Antimicrobial Resistance Pattern of Uropathogens Isolated from Rafha Central Hospital, Rafha, Kingdom of Saudi Arabia

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Antimicrobial resistance (AMR) is the capability of a microorganism to neutralize the harmful effects of a drug, which is used to stop their growth or kill them. AMR is considered as one of the utmost universal threats to human health. Microorganisms that are resistant to one or more drugs are harder to treat, necessitating the use of alternative drug or higher doses of the same drug, which can be expensive or even more toxic.

Urinary tract infections (UTI) are the most common and serious health problem among, both outpatients and hospitalized patients affecting millions of individuals worldwide. Because the urinary tract is in direct contact with the outer environment, it is more likely to get infected. About 150 million cases of UTI are estimated every year worldwide. The disease affects all age groups with manifestations varying from symptomatic cystitis to pyelonephritis and sepsis. Improperly treated UTI can result in substantial morbidity and mortality.

Women usually have more incidences than men due to the anatomical organization of their genitourinary tract. It has been estimated that at least 30% of all women get a UTI at some point during their lifetime. The other UTI causing factors consist of long time catheterization, poor hygiene, sexual intercourse during menstruation period and pregnancy.

The most common pathogen causing UTI is Escherichia coli followed by Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa. During laboratory investigation, a bacterial infection of the urinary tract is most often Escherichia coli. Resistance against 27 commonly used antibiotics was recorded. Among β-Lactam antibiotics, increasing resistance was observed except for Augmentin. The imipenem was relatively more effective. Among non-β-Lactam group, least resistance was seen against Vancomycin and Amikacin. Overall increase in antibiotic resistance was observed in the current study with some exceptions. It is therefore recommended that the routine urine cultures must be done and the resistance pattern in the region must regularly be monitored.

Keywords: Antimicrobial resistance (AMR), uropathogens, pattern, urinary tract infection (UTI), Escherichia coli, prevalence, susceptible.
The UTIs treatment depends on the age and sex of the patient, and the causative agent. It also depends on the site of infection i.e. lower or upper urinary tract infection. Cotrimoxazole (Trimethoprim/sulphamethoxazole) and ciprofloxacin are the most commonly used drugs for the treatment of UTI. The other commonly used drugs include fluoroquinolones, β-lactams (occasionally with β-lactamase inhibitors), cephalosporin and nitrofurantoin. Recent studies show that resistance to many antimicrobials including the fluoroquinolones is increasing. The increase in bacterial resistance to fluoroquinolone is multifactorial. With the increasing trend of antibiotic-resistance in E. coli, the management of urinary tract infections is likely to become complicated with limited therapeutic options.

This retrospective study was done to assess the current antibiotic resistance pattern among the UTI pathogens against commonly prescribed antibiotics in patients visiting Rafha central Hospital, Rafha city, Saudi Arabia.

**MATERIALS AND METHODS**

The study was done retrospectively on the antibiotic resistance pattern of uropathogens for 2 year (January 2016 to December 2017). The required culture and sensitivity data was collected from the records of Microbiology laboratory of Rafha Central Hospital, Rafha, Saudi Arabia. Approval from the Institutional Ethics Committee was obtained prior to the study.

A total of 2204 urine samples during the two years were brought to the lab for culture sensitivity testing. The samples were collected, cultured and antibiotic susceptibility was determined according to the Standard Clinical Laboratory procedures of the Lab. Briefly, urine samples were collected in specified sterile containers and brought to lab. Each sample was cultured on Blood Agar medium and MacConkey Agar medium using the calibrated loop technique. The plates were incubated overnight at 37°C. Bacterial growth > 10^5 cfu/ml was considered as significant. For identification of the bacterial isolates, conventional methods were used. The antimicrobial sensitivity testing was done using the standard Kirby-Bauer disc diffusion technique on Mueller Hinton agar medium according to the CLSI guidelines. The antibiotics discs used for the AST were from MASTRING-S™, Mast Diagnostics, UK and included: Penicillin G (10 units), Ampicillin (10 µg), Augmentin (30 µg), Oxacillin (1 µg), Piperacillin (100 µg), Cephalothin (30 µg), Cefoxitin (30 µg), Cefuroxime (30 µg), Ceftazidine (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Aztreonam (30 µg), Imipenem (10 µg), Tetracycline (30 µg), Amikacin (30 µg), Gentamicin (10 µg), Neomycin (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Nalidixic acid (30 µg), Cip/Norflaxacin (10 µg), Nitrofurantoin (300 µg), Cotrimoxazole (50 µg), Vancomycin (30 µg), Polymyxin B (300 units), Fusidic acid (10 µg)

