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Antibiotic-resistant bacteria isolated from selected urine and stool human specimens in Al-Fayoum, City Egypt

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During the current study, there 255 bacterial isolates were isolated from urine and stool samples, which collected from 255 patients (137 urine and 118 stool samples). They were consisted of 152 males and 103 females aged between 1-70 years. Out of the 255 specimens, 88 samples (34.5%) showed significant bacteriuria; 57.9% (51 samples) were from females, while 42.1% (37 samples) were from males. Out of the total isolates, 65 Gram negative and 23 Gram positive bacteria were detected. Also, the antibacterial susceptibility was tested for these 88 bacterial isolates. However, they were obtained from human feces specimens and human urine specimens as; 54 urine and 34 fecal specimens. Three fully identified bacteria from human samples (AM1, AM2, and AM3) were aligned against the 16S rRNA sequences as: *Escherichia coli*, *Klebsiella pneumonia,* and *Staphylococcus aureus*. They had a 100% identical counterpart with respect to their 16S rRNA sequences. In addition, data revealed that the isolated pathogens were resistant to various antibiotics. Some of them exhibited high resistance towards Duricef, Zithromax, Chloramphenicol, Amoxicillin, Unasyn, Flumox, Septrin, and Tetracycline. Moreover, they showed low resistance towards Amikacin, Vancomycin, and Ciprofloxacin.

Keywords: Antibiotic-resistant bacteria, *Escherichia coli*, *Klebsiella pneumonia, Staphylococcus aureus,* Human specimens.

INTRODUCTION

Pathogenic bacteria (e.g. *Staphylococcus sp*. and *E. coli*) make human ill because they reproduce quickly in human body and secret toxins, which can damage the tissue and make human sick (Diaz et al. 2008). Bacterial resistance to antibiotics poses a significant threat to public health (Morens et al. 2004). They have increased around the world, causing public health and medical concern because they threaten optimal care of patients with infection as well as the viability of the current health care system (Diaz et al. 2008).

Despite scientists' efforts to synthesize more potent antibiotics during the last five decades, the bacterial resistance continues to evolve in large part because of the overuse and misuse of antibiotics. The treatment of several pathogens, including methicillin resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae,* and vancomycin-resistant *enterococci*, is problematic (Lieberman, 2003). It has been observed that when bacteria are exposed to the same antibiotics repeatedly, the bacteria can change and are no longer affected by the drug (Pribis, 2019).

Antibiotic resistance is, sometimes, associated with significant morbidity, and excess costs, the excess cost related to resistant bacteria may be due to higher antibiotic acquisition costs and/or longer duration of hospitalization (Cosgrove, 2006) or due to delayed appropriate

antibiotic therapy or a necessity to perform surgery (Sipahi, 2008). The previous reasons were all significant factors for infections with multi drug resistant organisms (Kandemir et al. 2007).

Therefore, the present study was suggested to isolate and identify the most common pathogenic bacteria from the human specimens (urine and stool), in Al-Fayoum City, Egypt, in relation to their sensitivity and/or resistance to common commercial antibiotics.

MATERIALS AND METHODS

Collection of human specimens

Sampling was carried out from specimens collected from 255 patients who visited EL- Noor Laboratory at Al-Fayoum City during January and July 2018. They were made up of 151 males and 104 females and aged between 1-70 years. Faeces specimens were collected from 71 males and 47 females, while urine specimens were collected from 81 males and 56 females (Table 1).

Isolation of antibiotic-resistant bacteria

Urine specimens were obtained from patients via the clean-catch midstream technique. The urine specimens were plated within 2 h after collection and then incubated overnight at 37°C for 48 h (Murray et al. 1992). Faecal samples were placed in sterile plastic specimen tubes for bacterial isolation. The sample was directly cultured on MacConkey, EMB, MSA, and blood agar plates. The plates were incubated aerobically at 37ºC for up to 48 h (Murray et al. 2003). Grown colonies were isolated and identified by biochemical reaction tests (Rasha, 2011).

Preparation of the bacterial suspension

The bacterial inoculum was prepared by picking 5-10 colonies of each isolate with a sterile wire loop and suspended in into 2.5 ml of sterile distilled water. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 barium sulphate solution (CLSI, 2015).

Identification of antibiotic-resistant bacteria

Phenotypic characterization

Colony appearance was examined visually on fresh plates, while cell morphology was microscopically examined after Gram staining of 18 h old cultures. In addition, several biochemical tests were carried out to predict preliminarily the genus and species, to which these isolates are belonged. However, these biochemical tests were; catalase production test, oxidase production test, indole production, methyl red, and Voges-Proskaur tests (Cheesbrough, 1985), citrate utilization (Simmon's, 1926), and lactose fermentation (Hicks and Ryan, 1976).

Genotypic characterization

Sequencing 16S rRNA of the most potent bacterial isolate was done by Sigma Scientific Services Company, the genomic DNA of the isolate was extracted and sequencing was done. However, DNA extraction by use protocol of Gene Jet genomic DNA purification Kit (Thermo K0721) was done and primers used in PCR amplification and sequencing were; 16S 27F (5`AGAGTTTGATCCTGGCTCAG3`) and 16S 1492R (5`GGTTACCTTGTTACGACTT3`). The PCR products were purified and sequenced by the GATC-Biotech Co. (Germany). The sequences were compared with known sequences in the Gene Bank nucleotide database and identified as the nearest phylogenetic neighbor with the highest similarity percent (Hentschel et al. 2001).

Antibiotic susceptibility testing (AST)

Susceptibility testing was conducted using disc diffusion according to the guidelines of the CLSI (2009). However, sterile Mueller-Hinton agar plates were prepared and various antibiotic discs were selected. Bacterial suspension was taken by a sterile cotton swab then streaked the surface of all the plate in three different planes. The following 17 antibiotics were tested: SAM (Unasyn = Ampicillin/Sulbactam), AM (Ampicillin = Ampicillin/Penbritin), TE (Tetracycline), CN (Gentamicin), AK (Amikacin), CFR (Duricef), AZM (Zithromax), E (Erythromycin), CIP (Ciprofloxacin), OFX (Tarivid), CEP (Cefobid = Cefoperazone/Sulbactam), C (Chloramphenicol), AX (Amoxicillin), AMC (Augmentin = Amoxicillin/clavulanic acid), VA (Vancomycin), AF (Flumox = Amoxicillin/Flucloxacillin), SXT (Septrin = Sulfamethoxazole and Trimethoprim) were placed on the surface of the agar medium by gently pressing using a sterile forceps on the top of the discs (for better contact and effective diffusion of the antibiotics into the medium). The plates were incubated in an inverted position for 18 h at 37ºC (Baur et al. 1996). The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. After overnight incubation at 37˚C, inhibition zone diameters were read.

Table (1): Specimens collected from male and female patient for the current investigation.

