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African Journal of Bacteriology Research

*Full Length Research Paper*

# An overview of epidemiology and etiology of bacteria associated with diabetic injuries and their dominant infection at Central Region, Riyadh, Saudi Arabia

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**Diabetes mellitus (DM) is one of the most chronic and dangerous diseases worldwide and in the Saudi society in particular. Swabs (168) from DM injuries were collected from inpatient and outpatient departments. Analysis of variance revealed high incidence of diabetic foot infections compared to other injuries by an average of 51 and 33, respectively. Adults' category was significantly the highest age category in the incidence. Inpatients have recorded the highest incidence than outpatients, while routine cases have recorded the highest rates of infection compared to the urgent and very urgent cases. Etiology was confined in 210 bacterial isolates belonging to gram negative (G-ve), gram-positive (G+ve), aerobic and anaerobic bacteria. The study detected a high incidence in one genus/swab compared to two, three, four genera and polymicrobial/swab, with 67, 14, 1, 0.5 and 1.5%, respectively. Aerobic bacteria reached 98.5% compared to anaerobic bacteria (1.5%). G-ve aerobic bacteria were highly significant compared to G+ve. The dominant bacterial species in diabetic injuries was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* with a rate of 28 and 17%, respectively. It is worth noting that the antibacterial ability was evident in *Ps. aeruginosa* for the most bacterial isolates tested, and this reinforces the result found in the study of *Ps. aeruginosa's* dominance in diabetes compared to the rest of the isolated bacterial genera. Results of the study are considered unique in the epidemic spread of diabetic injuries for inpatients, outpatients, as well as the antagonistic relations of each bacterial etiology of diabetic injuries in Saudi Arabia.**

**Key words:** Epidemiology, etiology, bacteria, diabetic injuries, age, in-outpatients, priority, polymicrobial, aerobic, anaerobic, *Ps. aeruginosa, S. aureus.*

## INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that is widespread in the world. In Saudi society, in particular, the rate of diabetes in 1985 was estimated to be 5%, which reached 13% in 2000. Recent data from Saudi Arabia shows that the prevalence of type-2 DM reached 25.4% in 2014 (Al-Rubeaan et al., 2015). Moreover, there were 3.4 million cases of diabetes in 2015 (IDF, 2015), which means it is a continuous increase in Saudi Arabia, due to the lifestyle leading to higher incidence of DM (Al-Sobayel et al., 2014). Many studies have been conducted on the Saudi populations with risk factors for complications from diabetic foot (Al-Wakeel et al., 2009; Hu et al., 2014; Zahrani et al., 2014). Diabetic foot injuries are the most frequent(Currie et al.,

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2009), and lead to more severe complications for diabetic patients, by progressing to foot ulcers at some time in their lives (Singh et al., 2005). Moreover, diabetic injuries exposed to bacterial infection (Espinosa et al., 1999) mainly, compared to injured persons who are not diabetics (Reiber, 2001) lead to inflammation and destruction of the tissue, and can lead to amputation of the lower limbs.

The current research aims to study bacterial infections of diabetic injuries in a group of Saudi Arabia patients, represented in the Riyadh region by assessing the relationship between these injuries and the status of the patients from various aspects. The study also includes isolation and identification of bacteria associated with diabetic injuries in general, in addition to the production of “Bacteriocin” from bacterial species isolated from diabetic ulcers, for studying the dominance of these bacterial species in diabetic injuries.

## MATERIALS AND METHODS

Swabs were collected from 168 injuries who are suffering from diabetes mellitus in Riyadh Medical Complex, Ministry of Health, Central Region - Riyadh, Saudi Arabia, from September 5th, 2005 to January 30th, 2006.

Recommended methods in Medical Complex were followed such as sterilizing the place of taking the sample by using 70% of ethylic alcohol or iodine or quaternary ammonium compounds or chlorine hexedine. Swabs were cultured immediately at their arrival to Central bacteriological Laboratory on the following media: Blood agar, chocolate agar, MacConky in $CO_2$; the plates were incubated overnight at 37˚C in $O_2$ incubator. Pure colonies were checked in morphology, microscopic examination (gram stain, spores, and motility). Also, biochemical tests based on API-Staph system and API-tests 20E and API-20Strep (GHL, 2013) were used to identify the bacterial species. Furthermore, some differentiating tests were used such as: Catalase, Optochin Disk, Staphrex, Bile Aesculin hydrolysis, and Oxidase test by the methods used by Holt et al. (2000), and Murray et al. (2003).

It is worth mentioning, that wound swabs include the following data: Sex, age, place of isolation, hospital departments and bacterial isolates from injuries and on this basis we examined the different relationships associated with the epidemiological spread of the disease in the central region, Riyadh, Saudi Arabia.

Genera and species have been tested for their antagonistic effect depending on the method of Shoeib (1995). The antagonistic effect of the isolated bacterial species from the diabetics’ injuries was measured as the inhibition area resulting from growth.

Data were analyzed by SPSS (2006) Program: frequencies and percentages, the means and standard deviations, the analysis of variance and Duncan test.

## RESULTS

### The relationship between diabetic injuries and the general state of the patients

#### *Gender and DM foot infection*

Regarding the gender difference in the study, the results showed that the number of the males suffering from diabetic injuries was more than the females; where the number of males was 137 out of 168; while the number of females was 31 out of 168, at a rate of 4.4:1 respectively.

Patients’ reports also pointed the high incidence of diabetic foot injuries compared to the injuries in other areas of the body, where the incidence of diabetic foot reached 102 out of 168 representing 60.7%; while other injuries reached 66, representing 39.2% (Table 1).

Amputations cases reached 13 cases out of 168 representing 7.14%; 10 cases of them were males, and 3 were females; 10 cases were below the knee amputation and 3 cases of toe amputation.

Analysis of variance refers to the existence of significant difference P<0.01 where the incidence of diabetic foot was significantly more than other injuries by an average ratio of 51 and 33 respectively. The number of males was significantly P<0.0001 outperforming the number of females with an average ratio of 68.5 and 15.5, respectively (Table 1).

#### *Age categories*

Statistical analysis of the data showed that the proportion of males with diabetic injuries was significantly higher (P <0.01) than the proportion of females (27.4% and 6.2% respectively). There was also a significant effect (P <0.001) for age categories (Table 2), where the adult category was higher- according to the incidence (%55.5). Moreover, then came the four categories (adolescent, children, elderly, suckling) with an equal average statistically proportions (14.5, 7, 5.5 and 1.5%, respectively).

Considering the results of the analysis of variance, it was found that the number of infected males was significantly higher (P<0.05) than the number of females with an average of 27.6 and 6% respectively.

It was noted that the number of infected males was higher than the number of infected females in all age categories except the elderly category in which the number of females outranked the number of males at the rate of 1.75:1.

#### *Inpatients and outpatients*

According to the obtained results, it was found that there was a significant difference (P<0.05) in the incidence of diabetic injuries between inpatients (83.5%) and outpatients (0.5%) (Table 3). The number of male inpatients was 136 out of 167, and it was higher than the number of female inpatients (31 out of 167).