The results were calculated as frequency and percentage. Chi square test was used to find any significant correlation between different factors. The one tailed p values were calculated online at http://vassarstats.net/tab2x2.html

**PCR**

PCR was done to identify microbial strains and detect few resistant genes in some of the clinical isolates in order to compare our molecular methods with the standard microbial methods performed in hospital laboratory. For that purpose, ten samples of each of E. coli, Klebsiella, Pseudomonas and Coagulase negative Staphylococcus species were analyzed.

**DNA Extraction**

A single medium sized colony was suspended in 50 microliters of low TE buffer in a 200 microliter PCR tube and boiled at 95 °C for 5 minutes. It was then cooled and centrifuged at 5000 rpm for 1 minute and the supernatant containing DNA was transferred to a new tube. One microliter of this DNA was used in 20 microliter PCR mixture. The PCR mixture contained 1X PCR buffer (with KCl), 1.5mM MgCl₂, 0.2mM dNTPs, 0.5mM each primer (Table 1), 0.5 units of Taq polymerase (Thermo Scientific UK). Cycling conditions for PCR were: Initial denaturation at 95°C for 5 minutes and then 35 cycles of 95°C for 15 seconds, annealing (Table 1) for 15 seconds and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Five microliters
of PCR product were loaded on a 2% agarose gel in 1X TBE buffer to confirm the presence of PCR product.

**RESULTS**

During the two years study period, 2204 urine samples (1,028 in 2016 and 1,176 in 2017) were brought and processed in the lab. Out of which, 623 (28.27%) samples showed significant growth of pathogen. Remaining 1,581 samples were either sterile or had a very low bacterial count.

E. coli was the most prevalent UTI pathogen (43.3%) isolated during the study period followed by Klebsiella (15.9%) and Staphylococci (15.2%); Citrobacter was the least prevalent (1.1%). The prevalence of E. coli and Acinetobacter species isolated in 2016 were significantly higher than those of 2017 while on the other hand, the prevalence of Enterococci, Klebsiella and Pseudomonas species were higher during 2017 (Table 2).

In our lab E. coli, Klebsiella, Pseudomonas and Coagulase negative Staphylococcus species were detected by PCR amplification and on agarose gel electrophoresis along with five genes responsible for resistance to five common antibiotics (Fig. 1).

In the microbiology laboratory of the Rafha Central Hospital, a total of 27 antibiotics were used to study the antibiotic susceptibility patterns out of which 14 belonged to β-Lactam group (Table 3). The average resistance against penicillin group was 73.4% during 2016 and 78.4% during 2017. The resistance increased significantly against penicillin G \((p = 0.0128)\) and oxacillin \((p = 0.00507)\) while decreased against Augmentin \((p = 0.000011)\) during 2017. Among Cephalosporins, the resistance ranged from 35% against Cefoxitin to 76% against Cephalothin. A significant increase in resistance was seen against Cefoxitin \((p = 0.0036)\), cefuroxime \((p = 0.0411)\), Cefazidime \((p = 0.0221)\) and Cefotaxime \((p = 0.00001)\) during 2017. The monobactam Aztreonam also showed similar results \((p = 0.00034)\).

Among non-β-Lactam group, least resistance was seen against Amikacin (23.8%) and vancomycin (26.5%) during the two years whereas Fusidic acid and Erythromycin faced maximum resistance (92% each). Gentamicin showed decreased \((p = 0.026)\) while chloramphenicol and Ciprofloxacin /Norfloxacin showed increased resistance \((p = 0.0009\) and \(p = 0.006\) respectively) during 2017 (Table 4).

**The AMR pattern of tested antibiotics against various isolated strains E Coli**

A high percentage of isolated E. coli strains were resistance to penicillin group during the study period with average resistance of 78%. The resistance against Augmentin decreased significantly in 2017 \((p = 0.0011, Table 5)\). Similar results were also seen against Cephalothin among cephalosporin group \((p = <0.0001, Table 5)\).
5). Cefoxitin (second generation drug) faced the lowest resistance among cephalosporin during 2016 which increased slightly during 2017 but was statistically insignificant. The Cefepime (4th generation) showed relatively better results than most other Cephalosporins. The overall resistance against cephalosporin was >50%.