Age groups (years)	Male				Female			
	No. of urine sample examined	Urine positive records	No. of faeces sample examined	Faeces positive records	No. of urine sample examined	Urine positive records	No. of faeces sample examined	Faeces positive records
1-10	20	1	13	1	6	4	5	4
11- 20	10	4	10	1	15	8	10	5
21- 30	6	4	6	2	4	2	3	1
31- 40	5	3	4	1	14	12	14	5
41- 50	22	6	20	3	5	3	5	2
51- 60	16	3	16	2	6	3	6	1
60 - 70	2	1	2	1	6	4	5	1
Total	81	22	71	15	56	32	47	19

The results of a disc diffusion test are interpreted by comparing the measured zone diameter with the interpretive criteria recommended by CLSI, (2011)**.**

RESULTS

Isolation of antibiotic-resistant bacteria from human specimens

Two hundred fifty-five bacterial isolates were isolated from urine and feces samples, which collected from EL-Noor Laboratory at Al-Fayoum City from January to July 2018. Specimens were collected from 255 patients, who visited clinics they were made up of 137 urine samples, 118 stool samples and they were made up of 152 males, 103 females and aged between 1-70 years. Urine specimens were collected from 81 males and 56 females, while stool specimens were collected from 71 males and 47 females.

Out of the 81 specimens isolated from urine samples of male, 22 recorded significant bacteriuria. While 71 specimens examined in this study isolated from feces samples of male, 15 exhibited significant bacteriuria. Similarity, of the 56 specimens examined in this study isolated from urine samples of female, 32 showed significant bacteriuria. While 47 specimens isolated from female feces samples, 19 detected significant bacteriuria. Out of the 255 specimens examined in this study, 88 (34.5%) showed significant bacteriuria. They were 51 (57.9%) in females, while 37 (42.0%) were in males. However, data are shown in Table 2.

Gram staining and morphological examination of the 255 urine and feces suspected cases 88 samples (54 urine samples and 34 fecal samples) exhibited significant bacterial growth, and then they were examined. Gram negative bacteria had a higher frequency of occurrence than that of Gram positive; constituting 65 isolates (73.9%) of the total isolates. These included; *Escherichia coli* 48 isolate (54.6%) and *Klebsiella pneumoniae* 17 isolates (19.3%). On the other side, Gram positive bacteria, including only *Staphylococcus aureus*, accounted for 23 isolates (26.1%) of the isolates. It was also found that the rate of isolates of *E. coli* and *S. aureus* were higher in isolates exclusively from females. However, data are presented in Table 3.

Table (2): Prevalence of urinary tract and faces infection in relation to age and sex of patients.

Age groups (years)	Males examined samples	Males positive records	Positivity %	Females examined samples	Females positive records	Positivity %	Total positive records with positivity %
1-10	32	3	9.4	9	7	77.8	10 (11.4%)
11- 20	20	6	30	25	12	48.0	18 (20.5%)
21- 30	12	7	58.3	6	3	50.0	10 (11.4%)
31- 40	8	4	50.0	28	16	57.1	20 (22.7%)
41- 50	42	10	23.8	10	5	50	15 (17.0%)
51- 60	32	5	16	12	4	33.3	9 (10.2%)
60 – 70	4	2	50	11	4	33.3	6 (6.8%)
Total	152	37	24.5	103	51	51.9	88 (100%)

Table (3): Isolation frequency of bacteria in relation to patient sex and type of sample (urine/stool).

Bacterium	Sample sex				Sample type			
	Isolates from males	Isolates from females	Total isolates sum	Total frequency (%)	Isolates from urine	Isolates from stool	Total isolates sum	Total frequency (%)
E. coli	21	27	48	54.6	29	19	48	54.6
K. pneumonia	7	10	17	19.3	11	6	17	19.3
S. aureus	9	14	23	26.1	14	9	23	26.1
Total	37	51	88	100	54	34	88	100

Identification of common antibiotic-resistant bacteria

Isolates coded as; AM1, AM2, and AM3 were subjected to morphological and physiological tests alongside genotypic characterization through 16S rRNA technique.

Phenotypic characterization

The results presented in Table 4 show some phenotypic characteristics of the selected isolates. These included colony and cell morphology, Gram reaction, catalase and oxidase test, as well as, some physiological and biochemical experiments. Data conducted that isolate AM1 is a Gram-negative, short rods shape, motile bacterium. It grew at 30-37ºC, pH range (6-8) and catalase, indole production, lactose utilization and MR test are positive. The results also showed that isolate AM2 is a Gram-negative, short rods shape, non-motile bacterium. It grew at 30-35ºC, pH range (6-8) and catalase, VP test, lactose utilization and citrate utilization are positive. Furthermore, these results exhibited that isolate AM3 is a Gram-Positive, *Staphylococcus* shape, non-motile bacterium. It grew at 35-37ºC, pH range (7-8) and coagulase test, lactose utilization, Catalase and MR test are positive. Lactose fermenting coliforms formed pink colored colonies with Macconkey[s] agar, and blood agar showed haemolytic properties of bacteria with *Staphylococcus aureus*. Positive catalase test produced bubbles indicating Gram +ve cocci growth; methyl red test produced red color; Voges-Proskauer test showed pink color and citrate utilization test gave blue color. Its streak of growth indicated growth of Gram -ve bacilli.

Genotypic characterization

Genomic DNA of the bacterial isolates AM1, AM2, and AM3 were prepared, and the gene coding for the 16S rRNA was partially amplified using the universal primers (16S 27F and 16S 1492R). The amplified PCR fragment were purified and then sequenced. A valid sequencing fragment data (1313 bps, 1307 bps, and 1220 bps) were aligned against the 16S rRNA sequences of (http://blast.ncbi.nlm.nih.gov/Blast.cgi). It has been found that the bacterial isolates AM1, AM2, and AM3 had a 100% identical counterpart with respect to their 16S rRNA sequences. The most closely related species and the percentages of identity are presented in Table 5. The sequence of the isolates; AM1, AM2, and AM3 were affiliated according to 100% sequence homology to the genus *Escherichia coli* strain Amer1, *Klebsiella pneumoniae* strain Amer2 and *Staphylococcus aureus* strain Amer3, respectively. Moreover, their phylogenetic analyses of *E.* were obtained based on partial sequencing of 16S rRNA (Figure 1, 2, and 3).

Antibacterial susceptibility testing (AST)

Based on locations, 88 bacterial isolates were isolated from human feces samples and human urine samples (54 urine and 34 fecal samples), which were collected during this study. The isolated pathogens were resistance to the various antibiotics. Some of them exhibited high resistance towards Duricef, Zithromax, Chloramphenicol, Amoxicillin, Unasyn, Flumox, Septrin, and Tetracycline, while they showed low resistance to Amikacin, Vancomycin, and Ciprofloxacin. However, more details are explained below.

AST for pathogenic bacteria isolated from urine samples

Generally, there 54 isolates were obtained

from urine culture. Gram negative bacteria had a higher frequency of occurrence than Gram positive constituting 40 of the total isolates as 74.0%. These included; 29 isolates of *E. coli* (53.7%) and 11 of *K. pneumoniae* (20.4%). Gram positive bacteria, including *S. aureus,* accounted for 14 of the isolates as 25.9%. It was also found that the rate of isolates of *E. coli* was higher than isolated other bacteria.