#### *Priority*

Patients’ cases were divided in terms of their importance into three categories of cases: Routine, urgent, and very urgent. Analysis of variance (Table 4) has shown that there is a significant difference (P<0.01) in the proportion of the importance of the condition of

**Table 1.** The distribution of patients with diabetes, males and females, who have infected diabetic foot resulting from the bacterial infection as well as infection's average rates (± standard deviation), and analysis of variance.

| Infection | Sex | | | | Total (%) |
|---|---|---|---|---|---|
| | **Male** | **%** | **Female** | **%** | **Mean±S.D** |
| DM foot infection | 83 | 49.4 | 19 | 11.3 | 51±36.9 |
| Other infection | 54 | 32.1 | 12 | 7.1 | 33±24.2 |
| **Total (%) Mean±S.D** | **68.5±16.7** | | **15.5±4** | | |

**P<0.01; ****P<0.0001; the mean difference is significant at the 0.05 level.

**Table 2.** The distribution of patients with diabetes of male and female patients' with injuries according to different age categories as well as infection's average rates (± standard deviation), and analysis of variance.

| Age (year) | Sex | | | | Total (%) Mean±S.D |
|---|---|---|---|---|---|
| | **Male** | | **Female** | | |
| | **No.** | **%** | **No.** | **%** | |
| Suckling [b] (0-2) | 3 | 2.2 | 0.0 | 0.0 | 1.5±1.7 |
| Children [b] (3-11) | 14 | 10.2 | 0.0 | 0.0 | 7±8.1 |
| Adolescent [b] (12-21) | 25 | 18.2 | 4 | 2.9 | 14.5±12.1 |
| Adults [a] (22-60) | 91 | 66.4 | 20 | 64.5 | 55.5±40.9 |
| Elderly [b] ( ≥61) | 4 | 2.9 | 7 | 22.6 | 5.5±1.7 |
| **Total (%) (Mean±S.D)** | 27.4±34.5 | | 6.2±7.7 | | |

**P<0.01; *P<0.05, the mean difference is significant at the 0.05 level.

**Table 3.** Distribution of patients with diabetes injuries of male and female in an inpatient and outpatient departments as well as infection's average rates (± standard deviation), and analysis of variance.

| Department | Sex | | | | Total (%) |
|---|---|---|---|---|---|
| | **Male** | **%** | **Female** | **%** | **Mean±S.D** |
| Outpatient [b] | 1 | 0.6 | 0 | 0.0 | 0.5±0.6 |
| Inpatient [a] | 136 | 80.9 | 31 | 18.5 | 83.5±60.6 |
| **Total (%) (Mean±S.D)** | **68.5±77.9** | | **15.5±17.8** | | |

*P<0.05, the mean difference is significant at the 0.05 level.

patients with diabetes in Riyadh Medical Complex. The routine cases were significantly higher (72%) followed by the other two types (Urgent and Very Urgent) in static equal proportions (10.5 and 1.5%, respectively) where there is no significant difference between these two categories. It has been observed that the number of infected males was significantly higher (P<0.05) than the number of infected females
(45.6 and 10.3%, respectively).

## The relationship between diabetic injuries and bacterial etiology

### *Multiple bacterial species for each swab*

Statistical analysis has shown that the incidence of one genus of aerobic bacteria/swab was highly significant (67%). Then came the incidence of two, three, four aerobic bacterial species and polymicrobial (Mixed from aerobic and anaerobic bacteria)/swab, with an equal average of proportions statistically (14, 1, 0.5 and 1.5%, respectively) (Table 5).

### *Aerobic and anaerobic bacteria*

All tested 168 swabs contained aerobic bacteria, where the number of aerobic isolates was 207 out of 210 represented by 98.5%; while the number of anaerobic isolates reached 3 represented by 1.5%. It is worth mentioning that anaerobic bacteria are isolated independently, but are associated with aerobic bacteria as well (Figure 1).

### *Aerobic G-ve and G+ve bacteria*

Analysis of variance indicates a significant difference (P<0.05) between the incidence of aerobic G-ve bacteria (76.5%) and the incidence of aerobic G+ve

**Table 4.** Distribution of patients with diabetes, male and female infected with wound according to the importance of the pathological condition (Priority) along with infection's average rates (± standard deviation), and analysis of variance.

| Priority | Sex | | | | Total (%) |
|---|---|---|---|---|---|
| | Male | % | Female | % | Mean±S.D |
| Routine [a] | 116 | 69 | 28 | 16.7 | 72±50.6 |
| Urgent [b] | 18 | 10.7 | 3 | 1.8 | 10.5±8.6 |
| Very urgent [b] | 3 | 1.8 | 0.0 | 0.0 | 1.5±1.7 |
| **Total (Mean±S.D)** | 45.6±54.8 | | 10.33±13.7 | | |

* P<0.05 , the mean difference is significant at the 0.05 level-

**Table 5.** Distribution of the multiplicity of infection cases with more than one genus in the wounds of patients with diabetes, male and female along with average rates of infection (± standard deviation), and analysis of variance..

| Type of bacteria | Genus | Sex | | | | Total (%) |
|---|---|---|---|---|---|---|
| | | Male | % | Female | % | Mean±S.D |
| Aerobic bacteria | One genus[a] | 107 | 63.6 | 27 | 16.1 | 67±46.2 |
| | Tow genus[b] | 26 | 15.5 | 2 | 1.2 | 14±13.8 |
| | Three genus[b] | 2 | 1.2 | 0 | 0.0 | 1 ±1.2 |
| | Four genus[b] | 1 | 0.6 | 0 | 0.0 | 0.5±0.5 |
| Mixed (aerobic and anaerobic bacteria) | Polymicrobial[b] | 2 | 1.2 | 1 | 0.6 | 1.5±0.5 |
| **Total (Mean±S.D)** | | **27.6±43** | | **6±11.9** | | |

**P<0.01, *P<0.05, the mean difference is significant at the 0.05 level.

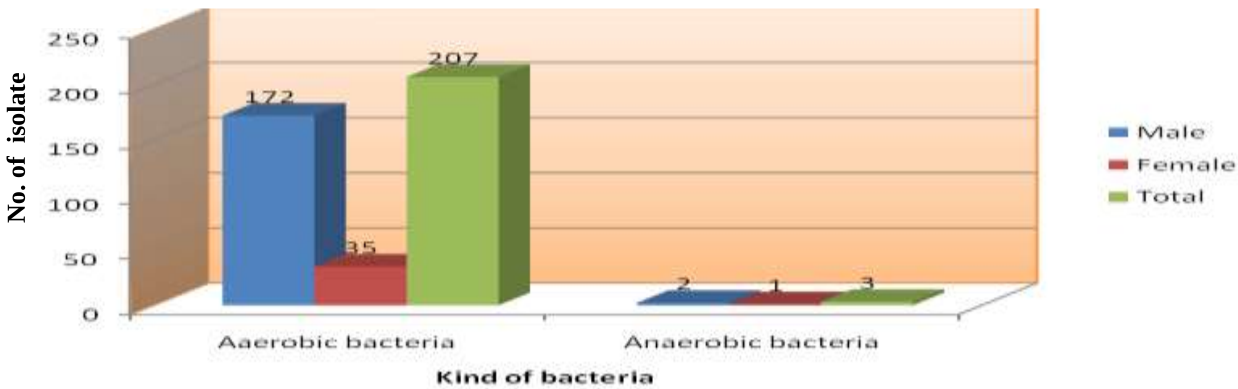


**Figure 1.** The number of aerobic and anaerobic bacteria isolates isolated from wounds of patients with diabetes, males and female.

bacteria (28.5%). Analysis of variance also refers to the high incidence for males than females significantly, and is also P<0.01, with an average represented by 87% compared to 18% for male and females respectively (Table 6).

### *The dominance of bacterial species*

According to the standard tests recommended, G-ve and G+ve bacteria genera and species had been identified (Table 7); also the fungus *Candida tropicalis* of the yeasts was isolated. Throughout the study, it was found that the bacterial species mostly associated with injuries was the rod bacterium of *Ps. aeruginosa* (G-ve); it amounted to 56 out of 210 isolates with an average of 26.7%, followed by spherical bacteria of *S. aureus* (G+ve), comprising 34 isolates out of 210 with an average of 16.2% (Table 7).

### *Diabetic injuries and its associated genera with Ps. aeruginosa*

Due to the dominance of *Ps. aeruginosa* in diabetic injuries, species associated with it have been studied (Table 8). Analysis of variance showed significant difference (P<0.00001) of the presence of *Ps. aeruginosa*

**Table 6.** Distribution of aerobic bacteria isolates (negative and positive to gram) in wounds of patients with diabetes, male and female along with average rates of infection (± standard deviation), and analysis of variance.

| Kind of bacteria | Sex | | | | Total (%) (Mean±S.D) |
|---|---|---|---|---|---|
| | Male | | Female | | |
| | No. | % | No. | % | |
| Gram negative/ aerobic[a] | 129 | 62.3 | 21 | 10.1 | **76.5±62.9** |
| Gram positive/ aerobic[b] | 43 | 20.8 | 14 | 6.8 | **28.5±16.4** |
| **Total (%) (Mean±S.D)** | **87±50.8** | | **18±4.6** | | |

*P<0.05, **P<0.01, the mean difference is significant at the 0.05 level.