Imipenem was the drug of choice followed by Amikacin and Nitrofurantoin during the study period as they were effective against most of the isolated E.coli strains. Maximum (100%) resistance was seen against Tetracycline, erythromycin and clindamycin. Resistance against imipenem, Aztreonam and Cip/Norfloxacin increased significantly during the study period.

Klebsiella spp.

Penicillin group was poor against isolated klebsiella species facing 84% resistance overall with penicillin G being 100 ineffective. Cephalosporins were the same with more than

### Table 1. Oligonucleotide primers used.

| Primer Name | Primer Sequence | Target                  | Product Size | Annealing temp |
|-------------|-----------------|-------------------------|--------------|----------------|
| M12-F       | 5' - GTGATCTCCAGCTACCGCTA-3' | E. coli               | 200          | 55° C          |
| M12-R       | 5' - CGTTGCAAACCTGACGGTCTCTT-3' |                |              |                |
| PsA-F       | 5' - TTCGGTGAGAGTGGCCTAAGT-3' | Pseudomonas           | 297          | 57° C          |
| PsA-R       | 5' - AGGTAGCCGTGAACGCGCCCTT-3' | Aeruginosa            |              |                |
| KN-F        | 5' - GTCATGCTCTCGTGCTGTTT-3' | Klebsiella pneumoniae | 236          | 55° C          |
| KN-R        | 5' - GACACCGCGGTCATCATATT-3' |                      |              |                |
| CNS-F       | 5' - TATCCACGAAACTCTCTAAAA CAACGTATTAC-3' | Coagulase Negative Staphylococcus | 204          | 57° C          |
| CNS-R       | 5' - TCTTTTAGATAATACGTATACTT CAGCTTTGAATTT-3' |             |              |                |
| 16s-F       | 5' - CTAGTAATCGCGGATCAGCAT -3' | 16s RNA            | 174          | 54° C          |
| 16s-R       | 5' - GATAACGGCTACCTTTGACTTAC-3' |                     |              |                |
| SUL1-F      | 5' - TTCGGCATCTGAATCTCTCA-3' | Sulphonamide resistant gene | 822          | 54° C          |
| SUL1-R      | 5' - ATGATCTTACCCCTCGGCTCTC-3' |                      |              |                |
| ere(A)-F    | 5' - GCCGGTGCTCATGAACCGTGAG-3' | Erythromycin resistant gene | 419          | 57° C          |
| ere(A)-R    | 5' - CGACTCTATTTCACTACGAGGC-3' |                      |              |                |
| tetA-F      | 5' - GGTTCACTGCAACGCTGCA-3' | Tetracycline resistant gene | 577          | 55° C          |
| tetA-R      | 5' - CGTCCGAGACGTGTAAGGA-3' |                      |              |                |
| DfrA1-F     | 5' - GGAGTGCCAAAGGTGACGC-3' | Trimethoprim resistant gene | 367          | 57° C          |
| DfrA1-R     | 5' - GAGGGGAAGTGTGGTGAAAAC-3' |                      |              |                |
| CatA1-F     | 5' - AGTGGCTAATGTCATATTACACCTAACC-3' | Chloramphenicol resistant gene | 547          | 54° C          |
| CatA1-R     | 5' - TTGTAATCTATTAAACGATCCTGCTGC-3' |                      |              |                |

### Table 2. Frequency and percentage of different bacterial strains isolated during the two years

|                      | Total N (%) | 2016 N (%) | 2017 N (%) | p value |
|----------------------|-------------|------------|------------|---------|
| Acinetobacter        | 20 (3.2)    | 19 (7.1)   | 1 (0.5)    | <0.0000 |
| Citrobacter          | 7 (1.1)     | 5 (1.9)    | 2 (0.6)    | —       |
| E. coli              | 270 (43.3)  | 130 (48.5) | 140 (39.4) | 0.0146  |
| Enterobacter         | 10 (1.6)    | 3 (1.1)    | 7 (2)      | —       |
| Enterococci          | 55 (8.8)    | 14 (5.2)   | 41 (11.5)  | 0.0038  |
| Klebsiella           | 99 (15.9)   | 33 (12.3)  | 66 (18.6)  | 0.0213  |
| Proteus              | 25 (4)      | 9 (3.4)    | 16 (4.5)   | —       |
| Pseudomonas          | 42 (6.7)    | 10 (3.7)   | 32 (9)     | 0.0062  |
| Staphylococci        | 95 (15.2)   | 45 (16.8)  | 50 (14.1)  | —       |
| Total                | 623         | 268        | 355        | —       |
Table 3. AMR pattern against β-Lactam antibiotics

