Incidence and Antibiotics Resistance of *Staphylococci* and *Escherichia coli* Isolated from Diabetic Urinary Tract Infection Patients in Egypt

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**Abstract**

This study investigated the prevalence, risk factors and antimicrobial resistance of *Staphylococci* and *E. coli* isolated from diabetic urinary tract infection patients. Urine samples collected from 694 cases admitted to the General Health Insurance Authority Hospital in Port Said City, Egypt. A total of 302 bacterial strains were isolated from 292 urine samples with glucosuria and infection positive. The prevalence of the *S. saprophyticus*, *S. aureus*, *E. coli* and other species isolates from the urine samples are 54.7%, 34%, 7.6% and 3.7%, respectively. The antibacterial resistance profile of the isolated bacteria was investigated against different antibiotics. Most bacterial isolates were more susceptible to ofloxacin antibiotic while resistance to ampicillin. Multiple antibiotic resistance (MAR) was found highest for bacterial isolates obtained from urine samples. *S. aureus* showed high resistance percentage than other bacterial strains to studied antibiotics. Vancomycin resistance was detected in 23% of all *S. aureus* isolates. Out of 165 isolates *S. saprophyticus*, 71% were β-lactamase producers, while all *S. aureus* and *E. coli* isolates were β-lactamase positive. Also the purpose of this study was to study any relative between the recognized antibiotic resistance of bacteria and the occurrence of plasmids. Molecular sizes of the detected plasmids were 39.306 kbp in *S. aureus* and 44.640 kbp in *E. coli*. Plasmid curing in combination with MIC determination revealed that resistance of staphylococci and *E. coli* isolates was plasmid-mediated linked. The high MAR recognized makes it essential for antibiotic resistance testing to be accompanied prior to antibiotics remedy for diabetic urinary tract infection patients.

**Keywords**: Antibiotics Resistance, *Staphylococci*, *E. coli*, Diabetic Urinary Tract Infection.

**Abbreviations**: UTI, urinary tract infection; M, male Patients; F, Female Patients; +, Glucosuria concentration 180-200 mg/dl; ++, Glucosuria concentration 200-250 mg/dl; ++++, Glucosuria concentration 250-350 mg/dl; ++++, Glucosuria concentration over 350 mg/dl.

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(Received: 02 August 2019; accepted: 08 September 2019)
INTRODUCTION

Diabetes is very public health difficulties that evidently rise in occurrence and frequency with progressing age. Besides organ complications, diabetic patients also hurt extra recurrently after problematical contagions equated with non-diabetic patients (Carten et al., 1992; Foxman, 2002; Geerlings et al., 2002; Hoepelman et al., 2003; Brown et al., 2004; Brown et al., 2005). To complicate substances further, diabetes also may be a risk factor for single and multi-drug resistance. However, all investigators have found increased uropathogen resistance to antibiotics in diabetic patients (Stapleton and stamm, 1997; Fedele et al., 2000; Geerlings et al., 2001; Wullt et al., 2003). Insufficient articles have observed endemic antimicrobial resistance in Egypt, especially UTI in diabetic patients (Haberberger et al., 1994; Oyofo et al. 1995; Ostroff et al. 1996; Hilal et al. 1997; El-Teheawy et al., 1988; El Kholy and Nassar, 1996; Samuel et al., 1996; El Kholy et al., 2003). This study was therefore supposed to study the occurrence, risk factors and antibiotic resistance of staphylococci and E. coli isolated from diabetic urinary tract infection patients in Egypt.

MATERIALS AND METHODS

Sample collection and identification of bacteria

Urine samples were collected as described by Collins et al. 1998 from 694 cases admitted to the General Health Insurance Authority Hospital in Port Said city, Egypt. A total of 388 DM patient’s urine samples with glucosuria positive concerned for further bacterial examination. One ml aliquots from urine suspensions were dispensed into CLED and MacConkey media. All plates were incubated face down and the bacteria were allowed to grow at 37°C for 24-48 hours prior to enumeration and further identification. Pure well-isolated colonies were preceded for their biochemical tests according to Bergeys manual of determinative bacteriology (Holt et al., 1994).