More specifically, the isolates of *E. coli* represented 53.7% from total isolates. However, they were resistance to the various antibiotics (Figures 4). Actually, they showed 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Tetracycline, and Vancomycin, followed by Flumox (96.6%) and Unasyn (82.8%), while they exhibited low resistance to Amikacin 10.4% and Ciprofloxacin 13.8. However, data are illustrated in Figure 5.

Also, the isolates of *K. pneumoniae* represented 20.4% from total isolates. The isolated *K. pneumoniae* strains were resistance to the various antibiotics (Figure 4). Indeed, they exhibited 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Tetracycline, Flumox, Septrin, and Vancomycin, followed by Erythromycin (81.8%) and Unasyn (81.8%), while they showed low resistance to Amikacin 18.1% and Ciprofloxacin 27.3% (Figure 5).

Additionally, the isolates of *S. aureus* represented 25.9% from total isolates. Its isolated strains were resistance to the various antibiotics (Figure 4). They showed 100% resistance towards Duricef, Zithromax, Ampicilin, Chloramphenicol, Gentamicin, Amoxicillin, Tetracycline, Flumox, Septrin, and Cefobid, followed by Erythromycin (78.5%), while they exhibited low resistance to Vancomycin (14.3%) and Amikacin (28.6%) (Figure 5).

Table (4): Phenotypic characterization of the most common bacterial isolates from human urine and stool specimens.

Test	Isolate		
	AM1	**AM2**	**AM3**
Gram reaction	Gram -ve	Gram -ve	Gram +ve
Motile	Motile	Non-motile	Non-motile
Temperature	30-37ºC	30-35ºC	35-37ºC
pH	6-8	6-8	7-8
Catalase	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve
Indole production test	+ve	-ve	-ve
Methyl red test	+ve	-ve	+ve
Voges Proskaur test	-ve	+ve	-ve
Citrate test	-ve	+ve	-ve
Lactose Utilization	+ve	+ve	+ve
Coagulase test	-ve	-ve	+ve
Result	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*

Table (5): Accession number of the experimental 16S rRNA sequence and similarity percentage to the closest known species.

Isolate code	Accession no.	Most related species	Similarity[1] (%)
AM1	MK106369.1	*Escherichia coli*	100
AM2	MK106370.1	*Klebsiella pneumoniae*	100
AM3	MK106371.1	*Staphylococcus aureus*	100

Genbank database was as follows: https://www.ncbi.nlm.nih.gov/nuccore/MK106369.1, https://www.ncbi.nlm.nih.gov/nuccore/MK106370.1, https://www.ncbi.nlm.nih.gov/nuccore/MK106371.1.

ACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAAC
GGTAGCTAATACCGCATAACGTCGCAAGCACAAAGAGGGGGACCTTAGGGCCTCTTGCCATCGGAT
GTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTC
TGAGAGGATGACCAGCAACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGTGGGGA
ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCNGCGTGTATGAAGAAGGCCTTCGGGTTGT
AAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGG
AACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGATAGAATTCCAGGTGTAGCGGTG
AAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTC
AGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCG
ACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGANNTAACGCGTTAAGTCGACCGCCTGGGGAG
TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGGTGGTT
TAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
GTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGGGTCCGGCCGGGAACTCAAAGGAG
ACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGG
CTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAA
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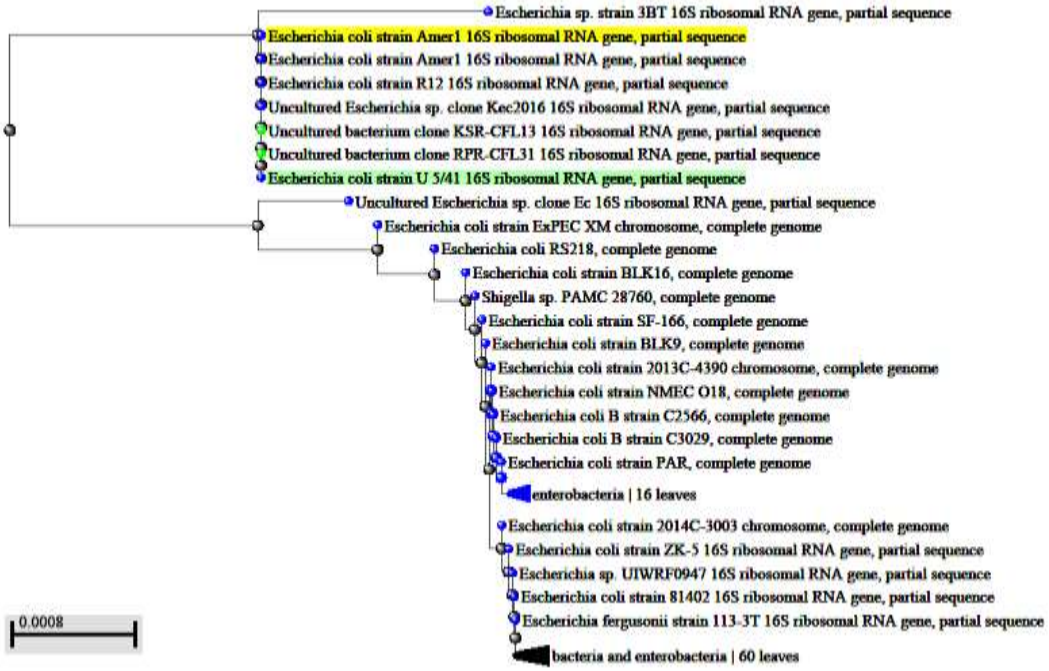

Figure (1): Nucleotide sequence of the 16S rRNA gene of *E. coli* isolate Amer1 (Upper). Phylogenetic analysis of *E. coli strain Amer1* based on its partial sequencing of 16S rRNA (Lower).

TCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCAT
GCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCC
CTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAA
GGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGATGAGGTTAATAACCTCATCGATTG
ACGTTACCCTGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATAGTGAAAT
CCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAA
TTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG

GACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
CGCCGTTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTA
AATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACCCACAGAACT
TTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGC
CGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAG
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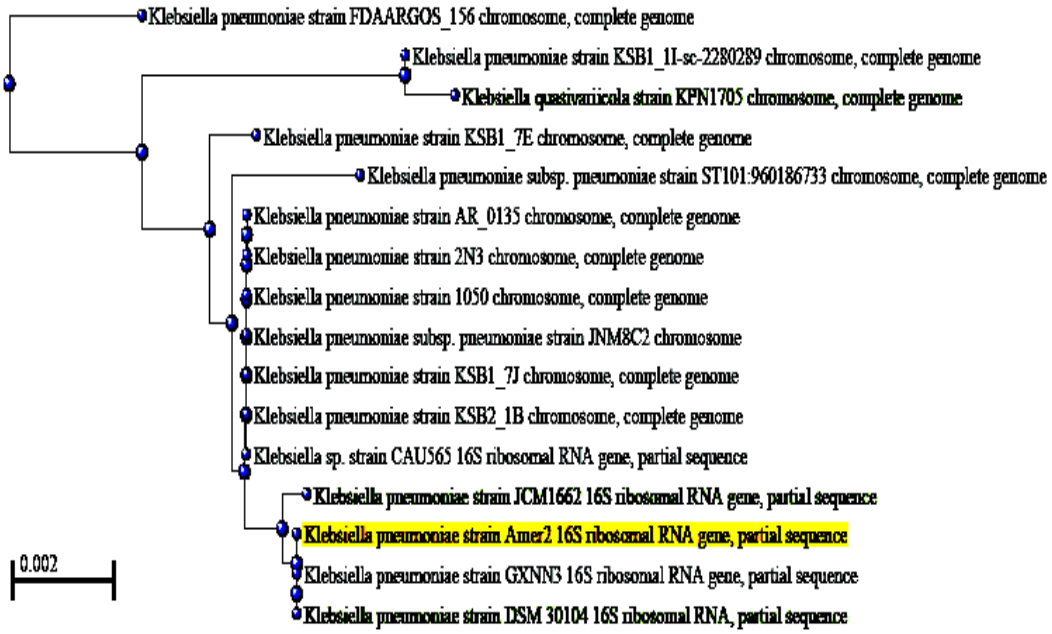

Figure (2): Nucleotide sequence of the 16S rRNA gene of *K. pneumoniae* isolate Amer2 (Upper). Phylogenetic analysis of *K. pneumoniae* strain Amer2 based on its partial sequencing of 16S rRNA (Lower).

GGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCG
GATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGC
GCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGG
TGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCCCGTGAGTGATGAAGGTCTTCGGATCGTAAAA
CTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGC
CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGG
GCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG
TCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGC
GCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCG
AAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGT
GTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTC
GAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCC
TTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGC
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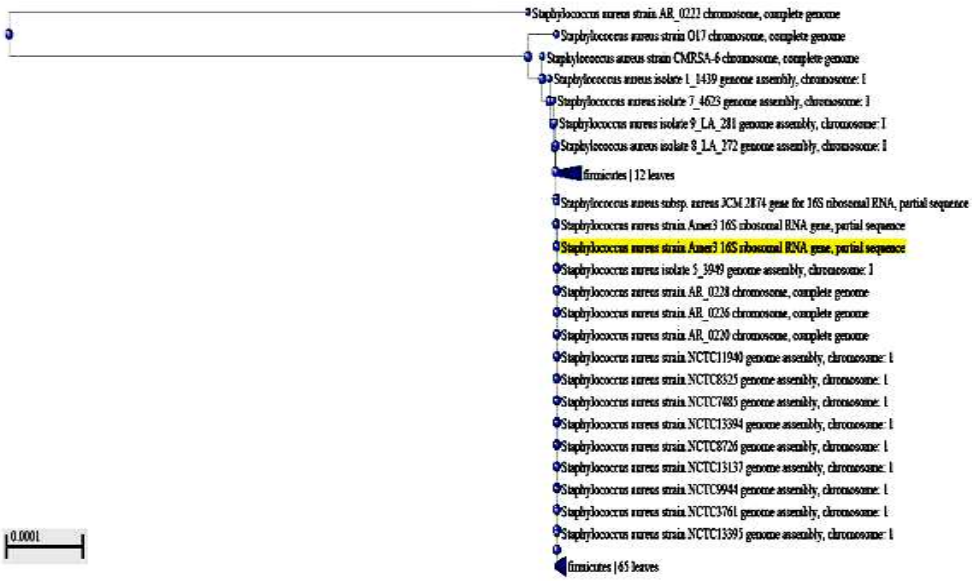

Figure (3): Nucleotide sequence of the 16S rRNA gene of *S. aureus* isolate Amer3 (Upper). Phylogenetic analysis of *S. aureus* strain Amer3 based on partial sequencing of 16S rRNA (Lower).

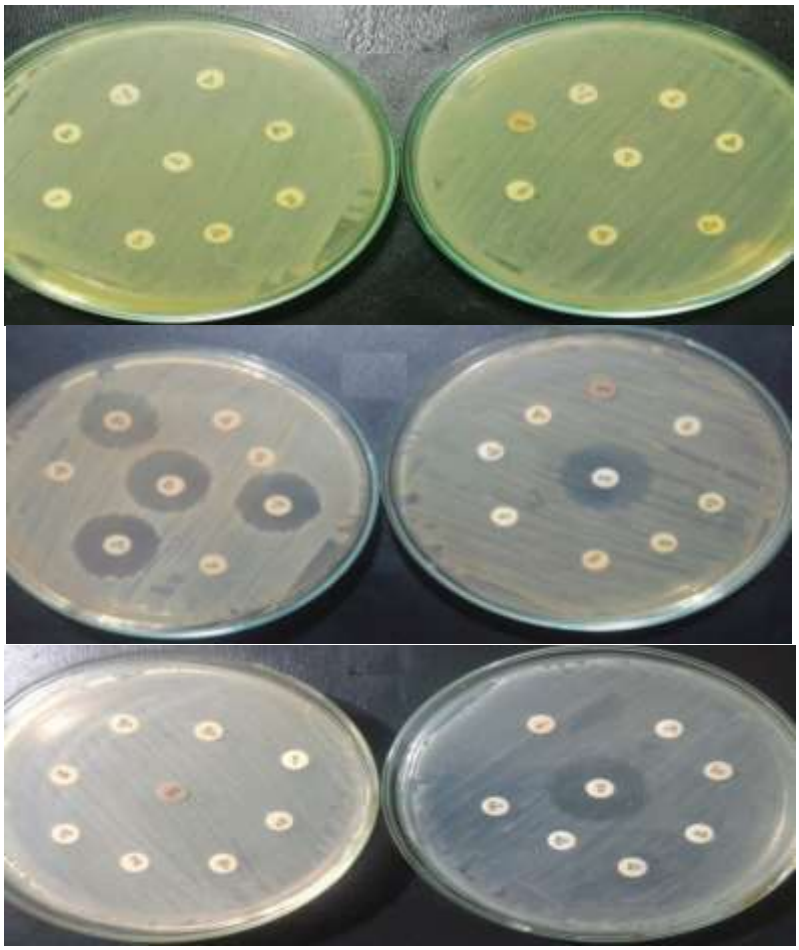

Figure (4): Sensitivity and resistance of *E. coli* (Upper), *K. pneumoniae* (Middle) and *S. aureus* (Lower) for different antibiotics in urine culture.