| Antibiotic class     | Penicillin group | 1st generation Cephalothin | 2nd generation Cefoxitin | 2nd generation Cefuroxime | Cephalosporins 3rd generation Cefazidime | Cephalosporins 3rd generation Ceftriaxone | Cephalosporins 3rd generation Cefotaxime | 4th generation Monobactam Carbapenem |
|----------------------|------------------|-----------------------------|--------------------------|---------------------------|-----------------------------------------|------------------------------------------|----------------------------------------|----------------------------------------|
| Agent                | Penicillin G     | Ampicillin                  | Oxacillin                | Piperacillin               |                                        |                                          |                                        |                                        |
| 2016                 |                  |                             |                          |                           |                                        |                                          |                                        |                                        |
| Resistant            | 17               | 179                         | 125                     | 30                        | 143                                    | 167                                      | 57                                     | 8                                      | 52                                     | 26                                     | 25                                     | 45                                     | 59                                     | 18                                     |
| Susceptible          | 5                | 49                          | 44                      | 14                        | 63                                     | 54                                       | 108                                    | 9                                      | 71                                     | 21                                     | 23                                     | 73                                     | 73                                     | 190                                    |
| Total                | 22               | 228                         | 169                     | 44                        | 206                                    | 221                                      | 165                                    | 17                                     | 123                                    | 47                                     | 48                                     | 118                                    | 132                                    | 208                                    |
| %age                 | 77%              | 79%                         | 74%                     | 68%                       | 69%                                     | 76%                                      | 35%                                     | 47%                                     | 42%                                     | 55%                                     | 52%                                     | 38%                                     | 45%                                     | 9%                                     |
| 2017                 |                  |                             |                          |                           |                                        |                                          |                                        |                                        |                                        |
| Resistant            | 174              | 257                         | 131                     | 55                        | 158                                    | 212                                      | 84                                     | 29                                     | 35                                     | 35                                     | 14                                     | 135                                    | 22                                     |                                        |
| Susceptible          | 10               | 52                          | 116                     | 6                         | 63                                     | 91                                       | 121                                    | 33                                     | 94                                     | 10                                     | 1                                      | 29                                     | 76                                     | 174                                    |
| Total                | 184              | 309                         | 247                     | 61                        | 303                                    | 332                                      | 206                                    | 39                                     | 36                                     | 43                                     | 43                                     | 111                                    | 196                                    |                                        |
| %age                 | 95%              | 83%                         | 53%                     | 90%                       | 71%                                     | 70%                                      | 49%                                     | 72%                                     | 54%                                     | 74%                                     | 97%                                     | 33%                                     | 64%                                     | 11%                                    |
| p value              | 0.0128           | 0.105                       | 0.000011                | 0.00507                   | 0.35739                                 | 0.0937                                   | 0.0036                                 | 0.0411                                 | 0.0221                                 | 0.0535                                 | 0.00001                                | 0.3233                                 | 0.00034                               | 0.2425                                |