**Table 7.** The number of bacteria isolates (negative and positive to gram) isolated from wounds of patients with diabetes in both sexes, male and female, along with the average rates of infection (± standard deviation), and analysis of variance.

| Bacteria | Sex | | | | Total (%) Mean±S.D |
|---|---|---|---|---|---|
| | Male | % | Female | % | |
| *Ps. aeruginosa* [a] | 50 | 23.8 | 6 | 2.9 | 28±25.4 |
| *S. aureus* [b] | 24 | 11.4 | 10 | 4.8 | 17±8.1 |
| *Ac. baumannii*[b,c] | 18 | 8.6 | 5 | 2.4 | 11.50 ±7.5 |
| *E. coli*[c,d] | 12 | 5.7 | 4 | 1.9 | 8±4.6 |
| *K. pneumoniae*[c,d] | 12 | 5.7 | 3 | 1.4 | 7.50±5.2 |
| *En. cloacae* [c,d] | 13 | 6.1 | 1 | 0.5 | 7 ±6.9 |
| *S.aureus* (MRSA) [c,d] | 8 | 3.8 | 3 | 1.4 | 15.5±7.5 |
| *Acinetobacter* spp [c,d] | 6 | 2.9 | 1 | 0.5 | 3.5±2.8 |
| *M. morganii*[d] | 5 | 2.4 | 0 | 0.0 | 2.5±2.8 |
| *P. mirabilis* [d] | 4 | 1.9 | 1 | 0.5 | 2.5±1.7 |
| *Klepsiella* spp. [d] | 3 | 1.4 | 0 | 0.0 | 1.5±1.7 |
| *S. epidermidis*[d] | 3 | 1.4 | 0 | 0.0 | 1.5±1.7 |
| *St. agalactiae* (group B) [d] | 3 | 1.4 | 0 | 0.0 | 1.5±1.7 |
| *Ent. fecalis*[d] | 2 | 0.9 | 0 | 0.0 | 1±1.2 |
| *Pr. stuartii*[d] | 2 | 0.9 | 0 | 0.0 | 1±1.2 |
| *Ci. koseri*[d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *Enterococcus* spp. [d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *K. oxytoca*[d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *Proteus* spp. [d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *Se. moarceus*[d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *S. cohnii* subsp*. cohnii* [d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *S. xylosus*[d] | 0 | 0.0 | 1 | 0.5 | 0.5±0.5 |
| St. (group A) [d] | 1 | 0.5 | 0 | 0.0 | 0.5±0.5 |
| *B. fragile* [d] | 2 | 0.9 | 1 | 0.5 | 1.5±0.5 |
| **Total (Mean±S.D)** | **7.3±10.8** | | **1.5±2.5** | | |

*****P<0.00001, the mean difference is significant at the 0.05 level.

by repeating 33 cases out of 56 cases in this bacterium alone (58.8%). *Ps. aeruginosa* was associated with *En. cloacae*, where the number of cases was 8 cases representing 14.2%. This bacterium was accompanied by other bacterial species (*K. pneumonia, Klebsiella spp, P. mirabilis, S. aureus + Bacteroides fragilis, S. aureus + Ac. baumannii + B. fragilis*) separately in one case at a rate of 1.8% and in equal proportions that were statistically significant. The high incidence in males than female significantly (P<0.00001) [average incidence in males (4.5) compared to 0.5 in females] was recorded (Table 8).

***Diabetic injuries and its associated genera with S. aureus***

*S. aureus* (G+ve) was the second genus that recorded the highest rates of bacterial diabetic injuries significantly (Table 7), where it was isolated solely by repeating 22 cases out of 34 cases, with a percentage of 65.1%. Moreover, while it was associated with other species it ranged between 5.9% and 2.9% (Table 9).

Results of the analysis of variance showed that the percentage of the presence of spherical bacteria (G+ve) of *S. aureus* solely is significantly higher (P<0.00001)

**Table 8.** Distribution of *Ps. aeruginosa* cases and its occurence with other sp./sexes in the diabetics injuries, males and female, in a single swab along with average rates of infection (± standard deviation), and analysis of variance.

| Bacteria | Sex | | | | Total (%) |
|---|---|---|---|---|---|
| | Male | % | Female | % | Mean±S.D |
| *Ps. aeruginosa*[a] | 29 | 51.7 | 4 | 7.1 | 16.5±14.4 |
| *Ps. aeruginosa+Ac. baumannii*[b] | 3 | 5.4 | 0 | 0.0 | 1.5±1.7 |
| *Ps. aeruginosa+Acinetobacter* spp.[b] | 2 | 3.6 | 0 | 0.0 | 1±1.2 |
| *Ps. aeruginosa+En. cloacae*[b] | 8 | 14.2 | 0 | 0.0 | 4±4.6 |
| *Ps. aeruginosa+K. pneumoniae*[b] | 1 | 1.8 | 0 | 0.0 | 0.5±0.5 |
| *Ps. aeruginosa+Klebsiella* spp.[b] | 1 | 1.8 | 0 | 0.0 | 0.5±0.5 |
| *Ps .aeruginosa+P. mirabilis*[b] | 0 | 0.0 | 1 | 1.8 | 0.5±0.5 |
| *Ps. aeruginosa+Pr.stuartii*[b] | 3 | 5.4 | 0 | 0.0 | 1.5±1.7 |
| *Ps. aeruginosa+S. aureus*[b] | 1 | 1.8 | 1 | 1.8 | 1±0.0 |
| *Ps. aeruginosa+S. aureus+B. fragilis*[b] | 1 | 1.8 | 0 | 0.0 | 0.5±0.5 |
| *Ps. aeruginosa+S. aureus+Ac. baumannii+B. fragilis*[b] | 1 | 1.8 | 0 | 0.0 | 0.50±0.57 |
| **Total (Mean±S.D)** | **4.5±1.2** | | **0.5±1.2** | | |

*****P<0.00001, **P<0.01, The mean difference is significant at the 0.05 level.

**Table 9.** Distribution of *S. aureus* cases and its presence with other sp./sexes in diabetics injuries, males, and female, in a single swab along with average rates of infection (± standard deviation), and analysis of variance.

| Bacteria | Sex | | | | Total |
|---|---|---|---|---|---|
| | Male | % | Female | % | Mean±S.D |
| *S. aureus* [a] | 14 | 41.1 | 8 | 24 | **11±3.5** |
| *S. aureus+E. coli* [b] | 2 | 5.9 | 0 | 0.0 | **1.2±1** |
| *S. aureus+E. coli+Ac. baumannii* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+Ci. koseri* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+K. oxytoca+M. morganii* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+M. morganii* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+Ps. aeruginosa* [b] | 1 | 2.9 | 1 | 2.9 | **0.0± 1** |
| *S. aureus+E. coli+ K. pneumoniae +Ac. baumannii* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+Ac. baumannii+Ps. aeruginosa+B. fragilis* [b] | 1 | 2.9 | 0 | 0.0 | **0.5±0.5** |
| *S. aureus+Ps. aeruginosa+B. fragilis* [b] | 1 | 2.9 | 0 | 0 | **0.5±0.5** |
| *S. aureus+Ac. baumannii+E. coli+B. fragilis* [b] | 0 | 0 | 1 | 2.9 | **0.5±0.5** |
| Total (Mean±S.D) | **2.2±3.8** | | **0.9±2.3** | | |

*****P<0.00001, the mean difference is significant at the 0.05 level.

than its presence associated with other bacterial species with an equal average ratio of 11%. While, the other species was associated with an equal average statistic of 1% and 0.5%. There was a higher incidence for males than females (P<0.00001) with an average infection rate of 2.2% and 0.9% respectively (Table 9, Figure 2).