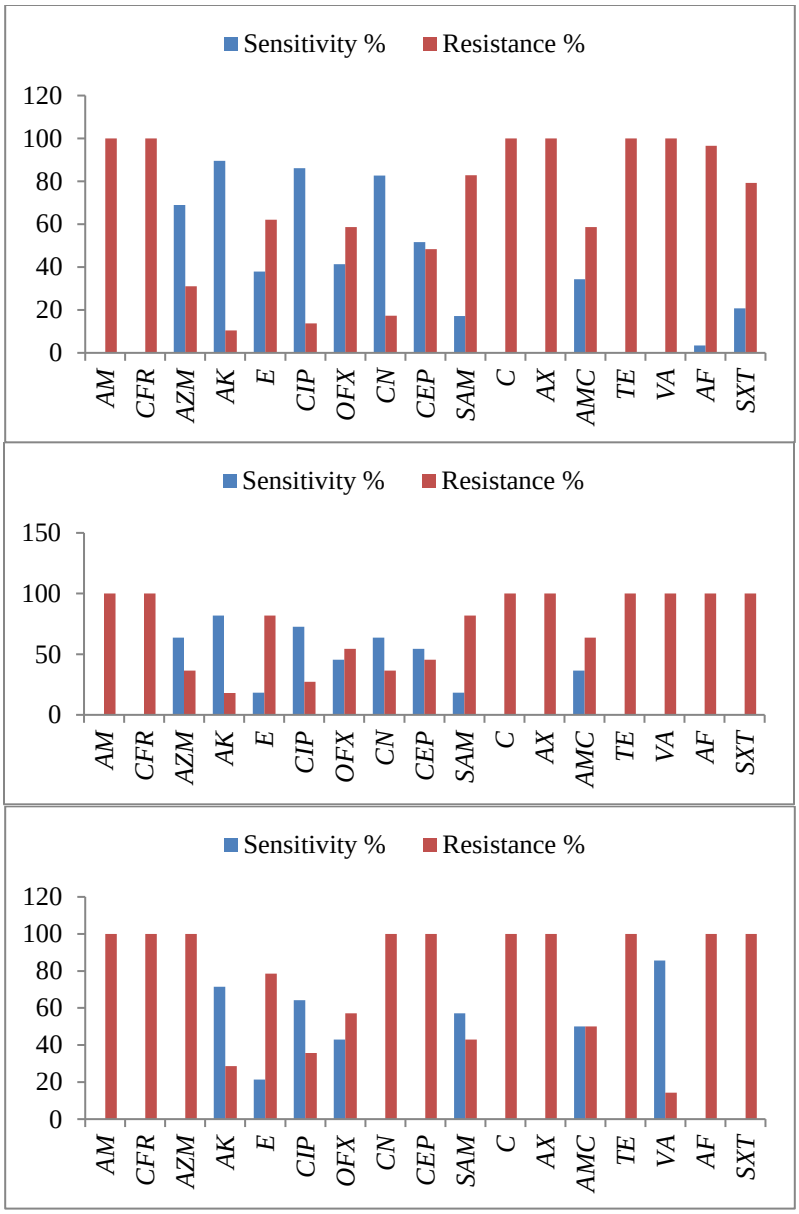

Figure (5): AST against isolated *E. coli* (Upper), *K. pneumoniae* (Middle) and *S. aureus* (Lower) in urine culture.

AST for pathogenic bacteria isolated from stool samples

Thirty four isolates were obtained from stool culture. Results confirmed that Gram negative bacteria had a higher frequency of occurrence than Gram positive constituting 25 of the total isolates as 73.5%. These included; 19 isolates of *E. coli* (55.9%) and 6 of *K. pneumoniae* (17.6%). Gram positive bacteria accounted for 9 of the isolates as 26.5%. They included; *S. aureus*. It was also found that the rate of isolates of *E. coli* was higher than isolated other bacteria.

Clearly, the isolates of *Escherichia coli* represented 55.9% (19 isolates) from total isolates. The isolated *E. coli* strains were resistance to the various antibiotics, are shown in Figure 6. They exhibited 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Unasyn, Amoxicillin, Tetracycline, and Vancomycin, followed by Flumox (94.7%) and Augmentin (84.2%), while they showed low resistance to Amikacin (10.5%) and Ciprofloxacin (21.1%). However, data are illustrated in Figure 7.

Also, the isolates of *K. pneumonia*

represented 17.6% from total isolates. The isolated *K. pneumonia* strains were resistance to the various antibiotics (Figure 6). They recorded 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Flumox, and Vancomycin, followed by Erythromycin (83.3%), Tarivid (83.3%) and Tetracycline (83.3%). Obviously, they showed low resistance to Amikacin (16.7%) and Ciprofloxacin (33.3%) (Figure 7).

In addition, the isolates of *S. aureus* represented 26.5% from total isolates. The isolated *S. aureus* strains were resistance to the various antibiotics (Figure 6). Indeed, they showed 100% resistance towards Duricef, Zithromax, Ampicilin, Chloramphenicol, Cefobid, Gentamicin, Amoxicillin, Flumox, and Septrin, followed by Tetracycline 88.9%. As well as, they recorded low resistance to Vancomycin (11.1%) and Amikacin (22.2%) (Figure 7).