Table 4. AMR pattern against non-β-Lactam antibiotics

| Antibiotic class     | Tetracycline | Protein synthesis inhibitors | Aminglycosides | Macrolides | Quinolone | Glycopeptide | Polypeptide | Steroids |
|----------------------|-------------|------------------------------|----------------|------------|-----------|--------------|-------------|----------|
| Agent                | TC          | Amikacin                     | Gentamicin     | Neomycin   | Erythromycin | Chloramphenicol | Nalidixic acid | Cip/Norfloxacin | Nitrofurantoin | Cotrimoxazole | Vancomycin | Polymyxin B | Fusidic Acid |
| 2016                 |             |                              |                |            |            |               |             |          |            |             |            |            |                |            |
| Resistant            | 44          | 50                            | 92             | 8          | 24         | 15           | 171         | 123       | 81         | 173         | 9          | 6          | 26             |
| Susceptible          | 12          | 158                           | 119            | 1          | 2          | 22           | 51          | 116       | 133        | 59          | 38         | 3          | 3               |
| Total                | 56          | 208                           | 211            | 9          | 26         | 37           | 222         | 239       | 214        | 232         | 47         | 9          | 29              |
| %age                 | 79%         | 24%                           | 44%            | 89%        | 92%        | 41%          | 77%         | 51%       | 39%        | 75%         | 19%        | 67%        | 90%             |
| 2017                 |             |                              |                |            |            |               |             |           |            |             |            |            |                |            |
| Resistant            | 18          | 44                            | 88             | 29         | 81         | 71           | 219         | 174       | 113        | 235         | 18         | 26         | 21              |
| Susceptible          | 7           | 143                           | 168            | 14         | 7          | 28           | 52          | 103       | 155        | 79          | 37         | 15         | 1               |
| Total                | 25          | 187                           | 256            | 43         | 88         | 99           | 271         | 277       | 268        | 314         | 55         | 41         | 22              |
| %age                 | 72%         | 24%                           | 34%            | 67%        | 92%        | 72%          | 81%         | 63%       | 42%        | 75%         | 33%        | 63%        | 95%             |
| p value              | 0.3531      | 0.5003                        | 0.0260         | 0.1912     | 0.6643     | 0.0009       | 0.1794      | 0.0060    | 0.1933     | 0.5099      | 0.0920     | 0.5874     | 0.4167          |
### Table 5. The AMR pattern among different isolated bacteria:

|                      | E. coli | Klebsiella sp. | Enterococci | Sphingobacterium | Pseudomonas |
|----------------------|---------|----------------|-------------|------------------|------------|
|                      | 2016 Res/Sus (%) | 2017 Res/Sus (%) | 2016 Res/Sus (%) | 2017 Res/Sus (%) | 2016 Res/Sus (%) | 2017 Res/Sus (%) | 2016 Res/Sus (%) | 2017 Res/Sus (%) | 2016 Res/Sus (%) | 2017 Res/Sus (%) |
| Penicillin G         | 53/0 (100) | 53/0 (100) | 29/0 (100) | 29/0 (100) | 62/7 (92) | 62/7 (92) | 21/3 (88) | 21/3 (88) | 21/0 (100) | 21/0 (100) |
| Ampicillin           | 88/15/85 | 104/18/85 | 2/2/2 (92) | 2/2/2 (92) | 18/18 (100) | 18/18 (100) | 16/15/82 | 16/15/82 | 23/1/7 (30) | 23/1/7 (30) |
| Augmentin            | 68/21/76 | 47/41/53 | 0/0/0 (0)  | 0/0/0 (0)  | 4/4 (33)  | 4/4 (33)  | 6/22/21 | 6/22/21 | 3/1 (1)  | 3/1 (1)  |
| Oxacillin            | 139/22/80 | 72/25/74 | 19/11/63 | 19/11/63 | 3/2/1 (100) | 3/2/1 (100) | 6/55 | 6/55 | 3/7 (30) | 3/7 (30) |
| Piperacillin         | 94/9/91 | 82/36/69 | 18/6/75 | 18/6/75 | 3/2/1 (100) | 3/2/1 (100) | 4/2/1 (80) | 4/2/1 (80) | 0.0186 | 0.0186 |
| Cefotaxin            | 14/78/15 | 19/60/24 | 6/10/38 | 6/10/38 | 2/1/6 (100) | 2/1/6 (100) | 6/11/19 | 6/11/19 | 23/1/7 (30) | 23/1/7 (30) |
| Ceftriaxime (CXM)    | ——- | ——- | ——- | ——- | ——- | ——- | ——- | ——- | ——- | ——- |
| Cefazidime (CZ)      | 27/48/36 | 26/54/33 | 5/11/3 | 5/11/3 | 1/1/50 | 1/1/50 | 14/70 | 14/70 | 0.0499 | 0.0499 |
| Cefixime (CRO)       | ——- | 6/8 (43) | ——- | ——- | 6/1/66 | 6/1/66 | 7/1/35 | 7/1/35 | 10/0 (100) | 10/0 (100) |
| Cefotaxime (CTX)     | ——- | 6/0 (100) | ——- | ——- | 4/2/67 | 4/2/67 | 5/1/83 | 5/1/83 | 8/0 (100) | 8/0 (100) |
| Cefepime (CPM)       | 7/28/20 | 4/11/27 | 7/9/44 | 7/9/44 | 2/1/67 | 2/1/67 | 5/1/83 | 5/1/83 | 8/0 (100) | 8/0 (100) |
| Aztreonam            | 33/46/42 | 51/36/99 | 6/1/11 (35) | 6/1/11 (35) | 1/1/12 (8) | 1/1/12 (8) | 6/2/15 (70) | 6/2/15 (70) | 10/0 (100) | 10/0 (100) |
| Imipenem             | 3/113/3 | 8/77/9 | 0/0/0 (0) | 0/0/0 (0) | 1/1/12 (8) | 1/1/12 (8) | 4/2/1 (80) | 4/2/1 (80) | 8/0 (100) | 8/0 (100) |
| Cefpodoxime Proxetil| ——- | 7.0/100 | ——- | ——- | ——- | ——- | 2/1/67 | 2/1/67 | 10/0 (100) | 10/0 (100) |
| Tetracycline         | 22/4/85 | 7.0/100 | ——- | ——- | ——- | ——- | 5/1/83 | 5/1/83 | 8/0 (100) | 8/0 (100) |
| Amoxicillin          | 11/100/10 | 11/75/13 | 7/7/24 | 7/7/24 | 1/1/100 | 1/1/100 | 5/1/38 | 5/1/38 | 0.014 | 0.014 |
| Gentamycin           | 26/69/27 | 21/73/22 | 11/13/46 | 11/13/46 | 12/4/10 | 12/4/10 | 8/1/21 (30) | 8/1/21 (30) | 10/0 (100) | 10/0 (100) |
| Neomycin             | ——- | ——- | 7/3/70 | 7/3/70 | 1/1/40 | 1/1/40 | 12/4/16 (63) | 12/4/16 (63) | 10/0 (100) | 10/0 (100) |
| Tobramycin           | ——- | 5/10/33 | ——- | ——- | ——- | ——- | 3/5/30 | 3/5/30 | 0.051 | 0.051 |
| Erythromycin         | ——- | 160/100 | ——- | ——- | 1/1/50 | 1/1/50 | 15/4/79 | 15/4/79 | 2/3/40 | 2/3/40 |
| Chloramphenicol      | ——- | 24/8/75 | ——- | ——- | 1/3/100 | 1/3/100 | 1/1/50 | 1/1/50 | 0.001 | 0.001 |
| Nalidixic acid       | 67/33/67 | 81/26/76 | 15/13/54 | 15/13/54 | 1/1/50 | 1/1/50 | 6/9/40 | 6/9/40 | 10/0 (100) | 10/0 (100) |
| Ciprofloxacin        | 50/61/45 | 64/44/59 | 0/0/0 (0) | 0/0/0 (0) | 1/1/50 | 1/1/50 | 6/2/21 (22) | 6/2/21 (22) | 15/3 (83) | 15/3 (83) |
| Norfloxacin          | 54/37/65 | 64/44/59 | 0/0/0 (0) | 0/0/0 (0) | 1/1/50 | 1/1/50 | 6/2/21 (22) | 6/2/21 (22) | 15/3 (83) | 15/3 (83) |
| Nitrofurantoin       | 18/77/19 | 29/83/26 | 15/14/52 | 15/14/52 | 2/1/50 | 2/1/50 | 6/2/21 (22) | 6/2/21 (22) | 15/3 (83) | 15/3 (83) |
| Cotrimoxazole        | 73/31/71 | 88/35/72 | 19/7/73 | 19/7/73 | 10/0/100 | 10/0/100 | 6/2/21 (22) | 6/2/21 (22) | 15/3 (83) | 15/3 (83) |
| Chloramphenicol      | ——- | 11/0 (100) | ——- | ——- | 8/0 (100) | 8/0 (100) | 17/4/81 | 17/4/81 | 2/2/21 (51) | 2/2/21 (51) |
| Vancomycin           | ——- | ——- | 1/2/53 | 1/2/53 | 1/1/8 (8) | 1/1/8 (8) | 4/2/6 (13) | 4/2/6 (13) | 0.0252 | 0.0252 |