**The antagonistic effect and dominance of bacteria isolated from diabetic injuries**:

The antagonistic effect of isolated bacteria from diabetic injuries showed inhibition zone of some other isolated bacteria (Table 10) as follows: *S. aureus* (MRSA) showed antagonistic ability against *K. pneumoniae*, and the inhibition area was estimated by 5 mm; while *K. pneumoniae* showed antagonistic ability against *S. aureus* with an estimated inhibition area of 10 mm. *En. cloacae* showed antagonistic ability against *K. pneumoniae* by the emergence of the inhibitory area, and that was estimated by 4 mm. *Proteus* spp. showed antagonistic ability against *Klebsiella* spp. that was estimated by 17 mm..

While *Ps. aeruginosa* showed different antagonistic ability against many tested genera namely: *S. aureus*, *Proteus* spp, *K. pneumoniae*, *E. coli*, *Ac. baumannii*, *S. aureus* (MRSA), *Klebsiella* spp, while the antagonistic ability of *Ent. faecalis* was estimated by 31 mm. Moreover, some tested Genera showed antagonistic ability against other isolates represented in the emergence of a limited number of bacterial colonies along the inoculation line (Table 10).

## DISCUSSION

The results of the statistical analysis indicated that the

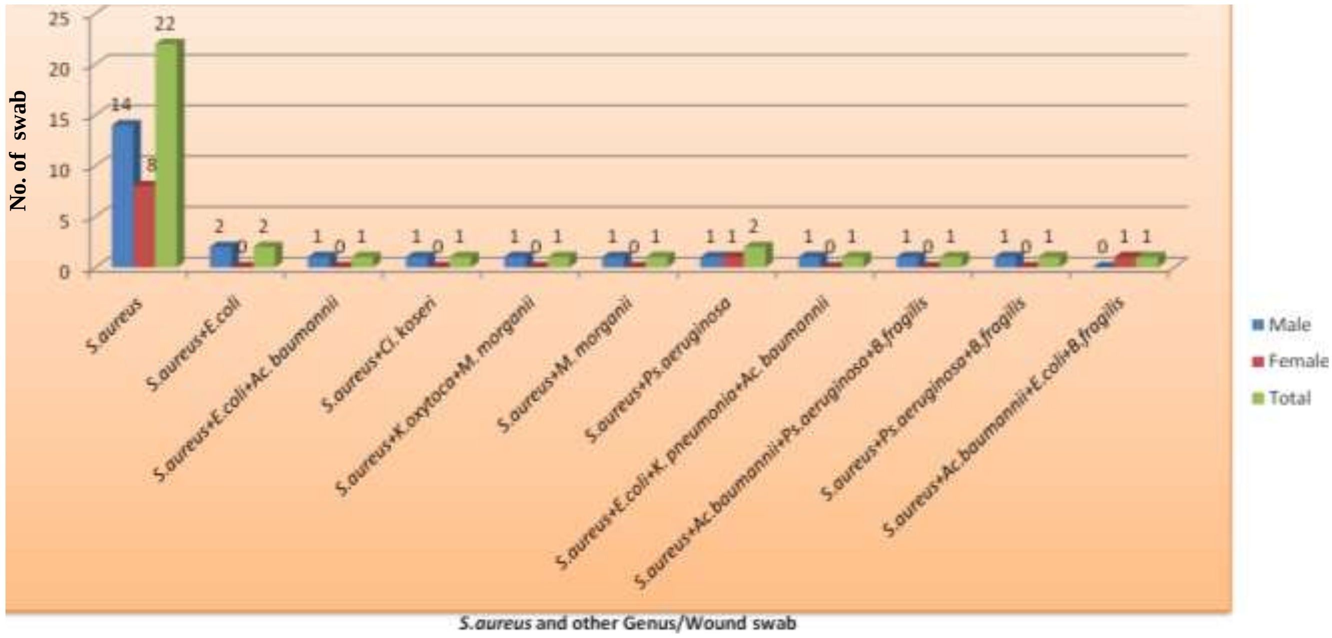


**Figure 2.** The number of infection cases with *S. aureus* and its other accompanying races/sexes in the wounds of diabetic patients, male and female, in a single swab.

highest infection rates of injuries for both males and females were confined in the adult category, while there was no significant difference between the other categories (Abdulrazak et al, 2005; Pemayun and Naibaho, 2017). A high incidence of diabetic foot compared to other injuries was in agreement with the findings of previous studies (Pecoraro et al, 1990; Reiber et al, 1995; Karchmer and Gibbons 1994; Boulton et al, 2006; Gonzalez et al, 2003; Pemayun and Naibaho, 2017).

The current study has shown that the number of inpatients and routine cases for patients scored significantly higher than the number of outpatients and both urgent and very urgent cases. The etiology of bacteria associated with diabetic injuries coincided with previous studies in the dominance of aerobic bacteria over the anaerobic ones (Ako-Nai et al, 2006; Akhi et al, 2015; Haldar et al, 2017) and that the G-ve were the most prevalent ones, followed by the G+ve aerobic bacterial species (Espinosa et al, 1999, Haldar et al, 2017).

Isolation of anaerobic *B. fragilis* with few repetition agrees with Ward (1982), and they were not isolated autonomously (Louie et al, 1976; Scher and Steel, 1988; Young et al, 1993).

Detection of Polymicrobial/swab in this study agreed with Brook and Frazier, (2000); Mottola et al (2016) and Haldar et al (2017). While, Cunha (2000) said that the most diabetic foot injuries, that are gravity ranging between being moderate to severe were caused by Polymicrobial which requires treatment with wide-ranging antibiotics.

*Ps. aeruginosa* and *S. aureus* were the dominant in diabetic injuries; this is in agreement with a study of Abdulrazak et al. (2005), Bansal et al. (2008) and Sánchez-Sánchez et al. (2017) who mentioned that *S. aureus* was isolated more frequently regarding individual species; however, it disagreed with *Proteus mirabilis* (Espinosa et al., 1999), *E. coli* (Ako-Nai et al., 2006) *Streptococcus* sp. and *Enterococcus* sp. (Gonzalez et al., 2003).

Antagonistic ability of *Ps. aeruginosa* for the most tested isolates namely: *S. aureus*, *Proteus* spp., *K. pneumoniae*, MRSA, *Klebsiella* spp., *E. coli*, *Ac. baumannii* were recorded, and what enhances the obtained result during the research is *Ps. aeruginosa* in diabetic injuries was dominant. Results from Hosono et al (2011) agreed with our findings which indicated that *Ps. aeruginosa* has antimicrobial activities against G-ve bacteria, including *E. coli* and *K. pneumoniae*, as well as *P. aeruginosa* itself. Moreover, Chacon et al. (1986) indicated that 90% of the isolates *Ps. aeruginosa* have the ability to produce “Bacteriocin”. Bauernfeind et al. (1981) also showed that *K. pneumoniae* has the ability to produce antioxidants and the inhibition of some genera. However, this result was explained partially with the current study, which showed that K. pneumoniae isolated from the diabetic injuries has antagonistic ability against *S. aureus* only. In addition, our results recorded the ability of MRSA, *En. cloacae* and *Proteus* sp., separately, to inhibit the growth of *K. pneumoniae*, while *Ent. faecalis* has antagonistic ability against itself.