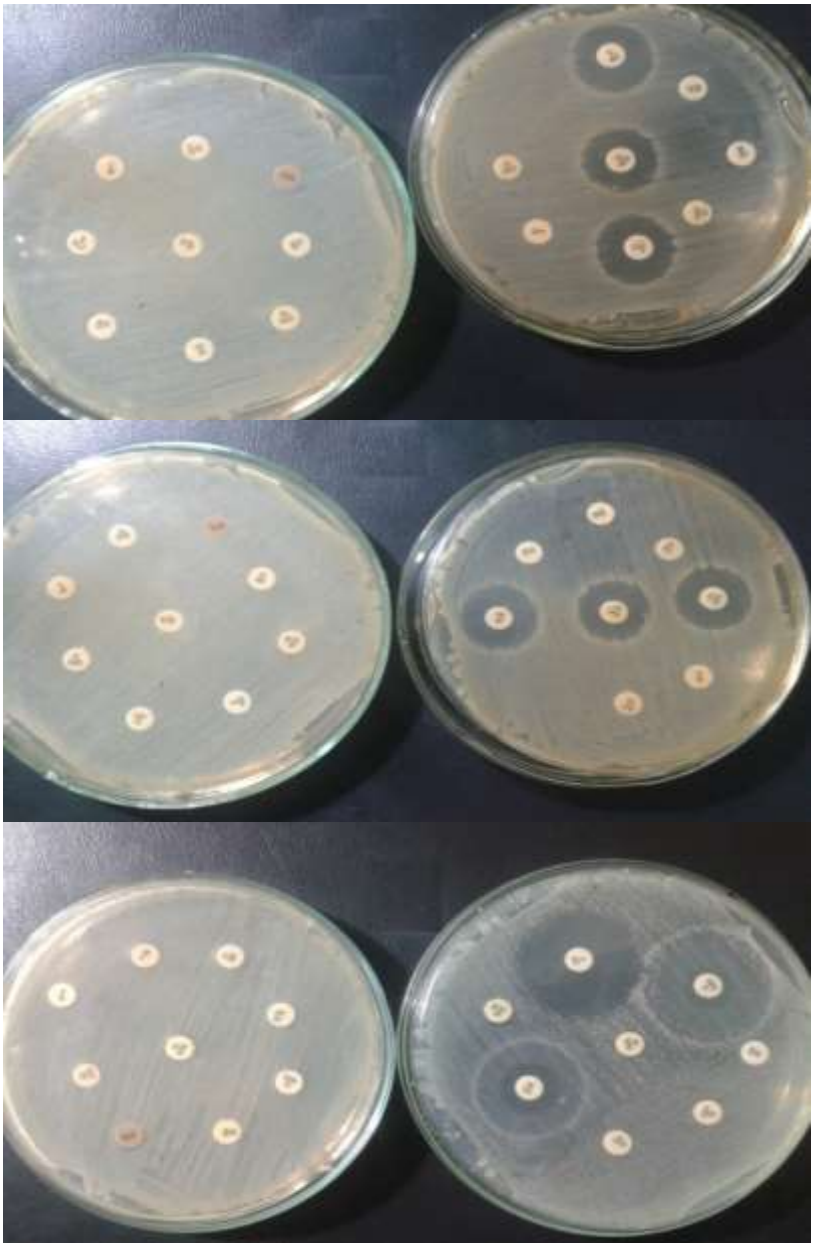

Figure (6): Sensitivity and resistance of *E. coli* (Upper), *K. pneumoniae* (Middle) and *S. aureus* (Lower) for different antibiotics in stool culture.

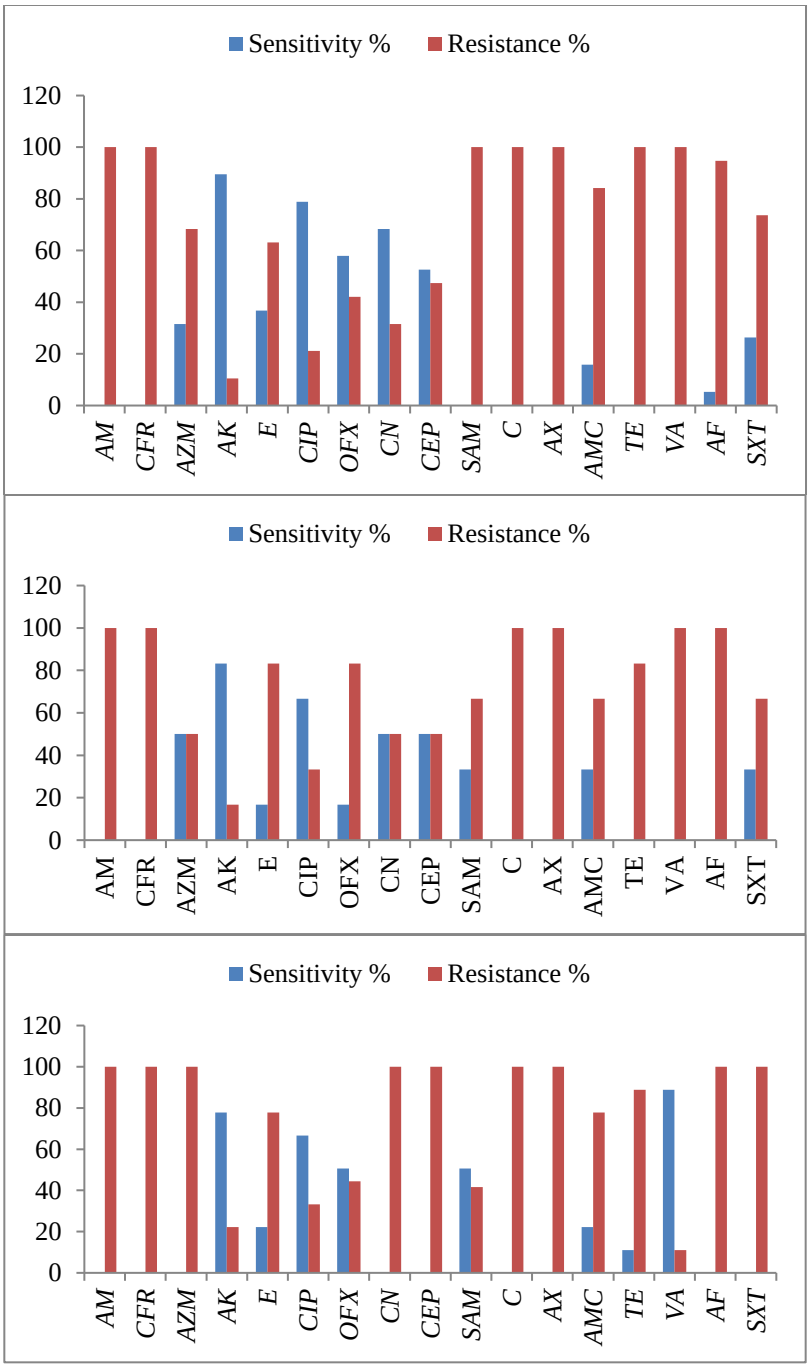

Figure (7): AST against isolated *E. coli* (Upper), *K. pneumoniae* (Middle) and *S. aureus* (Lower) from stool culture.

DISCUSSION

Examples of pathogenic bacteria that cause infections include *Staphylococcus sp*. and *E. coli*. The huge usage of antibiotics increase the chances of bacteria in human body to be more resistant them causing antibiotic resistance (Diaz et al. 2008). The treatment of methicillin resistant *S. aureus*, penicillin-resistant *Streptococcus pneumoniae,* and vancomycin-resistant *enterococci*, is considered a great problem (Lieberman, 2003).

Our data presented the prevalence of urinary tract and feces infection in relation to age and sex of patients. This result indicated that a high percentage of microorganisms were isolated from

both males and females within the age brackets of 11-20 years and 31-40. Comparatively, however, there were more cases in females than males.

The most common organisms isolated from patients during the current study were *E. coli*, *K. pneumoniae* and then *S. aureus*. This finding is similar to other reports, which indicated that a Gram negative bacterium, particularly *E. coli* (54.6%) was found to be the most frequent bacterial isolate in this study, which is in line with other reports from different areas (Njoku et al. 2001; Mbata, 2007). However, it was lower than reported in the previous studies conducted in different countries, which was 64.4% in Libya (Tamalli et al. 2013), 55% in Afikpo, Ebonyi State (Onuoha and Fatokun, 2014), and 57.1% in Italy (de Francesco et al. 2017). *Escherichia coli* is the most common microorganism in the vaginal and rectal area.

Also, Nicolle (2008) reported that *E. coli* is the cause of 80-85% of bacterial infections, with *S. aureus* being the cause of 5-10% and *Klebsiella sp.* being the cause of 8-9% of the infections. Moreover, Derese et al. (2016) reported that 14% Gram-negative bacteria were more prevalent (73%), *E. coli* (34.6%), coagulase-negative staphylococci (19.2%), *P. aeruginosa* (15.4%), and *Klebsiella* spp. (11.5%) were common bacterial isolates.