Res; Resistant, Sus; susceptible
50% resistance except Cefoxitin, ceftazidime and Cefepime (38%, 31% and 44% respectively) during 2016. Imipenem was much better with average 9% resistance followed by Amikacin with 19.5% average resistance. Erythromycin and clindamycin faced 100% resistance followed by chloramphenicol (92%) and Cotrimoxazole (73%)  

**Enterococci**  
Penicillins were less effective here too, facing 66% resistance overall. Resistance against Augmentin decreased significantly in 2017 from 80% to 21% (p = 0.0214). Cephalosporins also had poor efficacy facing 64% resistance. Among non-beta lactam group, imipenem was the most effective with 8% resistance only. The resistance against gentamicin decreased significantly in 2017 (p = 0.0026, Table 5)  

**Staphylococci**  
A higher proportion of staphylococcus species were resistant against Penicillins during the study period with average resistance of 63%; piperacillin faced least resistance (27%) in 2016 while oxacillin was almost ineffective with 96% resistance in 2017. The resistance increased significantly against Ampicillin and oxacillin during the study period (p = 0.0186 and p=0.002 respectively, Table 5).  
Staphylococci expressed a variety of resistance against Cephalosporins (average resistance 57%) from 28% against Cephalothin in 2016 to 100% against cefotaxime in 2017. Resistance against all the Cephalosporins used in the study increased during the two years but those Cephalothin and Cefotaxime were statistically significant (p=0.0287 and p=0.0014 respectively). Imipenem again was the most effective antibiotic among the non-β-lactams with 16.7% resistance followed by vancomycin (25.5), Amikacin and chloramphenicol (32% each). Nalidixic acid was the least effective against this group of pathogens facing average resistance of 95.7% followed by erythromycin (93%) and Aztreonam (88%, Table 5).  

**Pseudomonas**  
Most penicillin like penicillin G, ampicillin and oxacillin were totally useless against these pathogens while Augmentin faced 89% resistance. Only piperacillin was a little better facing 30% resistance which increased to 72% during 2017 which proved to be a significant change (p=0.0235). Cephalosporins also proved to be almost ineffective with significant increase in resistance against cefuroxime (p=0.0499) and ceftazidime (p=0.0473) during the study period. Imipenem was the most effective drug against Pseudomonas with 6.6% overall resistance followed by amikacin (22.6% resistance). Most other non-β-lactams faced 80-100% resistance (Table 5).  

**DISCUSSION**  
This is first study to appraise the antimicrobial resistance pattern among bacterial pathogens isolated from patients with urinary tract infections in Rafha, Kingdom of Saudi Arabia.  
After upper respiratory tract infections, UTI are the most common infections worldwide  

13, 14, 15, which are therefore, important cause of morbidity and mortality and cost over 6 billion US dollars annually worldwide  

13. For that reason, the uropathogens and their AMR pattern must be studied to decide effective treatment of the infection  

14.  
It is a well-known fact that the antibiotic resistance in community acquired pathogens is an ever increasing phenomenon  

16, 17, 18. Increasing rates of antibiotic resistance among most of the pathogenic bacteria, including Gram negative bacteria, decrease the options for the treatment of deadly infections. The widespread antibiotic resistant pathogenic bacteria are now a serious public health concern worldwide  

16. Infections caused by multidrug resistant bacteria can result in longer hospital stays and increased mortality  

19, 20, 21. The resistance was found to be highly prevalent during the current study. Many drugs showed increased resistance in 2017 as compared to that seen in 2016. These drugs include Penicillin G, Oxacillin, Cefoxitin, Ceftazidime, Cefotaxime, Aztreonam, Chloramphenicol and Ciprofloxacin/Norfloxacin. This is an alarming sign for the concerned authorities of the local health department and suggests changes in treatment options. Augmentin and gentamicin however showed an opposite trend and were relatively more effective during 2017 than 2016. Imipenem, a member of carbapenem group was found to be the most effective antibiotic especially among the gram negative organisms with resistance not exceeding...
15%. El-Kersh et al., 2015 has reported similar results.22

E. coli has been reported to be the most common uropathogens worldwide ranging from 36% to 77%.22, 23. During the study period, E. coli was the most common UTI pathogen (43.3%) followed by Klebsiella (15.9%) and Staphylococci (15.2%). Among the resistant E. coli, 22% were ESBL producers in 2016 and 28% in 2017.

