Med Microbiol 2018; 67(6): 790-797. https://doi.org/10.1099/jmm.0.000742
23. Bazaid AS, Saeed A, Alrashidi A, Alrashidi A, Alshaghdali K, Hammam AS, Alreshidi T, Alshammary M, Alarfaj A,

Thallab R, Aldarhami A. Antimicrobial Surveillance for Bacterial Uropathogens in Ha'il, Saudi Arabia: A Five-Year Multicenter Retrospective Study. Infect. Drug Resist 2021; 14: 1455–1465. https://doi.org/10.2147/idr.s299846

24. *Abalkhail A, AlYami AS, Alrashedi SF, Almushayqih KM, Alslamah T, Alsalamah YA, Elbehiry A. The Prevalence of Multidrug-Resistant Escherichia coli Producing ESBL among Male and Female Patients with Urinary Tract Infections in Riyadh Region, Saudi Arabia. Healthcare (Basel) 2022; 10(9): 1778. https://doi.org/10.3390/healthcare10091778*