## Conclusion

The increased number of males with diabetic injuries than females is probably due to the nature of the Saudi society, particularly the different activities of males, which exposes them to more injuries. Previous studies did not address the epidemic spread of diabetic

**Table 10.** The opposite ability for the types and bacteria genera isolated from wounds of patients with diabetes.

| Producer indicator | *Ac. baumannii* | *MRSA* | *K. pneumoniae* | *En. cloacae* | *E. coli* | *Proteus* spp. | *M. morganii* | *Ps. aeruginosa* | *Klebsiella sp.* | *S. aureus* | *Fecalis* | *P. mirballis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ac. baumannii* | - | - | - | - | - | - | - | 10 mm | - | - | - | - |
| *MRSA* | - | - | - | - | - | - | - | 52 mm | - | ± | - | - |
| *K. pneumoniae* | - | 5 mm* | ± | 4 mm | - | - | - | 11 mm | - | ± | - | - |
| *En. cloacae* | - | - | ± | - | - | - | - | - | - | - | - | - |
| *E. coli* | - | - | - | - | - | - | - | 18 mm | - | ± | - | - |
| *Proteus* spp. | - | - | - | - | - | - | - | 2 mm | - | - | - | - |
| *M. morganii* | - | - | - | - | - | - | ± | - | - | - | - | - |
| *Ps. aeruginosa* | - | - | - | - | - | - | ± | - | ± | - | - | - |
| *Klebsiella* spp. | - | - | - | - | - | 17 mm | ± | 18 mm | - | - | - | - |
| *S. aureus* | - | - | 10 mm | - | ± | - | - | 21 mm | - | ± | - | - |
| *Ent. faecalis* | ± | - | _ | - | - | - | - | ± | - | ± | 31 mm | - |
| *P. mirballis* | - | - | - | - | - | - | - | - | - | -- | - | - |

-, No growth; ±, Few colonies on the inoculated line; *Zone of Inhibition in mm.

injuries for inpatients, outpatients and priority, as well as the antagonistic relations of each bacterial etiology of diabetic injuries, subsequently dominance of its genus/genera. Therefore, data obtained from this study covered for first time the missing information in epidemic spread of inpatients, outpatients and priority, as well as the antagonistic relations of each bacterial etiology of diabetic injuries in Central Region of Riyadh, Saudi Arabia.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Bacterial identification and drug susceptibility pattern of urinary tract infection in pregnant Women at Karamara Hospital Jigjiga, Eastern Ethiopia

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**Urinary tract infections are the most common bacterial infections during pregnancy and if left untreated in pregnancy it may result in acute pyelonephritis, abortion, premature delivery, low birth baby and even still birth. Thus the aim of this study was to determine the bacterial profile and antibiotic susceptibility patterns of urinary tract infection in pregnant women at Karamara Hospital Jigjiga, Eastern Ethiopia. A cross-sectional study was carried out among 190 pregnant women at Karamara Hospital. Clean catch midstream urine was collected and cultured on MacConkey and Blood agar for isolation and on Muller Hinton agar media for antibiotic sensitivity tests. The present study showed that overall prevalence of UTI was 13.2%. *Escherichia coli* was the most frequently isolated organism [10 (40%)] followed by *Citrobacter spp.*, *Klebsiella spp.*, coagulase negative *Staphylococci* (CoNS) and *Staphylococcus aureus,* each 3 (12%), *Proteus species* [2 (8%)] and *Pseudomonas aeuroginosa* [*1* (4%)]. Gram-negative isolates showed high resistance rate of 89.5 and 84.2% to amoxicillin and ampicillin, respectively. All Gram-negative bacterial isolates revealed low level of resistance (26.3%) against ciprofloxacin. Among the total isolates (n=25), multi-drug resistance (resistance for two or more drugs) were observed in 24 (96%) of all bacterial pathogen from urine specimen. The present study revealed bacterial agents causing urinary tract infections amongst pregnant women were multidrug resistant. *E. coli* was the most common isolated bacteria from mid-stream urine specimen.**

**Key words:** Pregnancy, urinary tract infection, antimicrobial sensitivity pattern.

## INTRODUCTION

Urinary tract infections (UTI), which are caused by the presence and growth of microorganisms in the urinary tract, are perhaps the single commonest bacterial infections of mankind and in pregnancy which may involve the lower urinary tract or the bladder (Brook et al., 2001). UTI has been reported among 20% of the pregnant women and it is the most common cause of admission in obstetrical wards (Theodore, 2007).

Anatomically UTI can be classified into lower urinary tract infection which involve the bladder and urethra and

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upper urinary tract infection involving the kidneys, pelvis, and ureter (Delzell and Lefevre, 2000). The majority of the UTI occur due to ascending infection (Schaeffer et al., 2001). Three common clinical manifestations of UTIs in pregnancy are: asymptomatic bacteriuria, acute cystitis and acute pyelonephritis (Loh and Silvalingam, 2007).

Female gender itself is a risk factor because of short urethra, its proximity to vagina and anus and inability of women to empty their bladder completely. Sexual activity and certain contraceptive methods are also said to increase this risk. The anatomical relationship of female's urethra and the vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up the urethra into the bladder during pregnancy/child birth (Imade et al., 2010). Bacteriuria is said to be significant in the presence of $\geq 10^5$ colony forming units (CFU)/L. The danger with bacteriuria is that it is not always present with symptoms. Occult infection occurs in about 2 to 7% of pregnancies and 30 to 40% of cases develop acute pyelonephritis later in pregnancy. Also there are associations between maternal complications of pregnancy and pyelonephritis including hypertension, preeclampsia, anaemic, amnionitis, and endometritis. Pyelonephritis can lead to renal scarring, hypertension and renal failure in the long run (Gupta and Trautner, 2008). UTI (perhaps if untreated) can lead to serious obstetric complications, poor maternal and perinatal outcomes e.g intrauterine growth restriction, preeclampsia, caesarean delivery and preterm deliveries. Furthermore, it has been observed that asymptomatic bacteriuria can lead to cystitis and pyelonephritis which can lead to acute respiratory distress, transient renal failure, sepsis and shock during pregnancy. Screening of pregnant women for UTI can minimize these UTI associated complications (Mazor-Dray et al., 2009; Schnarr and Smaill, 2008).

Urinary tract infection in pregnancy is associated with significant morbidity for both the mother and the baby (Assefa et al., 2008). In most developing countries including Ethiopia, screening for UTI in pregnancy using microbial culture is not considered as an essential part of antenatal care and therefore the picture of microbial etiologies and their antimicrobial susceptibility patterns are not well known. Current management of UTIs is also usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI. Studies on early screening of pregnant woman for UTI causing bacterial uropathogens and determining their antibiotic susceptibility pattern is an important intervention to prevent complications that may endanger the life of both the pregnant women and the fetus. Therefore, the main objective of this study is to determine bacterial profile and antibiotic susceptibility patterns among pregnant women attending Karamara Hospital, Jigjiga, Eastern Ethiopia.

## MATERIALS AND METHODS

### Study site and design

A cross-sectional study was conducted at Karamara Hospital, Jigjiga, Ethiopia from September 2016 to December 2016. Karamara hospital is one of the public hospitals found in Jigjiga city and provides various health services for routine and referral cases. Jigjiga is one of the capital city of Ethio-Somali Regional States which is the second largest among the nine regions of Ethiopia. It is located 630 km away from Addis Ababa to eastern Ethiopia.

### Sample size

A single proportion formula (Naing et al., 2006) was used to calculate the sample size, $n = Z^2 p (1-p) / d^2$. Where: Z = Z score for 95% confidence interval = 1.96, p = prevalence, d = tolerable error = 5% taking proportion as reference a study found 11.6% in Addis Ababa (Assefa et al., 2008). There by $n = (1.96)^2 0.116(1–0.116)/ (0.05)^2=158$, adding 20% non-response rate giving the final sample size of 190.

### Sampling technique

A convenient sampling method was employed and at least 10 pregnant women were recruited daily until the sample size was reached. Pre-designed and structured questionnaires were used for the collection of data on socio-demographic and associated risk factors. Collection of information on sign and symptoms of UTI and physical examination of pregnant women were done by experienced nurses working in ANC of Karamara Hospital.

### Study population

The source population of this study was pregnant women of all age group attending antenatal care at Karamara, Hospital. The study subjects are those pregnant women who fulfill the inclusion criteria and willing for consent and sampling. Socio-demographic variables (Age, Sex, Education level, Residence and other relevant clinical data such as parity, gravidity, trimester, history of catheterization and UTI) were included in a pre-designed questionnaire.

### Inclusion and exclusion criteria

Pregnant women who were taking antibiotics within seven days at the time of recruitment and who were not willing to participate were excluded from this study due to the fact that the antibiotic must have inhibited or destroyed the pathogens.