During the present study, the isolates of AM1, AM2, and AM3 were full characterized and successfully affiliated according to 100% sequence homology to the species *E. coli* strain Amer1, *K. pneumoniae* strain Amer2 and *S. aureus* strain Amer3. All of them have been associated with different types of infections in hospitalized patients (Murray et al. 2005).

Upon the level of antibiotic resistance, our data confirmed that the isolated pathogens were resistance to the various antibiotics. Some of them recorded high resistance towards Duricef, Zithromax, Chloramphenicol, Amoxicillin, Unasyn, Flumox, Septrin, and Tetracycline, while they showed low resistance to Amikacin, Vancomycin, and Ciprofloxacin.

Actually, medical research has confirmed that these low-resistance antibiotics have harmful medical effects. Generally use of Macrolides, Quinolones, Tetracyclines, Sulfonamides, and Metronidazole during early pregnancy was associated with an increased risk of spontaneous abortion and causing fetal abnormalities (Muanda et al. 2017). It also has a detrimental effect on kidney patients (Gregory et al. 2018). So it is advised not to be used it in such cases.

The AST against *E. coli* isolated from urine samples showed 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Tetracycline, and Vancomycin, followed by Flumox (96.6%) and Unasyn (82.8%), while they showed low resistance to Amikacin 10.4% and Ciprofloxacin 13.8. Ampicillin remains the most useful antimicrobial agents was observed by (Ronald, 1987) and the findings by Ebie et al. (2001), while AST against *E. coli* isolated from stool samples showed 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Unasyn, Amoxicillin, Tetracycline, and Vancomycin, followed by Flumox (94.7%) and Augmentin (84.2%), while they exhibited low resistance to Amikacin (10.5%) and Ciprofloxacin (21.1%). *E. coli* displayed high percentage of resistance to test antibiotics and thus multiple drug resistance was observed in the strains (Hussain et al. 1982).

Indeed, high resistance of *E. coli* to antibacterial agents tested was observed in this study, which is similar to what was observed by Desenclos et al. (1988), who reported very high resistance of *E. coli* isolates to Ampicillin. Also, they found that Ciprofloxacin resistance in Portugal was 25.8 and 24.3% in Italy, respectively, while in Germany and Netherlands it was 15.2% and 6.8%, respectively. The percentage of Ciprofloxacin resistance to *E. coli* isolated from urine and stool samples observed, in this investigation, were 13.8 and 21.1%, respectively, which is on the high side. In previous years, *E. coli* was 100% susceptible to the Fluoroquinolones. Egri-Okwaji et al. (1996) reported 100% susceptibility of *E. coli* isolates to Ofloxacin. High resistance of *E. coli* to ciprofloxacin has also been documented by (Oteo et al. 2005); they observed that 24% of 189 *E. coli* isolates were resistant to ciprofloxacin.

In the present study, *K. pneumoniae* has shown high antibiotic resistance, which is in similarity with the study reported by (Tonkic and Goic, 2005). As well as, AST against *K. pneumoniae* isolated from urine samples showed 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Tetracycline, Flumox, Septrin and Vancomycin, followed by Erythromycin (81.8%) and Unasyn (81.8%), while they showed low resistance to Amikacin (18.1%) and Ciprofloxacin 27.3%. In addition, AST against *K. pneumoniae* isolated from stool samples recorded 100% resistance towards Duricef, Ampicilin, Chloramphenicol, Amoxicillin, Flumox, and Vancomycin, followed by Erythromycin (83.3%), Tarivid (83.3%) and Tetracycline

(83.3%), while they exhibited low resistance to Amikacin (16.7%) and Ciprofloxacin (33.3%). The isolate of *K. pneumoniae* was resistance to Ampicillin as comparable with other studies (Aktas et al. 2002). The resistance rate in *K. pneumoniae* isolates were 27.3% to Ciprofloxacin in urine and 33.3% in stool, which is lower than other studies conducted in India (Revathi, 1998) and higher than reported in USA (Fedler and Biedenbach, 2006).

The AST against *S. aureus* isolated from urine samples showed 100% resistance towards Duricef, Zithromax, Ampicilin, Chloramphenicol, Gentamicin, Amoxicillin, Tetracycline, Flumox, Septrin, and Cefobid, followed by Erythromycin (78.5%), while they exhibited low resistance to Vancomycin (14.3%) and Amikacin (28.6%). While, AST against *S. aureus* isolated from stool samples recorded 100% resistance towards Duricef, Zithromax, Ampicilin, Chloramphenicol, Cefobid, Gentamicin, Amoxicillin, Flumox, and Septrin, followed by Tetracycline 88.9%, while they were low resistant to Vancomycin (11.1%), and Amikacin (22.2%).This observed resistance to the abovementioned common antibiotics might be due to the earlier exposure of the isolates to these drugs. Over the last decade there has been a substantial increase in resistance of uropathogens to antibiotics. Resistance rates among *S. aureus* strains are increasing, and a major part of this species has become resistant to β-lactamase resistant penicillins (Bogaard et al. 2001; Karbasizaed et al. 2003). For such resistant species, Vancomycin is the effective choice of drug. Resistance to Vancomycin is reported among enterococci (Williams, 2001; Alebouyeh et al. 2005), but this resistance has also begun to develop among staphylococci (Murray, 1990).

CONCLUSION

The present study was undertaken with the major goal isolating and identifying the most common antibiotic-resistance bacteria from human urine and feces specimens.

There were 88 bacterial isolates were examined. Out of them, 65 Gram negative and 23 Gram positive bacteria were detected.

The 16S rRNA-based PCR assays provide rapid, simple and reliable identification of *Escherichia coli*, *Klebsiella pneumonia,* and *Staphylococcus aureus*, and its differentiation from other phylogenetically closely related species.

Antibacterial susceptibility testing (AST) was conducted that the isolated pathogens were resistance to the various antibiotics. Some of them showed high resistance towards Duricef, Zithromax, Chloramphenicol, Amoxicillin, Unasyn, Flumox, Septrin, and Tetracycline, while they showed low resistance to Amikacin, Vancomycin, and Ciprofloxacin.

Actually, the medical research has shown that these low-resistance antibiotics have harmful medical effects. Generally use of Macrolides, Quinolones, Tetracyclines, Sulfonamides, and Metronidazole during early pregnancy was associated with an increased risk of spontaneous abortion and causing fetal abnormalities. It also has a detrimental effect on kidney patients so it is advised not to be used it in such cases.

Continuous monitoring of the antibiotic-resistance bacteria encourages the scientists to discover much more new antibiotics and antimicrobial agents. In our next investigations we will introduce solution in such manner.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHORS CONTRIBUTIONS

MTS supervised the microbiology experiments. AAMH executed all of the experiments. HAHI supervised the molecular biology part and wrote the original manuscript. All authors read, revised, and approved the final version of the article.