Antibiotic resistance testing

All trials were piloted using the inventive typical cultures to escape the unstructured hurt of antibiotic resistance. Antibiotic resistance was tested using a modified Kirby-Bauer disc diffusion method (Robert et al., 2003). β-lactamase detection and curing test was carried out as described by Odugbemi et al., (1977).

Plasmid Analysis

The plasmid isolated by mini-prep alkaline extraction method (Sambrook et al., 1989). Concentrations and purity of DNA were assessed spectrophotometrically using Spectro 22, Labo Med, Inc., USA. Gels were arranged by adding 1% agarose and 5 µL ethidium bromide (10 mg/mL) to the TBE buffer. Pure DNA sample

Table 1. Showing distribution of collected urine samples during study

| Patients        | Age   | No. Cases | Urine samples with glucosuria positive | Urine samples with glucosuria and infection positive |
|-----------------|-------|-----------|---------------------------------------|-----------------------------------------------------|
| Male patients   | Less 30 | 1         | 1                                     | 1                                                   |
|                 | 30-    | 17        | 7                                     | 7                                                   |
|                 | 40-    | 96        | 52                                    | 34                                                  |
|                 | 50-    | 104       | 54                                    | 44                                                  |
|                 | 60-    | 118       | 59                                    | 48                                                  |
|                 | 70-    | 39        | 20                                    | 11                                                  |
|                 | 80 – more | 15       | 10                                    | 9                                                   |
| Total           | 390    | 203       | 154                                   |                                                     |
| Female patients | Less 30 | 1         | 0                                     | 0                                                   |
|                 | 30-    | 16        | 10                                    | 7                                                   |
|                 | 40-    | 95        | 54                                    | 41                                                  |
|                 | 50-    | 69        | 44                                    | 34                                                  |
|                 | 60-    | 81        | 52                                    | 39                                                  |
|                 | 70-    | 35        | 21                                    | 15                                                  |
|                 | 80 – more | 7        | 4                                     | 2                                                   |
| Total           | 304    | 185       | 138                                   |                                                     |

| Total           | 694    | 388       | 292                                   |                                                     |
(3µL) was added to 12µL deionized water and 1µL endonuclease (EcoR I, Hind III (Sigma Production), BamHI (Roche Diagnostics GmbH)). Sequential dilutions of acridine orange were used for curing of isolated plasmids.

RESULTS

A full of 292 urine trials were found infection positive from all different ages and cases admitted to the General Health Insurance Authority Hospital (Table 1). The percentage of infection raised by increase the concentration of glucosuria. We detected 100% a bacterial infection in urine samples of ++++ glucosuria level on CLED medium. Also percentage of counts on MacCkonky medium increased from 35.21% on + glucosuria level to 66.67% on ++++ glucosuria level.

A whole of 302 bacterial isolates were isolated from urine samples. The distribution of staphylococci and E. coli is shown in Table 2. The incidence of the S. saprophyticus, S. aureus, E. coli and other species isolates from the urine samples are 54.7%, 34%, 7.6% and 3.7%, respectively (Table 2). Bacterial counts had flocculation on + and ++ glucosuria concentration but other concentration in female patients recorded high bacterial counts than male patients (Table 2).