### Laboratory method

#### *Urine collection and analysis*

About 10 ml freshly voided midstream urine specimens were collected. The participants were instructed on how to collect the urine sample through cleansing the gentalia and voided using leak proof, wide mouth sterile plastic containers. The urine specimens were then delivered to microbiology laboratory, Jigjiga University and processed within two hours. Most of the specimens were analyzed within an hour of collection. Urinalysis using urine dipstick (Mannheim GmbH, Germany) was done following manufacturer's instructions. A 1 µl to 10 µl were used to inoculate urine samples on MacConkey and Blood agar plates (OXOID-England). Plates were incubated for 24 h at 37°C. A diagnosis of UTI was made when there were at least $10^5$ colony forming unit (CFU)/ml of urine. High colony counts with more than one species of bacteria were considered as contaminations. Identification was done using in house biochemical testing (Murray et al., 1995). All positive cultures with Stonebrink's (SB) were then identified at species level by their colony characteristics, gram-staining reaction and by the pattern of biochemical profiles using standard procedures. The enterobacteriaceae were identified by indole production, H2S production in KIA agar, citrate utilization, motility test, urease test, and oxidase and carbohydrate utilization tests. The gram-positive bacteria were identified using catalase and coagulase tests (Cheesbrough, 2006). All procedures were done as recommended by Clinical Laboratory Standard Institute (CLSI). For quality control, *E. coli* ATCC 25922 was used as control strains (Wayne, 2010).

### Antimicrobial susceptibility testing

Antimicrobial susceptibility pattern were determined by disk diffusion method using Muller Hinton according to CLSI (Wayne, 2010) using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar (Oxoid, Ltd, England). A loop full of bacteria were taken from a pure culture colony and transferred to a tube containing 5 ml of saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension adjusted to the turbidity of McFarland 0.5 standard in a tube and then swabbed on Muller Hinton medium. The following antimicrobial agents were tested for Gram-positive bacteria, "Gentamicin (10 µg), Penicillin G (10 µg), Ampicillin (10 µg), Erythromycin (15 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg), and Ceftriaxone (30 µg)". For Gram-negatives bacteria, "Ampicillin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Norfloxacin, Amoxicillin and ceftriaxone (30 µg)" discs were used.

### Ethical consideration

This study was conducted after formal approval of the protocol obtained from the Research and Ethical Review Committee of Jigjiga University. Prior to data collection, communication was made with hospital director and written informed consent was obtained from the participants through detail explanation of the study objective and the study procedures. All laboratory tests included in study were done for the participant free of any payments. Apart from the inconvenience of taking time to answer the research questionnaire, participants were not exposed to any undue risk. Positive results were sent immediately to the attending clinician for appropriate medical consideration according to culture results and sensitivity patterns.

### Quality control

The quality assurance of pre-analytical, analytical and post-analytical stages of urine culture was applied. All specimens were transported from the hospital to microbiology laboratory, Jigjiga University within cold box and those specimens which were not processed within 2 h were kept in refrigerator and processed no longer than 18 h after collection. Only specimens which produced $\geq 10^5$ CFU/ml of urine were considered significant but specimen that produced $<10^5$ colonies/ml of urine was considered insignificant. Culture media were sterilized based on the manufactures instruction. Then the sterility of culture media were checked by incubating 3 to 5% of the batch at 35 to 37°C overnight and observed for bacterial growth. Those media which showed growth were discarded. The standard reference strains; *E. coli* (ATCC25922) were used for testing quality of culture media and antibiotic discs. Generally Standard operating procedures (SOPs) prepared based on the national infectious disease control and prevention guideline as well as CLSI bacteriological techniques were followed strictly.

### Data processing and analysis

All data on each subject in the study were recorded on a standard registration format. Socio-demographic, clinical and laboratory data were entered and analyzed using SPSS version 23. Descriptive data was explained by tables and texts. Proportions for categorical variables were compared using Chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

## RESULTS

### *Socio-demographic characteristics of study participants*

A total of 190 pregnant women aged between 15 to 40 years were enrolled in this study with the mean plus standard deviation of 25 (+4.8) years. Among the study participants, 17 (8.9%) were educated up to secondary school and above. According to their monthly income about 108 (55.8%) of the study participants were in the category of medium income. Based on their family type, 171 (90.0%) belongs to monogamous family. 4 (38.9%), 47 (24.7%) and 69 (36.3%) of study subjects were in the 1st, 2nd and 3rd trimester of pregnancy. 45 (76.3%) of the study participants were multigravida. About 49.5% of the study participant had sexual intercourse for at least three times per week. 5 (2.6%), 34 (17.9%) and 39 (20.5%) of study subjects had history of previous catheterization, contraceptive use and urinary tract infection, respectively (Table 1).

### Prevalence of urinary tract infection

The overall prevalence of UTI as confirmed by urine culture was 13.2%. The prevalence of infection in relation to age showed that individuals of between the age group 25 to 34 years (8.9%) had the highest incidence of infection followed by age group 15 to 24 years (3.7%). About 21 (11.1%) and 17 (7.9%) UTI prevalence was observed among asymptomatic and illiterate participants, respectively. Of all considered variables monthly income and frequency of sexual per week were significantly associated with UTI (P<0.005). There was no association between maternal age, address, parity, gravidity, trimester, history of catheterization and education with bacteriuria (Table 2).

### Bacterial etiology of urinary tract infection

The number and percentage of each etiologic pathogen

**Table 1.** Prevalence of UTI associated with socio-demographic characteristics of pregnant women attending ANC in Karamara Hospital, Jigjiga, Ethiopia (September to December, 2016).

| Variable | Culture (+ve, %) | Culture (-ve, %) | Total (%) | P-value | OR (95%CI) |
|---|---|---|---|---|---|
| **Age of participants** | | | | | |
| 15-24 | 7 (3.7) | 63 (33.2) | 70 (36.8) | - | - |
| 25-34 | 17 (8.9) | 91 (47.9) | 108 (56.8) | 0.277 | .595 (0.233-1.518) |
| 35-44 | 1 (0.5) | 11 (5.8) | 12 (6.3) | 0.858 | 1.222 (0.137-10.932) |
| **Education status** | | | | | |
| Illiterate | 15 (7.9) | 88 (46.3) | 103 (54.2) | 0.998 | 0.000 (0.000) |
| primary(1-8) | 7 (3.7) | 41 (21.6) | 48 (25.3) | 0.998 | 0.000 (0.000) |
| Secondary(9-12) | 3 (1.6) | 19 (10.0) | 22 (11.6) | 0.998 | 0.000 (0.000) |
| Higher(12+) | 0 (0.0) | 17 (8.9) | 17 (8.9) | - | - |
| **Religion** | | | | | |
| Muslim | 23 (12.1) | 149 (78.4) | 172 (90.5) | | |
| Non-Muslim | 2 (1.1) | 16 (8.4) | 18 (9.5) | 0.788 | 1.235 (0.266-5.727) |
| **Residence** | | | | | |
| Urban | 25 (13.2) | 158 (83.2) | 183 (96.3) | - | - |
| Rural | 0 (0.0) | 7 (3.7) | 7 (3.7) | 0.999 | 0.000 (0.000) |
| **Participants monthly income** | | | | | |
| Low (<500 Birr) | 5 (2.6) | 10 (5.3) | 15 (7.9) | 0.032 | 0.233 (0.062-.881) |
| Medium (500-1000) | 13 (6.8) | 95 (50.0) | 108 (56.8) | 0.748 | 0.853 (0.322-2.258) |
| High (>1000) | 7 (3.7) | 60 (31.6) | 67 (35.3) | - | - |
| **Family type** | | | | | |
| Monogamy | 24 (12.6) | 147 (77.4) | 171 (90.0) | - | - |
| polygamy | 1 (0.5) | 18 (9.5) | 19 (10.0) | 0.305 | 2.939 (0.375-23.043) |
| **Gestation period** | | | | | |
| 1st trimester | 9 (4.7) | 65 (34.2) | 74 (38.9) | - | - |
| 2st trimester | 4 (2.1) | 43 (22.6) | 47 (24.7) | 0.529 | 1.488 (0.431-5.139) |
| 3rd trimester | 12 (6.3) | 57 (30.0) | 69 (36.3) | 0.380 | .658 (0.258-1.675) |
| **Gravidity** | | | | | |
| Prim gravid | 5 (2.6) | 40 (21.1) | 45 (23.7) | 0.643 | 1.280 (0.451-3.631) |
| Multigravid | 20 (10.5) | 125 (65.8) | 145 (76.3) | - | - |
| **History of UTI** | | | | | |
| No | 21 (11.1) | 130 (68.4) | 151 (79.5) | - | - |
| Yes | 4 (2.1) | 35 (18.4) | 39 (20.5) | 0.549 | 1.413 (0.455-4.387) |
| **History of catheterization** | | | | | |
| No | 24 (12.6) | 161 (84.7) | 185 (97.4) | - | - |
| Yes | 1 (0.5) | 4 (2.1) | 5 (2.6) | 0.650 | 0.596 (0.64-5.561) |
| **History of contraceptive use** | | | | | |
| No | 21 (11.1) | 135 (71.1) | 156 (82.1) | | |
| Yes | 4 (2.1) | 30 (15.8) | 34 (17.9) | 0.791 | 1.167 (0.373-3.648) |
| **History of sexual practice per week** | | | | | |
| Once | 6 (3.2) | 16 (8.4) | 22 (11.6) | | |
| Twice | 9 (4.7) | 57 (30.0) | 66 (34.7) | 0.148 | 2.375 (0.735-7.671) |
| Three times | 9 (4.7) | 85 (44.7) | 94 (49.5) | 0.033 | 3.542 (1.107-11.331) |
| More than three times | 1 (0.5) | 7 (3.7) | 8 (4.2) | 0.410 | 2.625 (0264-26.072) |