All the gram negative bacteria (E. coli, Klebsiella and Pseudomonas) isolated in the current study were 100% resistant to penicillin G. The E. coli, Klebsiella and Pseudomonas resistance to Ampicillin was 85%, 91% and 100% respectively. The addition of the β-lactamase inhibitors (clavulanic acid or tazobactam) increases ampicillin activity (Co-amoxiclav such as Augmentin). This however was found to be less effective as it could only reduce the resistance in E.coli from 85 to 65%, in Klebsiella from 91 to 70% and in pseudomonas from 100 to 89%. The co-amoxiclave combination normally is very effective and increases the efficacy of ampicillin/amoxicillin to a quite valuable percentage. In a recent study in Riyadh,24 it was reported that the addition of β-lactamase inhibitor (clavulanic acid) restored the ampicillin activity (amoxicillin/clavulanate) in almost 37% of Gram negative bacteria. In the current study however, the maximum restoration of activity of the antibiotic was 21%.

Among Escherichia coli, resistance to Aztreonam, Imipenem, and Ciprofloxacin/ Norfloxacin was significantly higher during the year 2017. The resistance to augmentin and cephalothin however decreased significantly during 2017. Being resistant to Aminoglycoside 6'-N-Acetyltransferase inactivation, the Amikacin was much more effective than gentamicin in E. coli (11% vs. 25%), Klebsiella (19% vs. 33%), and Pseudomonas (23% vs. 50%). Some other studies have also reported similar results.22, 24

Klebsiella pneumoniae is one of the multidrug resistant bacteria considered as a serious threat to human health by the WHO and CDC. Infections caused by klebsiella pose a serious threat particularly among children, elderly and immunocompromised patients.25, 26. Among the isolated Klebsiella spp. higher resistance levels were seen against most of the antibiotics. As described earlier, imipenem was the most effective antibiotic against klebsiella while Aztreonam faced significantly increased resistance during 2017.

Most common infections caused by enterococci are the UTI infections.77 Higher resistance levels were seen among enterococci against most of the antibiotics. Surprisingly, augmentin and gentamicin were significantly more effective against enterococci during 2017.

Staphylococcal infections, particularly those caused by methicillin resistant S. aureus (MRSA), are increasing worldwide.28-33 In the United States, MRSA is the most common cause of skin and soft-tissue infections.24, 35. During the current study period, high levels of resistance among staphylococcus spp. were seen against most of the common antibiotic with significant increase in the resistance against Ampicillin, oxacillin, Cefalothin, cefotaxime, gentamicin and vancomycin during 2017.

Pseudomonas spp. frequently causes respiratory and urinary tract infections. Increasing resistance to cephalosporins, fluoroquinolones, carbapenems and other antibiotics have been reported in many studies.16. In these studies, reported imipenem resistance was around 20% while in the current study it was found to be much less (<7%). The reported ciprofloxacin resistance (<30%) however was less than that found in the current study (46%). The resistance against Piperacillin, cefuroxime, ceftazidime and ciprofloxacin/Norfloxacin was found to be significantly increasing during 2017.

According to the US Centers for Disease Control and Prevention (CDC), P. aeruginosa is the third most common cause of UTI. Almost 7% UTI infections are caused by this organism.7 National Nosocomial Infections Surveillance (NNIS) system report: data summary from January 1992 through June 2003, issued August 2003.36

In Saudi Arabia, carbapenem resistant Acinetobacter baumannii (CRAB) have also increased vividly in the recent years. Al-Obeid et al., 201577 revealed A. baumannii resistance to meropenem and imipenem in 2006 was between 20-35%, which increased to almost 90% during 2012. In the current study, A. baumannii resistance to imipenem was found in 8 out of 19 isolates (42%) during 2016. Molecular studies done on CRAB isolates from the GCC region showed that
pathogenic isolates from different parts of the region have assembled together.

CONCLUSION

Increased overall antibiotic resistance was observed in the current study. E. coli is the most prevalent uropathogen found in the study and this must be considered while selecting antimicrobial treatment for the UTIs. The resistance in the region is increasing against β-Lactam group except Augmentin where it showed opposite trend. Imipenem, Amikacin and Vancomycin are overall the most effective antibiotics against the uropathogens in the city.

As the antibiotic resistance is increasing by the time, routine urine cultures must be done for deciding a proper treatment for the infection to avoid treatment failure. Also, the antibiotic resistance trends must regularly be monitored in the region under a well-defined surveillance programs.

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