Table 2. Showing distribution of identified bacterial species in male and female patients according to glucosuria level

| Glucosuria level | +   | ++  | +++ | ++++ | Total (%) |
|------------------|-----|-----|-----|------|-----------|
| Bacterial isolates | M  | F  | M  | F  | M  | F  | M  | F  | M  |  |
| S. saprophyticus  | 15  | 8  | 34  | 19 | 40 | 46 | 1  | 2  | 165 (54.7) |
| S. aureus        | 8   | 11 | 16  | 13 | 21 | 34 | 0  | 0  | 103 (34) |
| E. coli          | 4   | 1  | 3   | 4  | 5  | 6  | 0  | 0  | 23 (7.6)  |
| Other Species*   | 2   | 1  | 1   | 3  | 1  | 3  | 0  | 0  | 11 (3.7)  |
| Total            | 50  | 93 | 156 | 3  | 302 |

* Other species include: Pseudomonas aeruginosa and Streptococcus sp.

The antibiotic resistance profile of staphylococci and E. coli isolates from urine samples is shown in Table 3. Most bacterial isolates were more susceptible to oflaxacin antibiotic while resistance to ampicillin. MAR was main for bacterial isolates gained from urine samples. Greater degrees of resistance were confirmed for S. aureus as compared with S. Saprophyticus versus most studied antibiotics. S. aureus showed high resistance percentage than other bacterial strains. Alls of staphylococci isolates were susceptible to vancomycin, except 23% of S. aureus (Table 3). E. coli isolates showed low resistance pattern to studied antibiotics. Susceptibility to imipenem and septazole (trimethoprim and sulfamoxazole) antibiotics for E. coli was 100% (Table 3). β-lactamase enzyme manufacture was confirmed in all staphylococci and E. coli isolates. Out of 165 isolates S. saprophyticus, 71% were β-lactamase producers, while all S. aureus and E. coli isolates were β-lactamase positive.

Plasmid outlines of the three bacterial

Fig. 1. Agarose gel electrophoresis of pure plasmids preparation from isolated strains. (A) lanes 1-3 pure plasmids of E. coli, S. aureus and S. saprophyticus respectively. (B) The restriction patterns of plasmids isolated from E. coli and S. aureus bacterial strains (contain only one plasmid) digested with Hind III, EcoR I and BamHI respectively. Where: Lane M: Lambda (3) DNA + Hind III + EcoR I, lanes 1-3: E. coli and lanes 4-6: S. aureus
isolates below study were strongminded (Table 4). *E. coli* and *S. aureus* contain one plasmid, though *S. saprophyticus* were establish to three plasmids (Fig. 1A). Breakdown of isolated plasmids of *E. coli* and *S. aureus* isolates presented in fig. 1B. Quantity of recognition sites, amount of fragments and the estimated molecular size of restricted fragments were as shown in Table 4.

**Table 3.** Antibiotic resistance profiles of isolated bacteria from urine samples

| Antibiotics / Isolates | Bacterial isolates resistance to antibiotics (%) |
|------------------------|-----------------------------------------------|
|                        | *S. saprophyticus* (no. = 165) | *S. aureus* (no. = 103) | *E. coli* (no. = 23) |
| Nitrofurantoin (300 µg) | 70 | 79 | 95 |
| Imipenem (10 µg) | 40 | 19 | 100 |
| Aztreonam (30 µg) | 100 | 100 | 76 |
| OFLAXACIN (5 µg) | 50 | 56 | 60 |
| Amoxicillin (25 µg) | 0 | 20 | 0 |
| Erythromycin (5 µg) | 79 | 100 | 40 |
| Trimethoprim (125 µg) + Sulfamoxazole (23 µg) | 60 | 80 | 100 |
| Amikacin (30 µg) | 36 | 20 | 60 |
| Cefotaxime (30 µg) | 60 | 100 | 40 |
| Amoxicillin + Clavulanic acid (30 µg) | 60 | 100 | 80 |
| Ampicillin (10 µg) | 71 | 100 | 100 |
| Tobramycin (10 µg) | 53 | 17 | 39 |
| Norfloxacin (10 µg) | 40 | 54 | 29 |
| Vancomycin (30 µg) | 0 | 23 | ND |

N: Number of studied isolates; ND: Not Determined.