**Table 2.** Antimicrobial susceptibility patterns of Gram-negative bacteria and Gram positive bacteria isolated from urine culture in pregnant women at Karamara Hospital, East Ethiopia (September to December, 2016).

| **Gram negative bacteria, 19 (76%)** | **Pattern** | **AMP** | **CN** | **NOR** | **CRO** | **CIP** | **AMOX** |
|---|---|---|---|---|---|---|---|
| | S | 0 (0) | 3 (30) | 8 (80) | 2 (20) | 8 (80) | 1 (10) |
| *Escherichia coli (N=10)* | I | 0 (0) | 1 (10) | 0 (0) | 1 (10) | 0 (0) | 0 (0) |
| | R | 10 (100) | 6 (60) | 2 (20) | 7 (70) | 2 (20) | 9 (90) |
| | S | 0 (0) | 0 (0) | 1(33.3) | 2 (66.7) | 2 (66.7) | 0 (0) |
| *Citrobacter freundii (N=3)* | I | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | R | 3 (100) | 3 (100) | 2 (66.7) | 1 (33.3) | 1 (33.3) | 3 (100) |
| | S | 3 (100) | 2 (66.7) | 3 (100) | 2 (66.7) | 2 (66.7) | 1 (33.3) |
| *Klebsiella pneumonia (N=3)* | I | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | R | 0 (0) | 1 (33.3) | 0 (0) | 1 (33.3) | 1 (33.3) | 2 (66.7) |
| | S | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 0 (0) |
| *Proteus species (N=2)* | I | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | R | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 2 (100) |
| | S | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| *Pseudomonas aeuroginosa (N=1)* | I | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | R | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| | S | 3 (15.8) | 5 (26.3) | 12 (63.2) | 6 (31.6) | 14 (73.7) | 2 (10.5) |
| Total=19 | I | 0 (0) | 1 (5.3) | 0 (0) | 1 (5.3) | 0 (0) | 0 (0) |
| | R | 16 (84.2) | 13 (68.4) | 7 (36.8) | 12 (63.2) | 5 (26.3) | 17 (89.5) |
| **Gram positive bacteria, 6 (24%)** | **Pattern** | **AMP** | **CN** | **E** | **P** | **CRO** | **V** |
| *Staphylococcus aureus (N=3)* | S | 0 (0) | 1 (33.3) | 3 (100) | 0 (0) | 2 (66.7 | 3 (100) |
| | R | 3 (100) | 2 (66.7) | 0 (0) | 3 (100) | 1 (33.3) | 0 (0) |
| *CONS (N=3)* | S | 0 (0) | 1(33.3) | 3(100) | 1 (33.3) | 3 (100) | 3 (100) |
| | R | 3 (100) | 2 (66.7) | 0 (0) | 2 (66.7) | 0 (0) | 0 (0) |
| Total=6 | S | 0 (0) | 2 (33.3) | 6(100) | 1 (16.7) | 5 (83.3) | 6 (100) |
| | R | 6 (100) | 4 (66.7) | 0 (0) | 5 (83.3) | 1 (16.7) | 0 (0) |

R=Resistant, S=Sensitive, I=Intermediate.

isolated from mid-stream urine samples are presented in Figure 1. Of the total 25 isolates, Gram-negative bacteria were highly prevalent 19 (76%) than Gram-positive bacteria 6 (24%). The predominantly isolated bacteria were *E. coli* 10 (40%), followed by *Citrobacter spp.*, *Klebsiella* spp., coagulase negative Staphylococci (CoNS) and *Staphylococcus aureus* each 3 (12%), *Proteus* species 2 (8%) and *Pseudomonas aeuroginosa 1* (4%).

## Antimicrobial susceptibility pattern of bacterial pathogens of UTI

The susceptibility patterns of isolates from mid-stream urine against nine antimicrobial agents are presented (Table 3). The result of antimicrobial susceptibility pattern of the isolate is shown on rates of susceptibility range from 0 to 100%. Most Gram-positive and Gram-negative isolates were resistant to two or more antibiotics (multi-drug resistant). In general Gram-negative isolates showed resistance rate of 89.5% to amoxicillin, 84.2% to ampicillin, 68.4% to Gentamycin and 63.2% to ceftriaxone. Least Gram-negative bacteria resistance was observed against ciprofloxacin (26.3%) and Norfloxacin (36.8%). *E.coli* which constituted for 52.6% of the Gram-negative bacteria showed 100, 90, 70, and 60% resistance against Ampicillin, Amoxicillin, Ceftriaxone and Gentamycin, respectively. *Citrobacter freundii* the other Gram-negative bacteria showed 100% resistance to Ampicillin, Gentamicin and Amoxicillin. All *proteus spp* and *Pseudomonas aeroginosa* from Gram-negative showed resistance to all types of antibiotic used in this experiment. All staphylococcus from Gram-positive bacteria showed 100% sensitive for Erythromycin and Vancomycin while 100% resistance for Ampicillin. Among

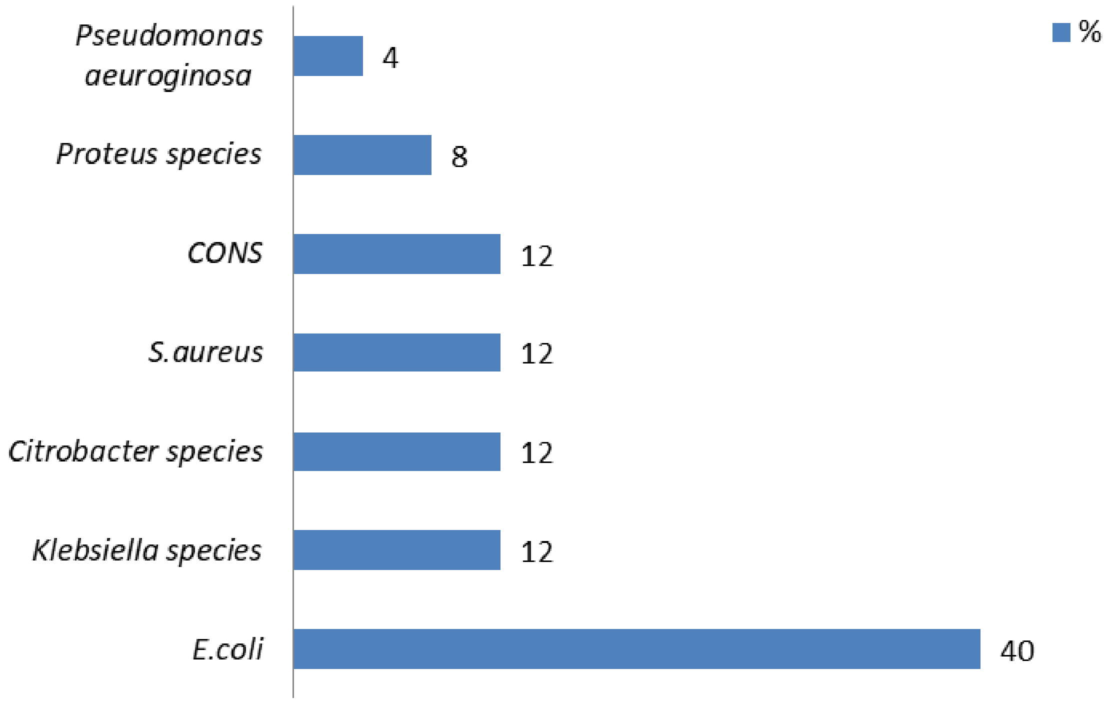


**Figure 1.** Distribution of Bacterial agent of urinary tract infection among pregnant women attending antenatal clinic at Karamara Hospital, East Ethiopia (September - December, 2016).