REFERENCES

Aktas E, Yigit N 2002. Detection of antimicrobial resistance and extended-spectrum beta-lactamase production in *Klebsiella* pneumonia strains from infected neonates. J Int Med Res 30(4): 445-448.

Alebouyeh M, Amirmozafari N, Forohesh H 2005. Evaluation of virulence factors and plasmid-related transmissibility among different isolates of enterococci. Iran Biomed 9(2): 51-

55.

Baur AW, Kirby WM, Sherris JC, Jurck M. 1996. Antibiotics susceptibility testing by a standard single disc method. Am J Clin Pathol 451: 493-496.

Bogaard AE, London N, Driessen C, Stobberingh EE 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 47: 763-771.

Cheesbrough M 1985. Medical Laboratory Manual for Tropical Countries. Vol. 2. Bullerworth and Co. London.

Clinical and Laboratory Standards Institute (CLSI) 2009. Performance standards for antimicrobial susceptibility testing. 19th Informational Supplement. CLSI Document M100eS19. Wayne, PA: CLSI; 2009.

Clinical and Laboratory Standards Institute (CLSI) 2011. Performance standards for antimicrobial susceptibility testing: 21st informational supplement M100–S21. Wayne (PA): CLSI; 2011.

Clinical and Laboratory Standards Institute (CLSI) 2015. Performance standards for antimicrobial susceptibility testing. 25th Informational supplements. CLSI document M 100- S 25, Wayne, P. A; Clinical laboratory standard institute; 35(3): 46-105.

Cosgrove SE 2006. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis 42(Suppl 2): S82- S89.

de Francesco MA, Guiseppe R, Laura P, Ricardo N, Nino M 2007. Urinary tract infection in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. Medi Scie Monit 13: 136-144.

Derese B, Kedir H, Teklemariam Z, Weldegebreal F, Balakrishnan S 2016. Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at antenatal clinic in DilChora Referral Hospital, Dire Dawa, eastern Ethiopia. Therapeutics and Clinical Risk Management 12: 251.

Desenclos JC, Zergabachew A, Desmoulins B, Chouteau L, Desve G, Admassu M 1988. Clinical, microbiological and antibiotic susceptibility patterns of diarrhoea in Korem, Ethiopia. J Trop Med Hyg 91(6): 296-301.

Diaz L, Granados CA, Cardo DM, McGowan Jr JE 2008. Antimicrobial resistance: international control strategies, with a focus on limited-resource settings. Int J Antimicrob Agents 32(1): 1-9.

Ebie M, Kandaki-Olukemi YT, Ayanbadejo J, Tanyigna KB 2001. UTI infections in a Nigerian Military Hospital. Nigerian J Microbiol 15(1): 31-37.

Egri-Okwaji MTC, Iroha EO, Kesah CN, Odugbemi T. 1996. Bacterial Pathogens causing neonatal sepsis in an out-born neonate unit in Lagos, Nigera. J Hosp Med 6: 149-152.

Fedler KA, Biedenbach DJ 2006. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. Diag Microbiol Infect Dis 56(4): 427-436.

Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C, Hacker J 2001. Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. FEMS Microbiol Ecol 35(3): 305-312.

Hicks MJ, Ryan, KJ 1976. Simplified scheme for identification of prompt lactose-fermenting members of the Enterobacteriaceae. J Clin Microbiol 4(6): 511-514.

Hussain MM, Glass RJ, Khan MR 1982. Antibiotic used in a rural community in Bangladesh. Int J Epidemiol 11: 402-405.

Kandemir Ö, Akbay E, Horasan ES, Gen R, 2007. Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. The J Inf 54(5): 439-445.

Karbasizaed V, Badami, N, Emtiazi G. 2003. Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. Afr J Biotechnol 2: 379-383.

Lieberman JM (2003). Appropriate antibiotic use and why it is important: the challenges of bacterial resistance. The Ped Infect Dis J 22(12): 1143-1151.

Mbata TI (2007). Prevalence and antibiogram of UTIs among prisons inmates in Nigeria. Int J Microbiol 1(5): 163-167.

Morens DM, Folkers GK, Fauci AS (2004). The challenge of emerging and re-emerging infectious diseases. Nat 8; 430(6996): 242-249.

Muanda FT, Sheehy O, Berard A 2017. Use of antibiotics during pregnancy and risk of spontaneous abortion. Cite as: *CMAJ* 2017 May 1; 189: E625-633.

Murray BG (1990). The life and times of the Enterococcus. Clin Microbiol Rev 3: 46-65.

Murray P, Traynor P, Hopson D 1992. Evaluation of microbiological processing of urine specimens: comparison of overnight versus two-day incubation. J Clin Microbiol 30: 1600-1601.

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken, RH 2003. Manual of clinical microbiology. 8th Ed. Washington, DC: American Society for Microbiology.

Murray PR, Holmes B, Aucken HM 2005. Microbiology and Microbial Infections. Topley & Wilsons (eds.), Volume 2, 10th edition. Salisbury, UK: Edward Arnold Ltd.

Nicolle LE 2008. Uncomplicated urinary tract infections in adults including uncomplicated pyelonephritis. Urol Clin North Amer 35 (1): 1-12.

Njoku CO, Ezissi NH, Amadi AN 2001. Observations on bacterial infections of urinary tract patients. Int J Environl Health and Human Develop 2: 57-61.

Onuoha SC, Fatokun K 2014. Prevalence and antimicrobial susceptibility pattern of urinary tract infection (uti) among pregnant women in Afikpo, Ebonyi State, Nigeria, Am J Life Sci 2(2): 46-52.

Oteo J, Lázaro E, de Abajo FJ, Baquero F, Campos J 2005. Spanish members of EARSS.Antimicrobial-resistant invasive *Escherichia coli*, Spain. Emer Infect Dis 11(4): 546-53.

Pribis JP 2019. An antibiotic-induced evolvable cell subpopulation differentiated by reactive-oxygen-induced general stress response. Molecular Cell 74: 1-16.

Rasha AE 2011. Different methods for detection of antibiotic sensitivity in bacterial urinary tract infection. Benha Faculty of Medicine Library 75-77.

Revathi G, Shannon KP, Stapleton PD Jain BK, French GL 1998. An outbreak of extended-spectrum, beta-lactamase-producing *Salmonella senftenberg* in a burns ward. J Hosp Infect 40(4): 295-302.

Simmon's, JS 1926. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J of Infect Dis 39:209.

Sipahi OR 2008. Economics of antibiotic resistance. Expert Rev Antibio and Infect Therapy 6(4): 523-539.

Tamalli M, Bioprabhu S, Alghazal, MA 2013. Urinary tract infection during pregnancy at Al-khoms, Libya. Int J of Med Sci 3(5): 455-459.

Tonkic M, Goic-Barisic I 2005. Prevalence and antimicrobial resistance of extended-spectrum beta-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, Croatia. Int Microbiol 8(2): 119–24.

Williams, JD (2001). Antibiotic resistance: who needs control over antibiotic use-community doctors, farmers or hospital practitioners? Newsletter of the International Society of Chemotherapy; 5:4.