**Table 3.** Antibiogram showing resistance of the most common isolates of urinary tract infection from pregnant women to one or more antibiotics in pregnant women at Karamara Hospital, East Ethiopia (September to December, 2016).

| Organism (Total No.) | No. | Pattern of resistance to antimicrobials |
|---|---|---|
| *Escherichia coli* (10) | 1 | AMP |
| | 1 | AMP, AMOX |
| | 4 | AMP, AMOX, CRO |
| | 1 | AMP, AMOX, CN |
| | 1 | AMP, AMOX, CRO,CIP |
| | 1 | AMP, AMOX, CN, CRO |
| | 1 | AMP, AMOX, CRO,GM, CIP, NOR |
| *Citrobacter freundii* (3) | 1 | AMP, AMOX, CN, CRO |
| | 2 | AMP, AMOX, CN, CRO, CIP, NOR |
| *Klebsiella pneumonia* (3) | 2 | AMP, AMOX |
| | 1 | AMP, AMOX, CN, CRO, CIP, NOR |
| *Staphylococcus aureus* (3) | 1 | AMP, CN |
| | 2 | AMP, CN, P |
| *CoNS* (3) | 2 | AMP, CN, P |
| | 1 | AMP, CRO |
| *Proteus species* (2) | 2 | AMP, AMOX, CN, CRO, CIP, NOR |
| *Psedomonas aeroginosa* (1) | 1 | AMP, AMOX, CN, CRO, CIP, NOR |

AMP: Ampicillin, CN: gentamicin, NOR: norfloxacin, CRO: ceftriaxone, CIP: ciprofloxacin, AMOX: amoxicillin, VA: vancomycin, P: penicillin, E: erythromycin.

the total isolates multi-drug resistance (MDR) were observed in 24 (96%) of all bacterial pathogen from urine specimen. All isolates of Gram-positive bacteria and 94.7% of Gram-negative bacteria showed resistance to two or more drugs.

## DISCUSSION

It is a potentially life threatening condition when the urinary tract is infected especially when it develops in pregnant women. Despite advances in diagnosis and treatment, bacterial urinary tract infection remains a major cause of pregnancy related morbidity and mortality worldwide (Rizvi et al., 2011). The causative agents of urinary tract infection and their antibiotic susceptibility patterns also become varying from time and geography hence this data is important to provide information used to formulate infection control measures and develop antibiotic polices everywhere. In this study, the overall prevalence of urinary tract infection in pregnant women attending antenatal clinic was 13.2%. Similar findings of 10.4% have been reported in previous study conducted at University of Gonder Teaching Hospital, Ethiopia (Alemu et al., 2012) and other countries at Tanzania (14.6%) (Masinde et al., 2009) and Khartoum (14%) (Hamdan et al., 2011).

However lower prevalence was reported by different studies conducted on pregnant women from FelegeHiwot Referral Hospital (9.5%) (Tazebew et al., 2012) and Nepal (9.8%) (Marahatta et al., 2012). There were other studies which reported higher prevalence of UTI among pregnant women from Southern Nigeria (25%) (Lawani et al., 2015), Nairobi (26.7%) (Fred et al., 2015) and Benin City, Nigeria (55%) (Oladeinde et al., 2015). Variations in prevalence of urinary tract infections may be explained by differences exist in socio-economic of the community, pregnancy associated physiological changes, educational level, environmental and personal hygiene.

In the present study, there was no statistical significant association between culture positive urine (UTI) and maternal age, religion, residence, education, gestational period, history of catheterization and gravidity. This is in agreement with several studies in Ethiopia (Tazebew et al., 2012; Alemu et al., 2012) and Sudan (Hamdan et al., 2011). However, a previous finding showed that maternal age and gravidity are risk factors for UTI among pregnant women (Haider et al., 2010). Prevalence of UTI in pregnant women with previous history of urinary tract infection not showed a significant association. In contrast to this finding, previous studies conducted in Ethiopia (Alemu et al., 2012) and Pakistan (Sheikh et al., 2000) confirmed significant association with previous history of UTI. This might be due to effective treatment or presence of resistance strains from various environments. In this study, gram-negative bacterial isolates were more prevalent than gram-positive bacterial isolates (76 and 24%, respectively). Similar findings were reported by previous studies in TikurAnbessa Specialized Hospital Addis Ababa (60 and 40%) (Assefa et al., 2008), in Gondar University Hospital (58.4 and 41.6%) (Alemu et al., 2012) and elsewhere in the world (Delzell and Lefevre, 2000). This finding also showed that the most common bacteria isolated from the mid-stream urine samples of the pregnant women was *E. coli* (40%), followed by *Klebsiella spp.* (12%), *Citrobacter spp.* (12%), *Stapylococcus aureus* (12%) and *Staphylococcus coagulase negative* (12%). This result is similar in majority of isolates to the separate findings of other study in Ethiopia and elsewhere (Tazebew et al., 2012; Alemu et al., 2012).

Currently many microorganisms have become resistant to different antimicrobial agents and in some cases to nearly all agents. Antibiotic resistance is a problem that has been caused by ineffectiveness of current empirical treatment against bacterial infections of the urinary tract infection. Based on *in-vitro* susceptibility tests in the present study; we observed that, *E. coli*, *Citrobacter spp., Proteus spp.* and *Pseudomonas aeuroginosa* isolates were resistant to Ampicillin (100%) and this implies that ampicillin cannot be used as empirical therapy for urinary tract infection particularly in the study area. Similarly high resistance was also observed against gentamycin. On the other hand, low levels of resistance were observed against ciprofloxacin and norfloxacillin. Similar findings have been reported in previous studies done at Ethiopia (Tazebew et al., 2012; Alemu et al., 2012). Tanzania (Raka et al., 2004) and Iran (Farajnia et al., 2009). Among Gram-positive bacteria tested all were sensitive to vancomycin and erythromycin (100%). However, more resistance was observed against ampicillin (100%) and gentamycin (66.7%). The possible explanation for different level of resistance may be due to frequent usage of antibiotic. Thus, Ciprofloxacillin and norfloxaclilin could be considered as alternative options in the treatment of UTIs.

Multi-drug resistance was observed in 96% of bacterial isolates from mid-stream urine of pregnant women. This finding is comparable with the study findings from Gondar 95% (Alemu et al., 2012) and Tikur Anbessa Specialized Hospital, Addis Ababa 74% (Assefa et al., 2008). Reasons for such alarming MDR might be inappropriate and incorrect administration of antimicrobial agents as empirical treatment, lack of health education and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community.

### Conclusion

The finding of this study revealed that urinary tract infection is common during pregnancy as it enhanced due to a variety of physiological changes during the period of pregnancy. This study has shown that the prevalence of UTI in pregnant women at Karamara

Hospital is similar with other previous studies in Ethiopia and other developing countries elsewhere in the world. The present study has shown the isolated bacterial pathogens of urinary tract infection are resistance for commonly used antimicrobial agents. Therefore, early screening of pregnant woman for UTI causing bacterial pathogens and determining their antibiotic susceptibility pattern is an important intervention to prevent complications that may endanger the life of both the pregnant women and the foetus. Health education about personal hygiene and antibiotic resistance should be emphasized by the antenatal care physician to all pregnant women.

## CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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