**Table 4.** The restriction patterns of plasmids from *E. coli* and *S. aureus* isolates

| Plasmids | Restriction Enzymes | No. of Recognition Sites | No. of Fragments | Size of Fragments (Kbp) | Plasmid Size (Kbp) |
|----------|---------------------|--------------------------|-----------------|-------------------------|------------------|
| *E. coli* | Hind III            | 6                        | 5               | 23000-9416-5148-4361 and 3530 | 44.64 |
|          | EcoR I              | 6                        | 5               | 23100-9416-5148-3410 and 3410 | 39.306 |
|          | BamH I              | 5                        | 4               | 23000-9416-5804 and 5643 | 2322 |
| *S. aureus* | Hind III          | 6                        | 5               | 23000-6557-5148-4973 and 3530 | 39.306 |
|          | EcoR I              | 6                        | 5               | 22000-5804-4973-3530 and 2037 | 39.306 |
|          | BamH I              | 8                        | 7               | 9416-7421-5148-5100-3430-3530 and 2322 | 2322 |

**DISCUSSION**

Patients with diabetes frequently have enlarged difficulties of UTI, harshness and unfamiliar expressions (Stepleton, 2002). The risk for UTI complications is higher in people with DM. The foremost reasons of severe and straightforward UTI in ambulatory patients have been informed to be due to *E. coli, S. aureus* and *Pseud. Aeruginosa* (Foxman et al., 2000; Hoepelman et al., 2003). *E. coli* origins the common of UTIs between utmost clusters of patients deprived of diabetes and is likewise the maximum joint cause of UTI between men or women with diabetes (Parker et al., 2004). In our studies find similar results.

From the above data it can be mostly settled that staphylococci and *E. coli* isolates are relatively resistant to commonly used antibiotics. Resistance rates between staphylococci have been testified from other domestic geographical areas with results similar to ours (HEl-Kholy et al., 2003H). Amongst isolates of *E. coli*, 100% were resistance to ampicillin. Completely isolates were
susceptible to imipenem and septazole. *E. coli* isolates in our study were have resistant outline to the antibiotics like to those from the El-Kholy *et al.* 2003. The occurrence of MAR organisms has attracted the attention of many workers and increasing antibiotic resistance is a factual problem in UTI (Foxman, 2002). It occurs from the infected patients who respond poorly to treatment and in a hospital, which may perform infection control and prevention programs. By definition, patients with complex UTI have a superior possibility of infection with an antimicrobial-resistant organism and/or an uncommon organism and also have a larger hazard of treatment disappointment (Patterson and Andriole, 1995; Ronald and Harding, 1997).

Most antibiotic-resistant microbes develop as a consequence of genetic change and following assortment courses by antibiotics. The resistance factor may be chromosomal, that developed as a result of spontaneous mutations and extrachromosomal resistance (Bekowitz, 1995; Selim, 2003). Plasmid-mediated antibiotic resistance in *S. aureus* has been described by many authors (Archer *et al.*, 1986; Lyon and Skurray, 1987; Mansouri and Khaleghi, 1997; Shakibaie *et al.*, 1999; Selim, 2003). The plasmid-linked resistance outlines of three bacterial ampicillin-resistance isolates were examined. For them plasmids were experimental and plasmid-linked resistance to ampicillin was established with relatively high MIC(S). The frequency of penicillin and cephalosporin-resistant clinical isolates of *S. aureus* has enlarged quickly and most of the isolates are -lactamase producers. In greatest strains, the enzyme is determined by plasmid but can also be establish on the chromosome (Shakibaie *et al.*, 1999; Ako-Nai *et al.*, 2005).

In, our data propose that antimicrobial resistance amongst staphylococci and *E. coli* is joint and important in diabetic patients in Egypt. The results obtained here, we confidence, will improve clinicians’ ability to improved achieve these diseases and decrease probable development. Our outcomes request for additional molecular biology studies to describe genes involved in antimicrobial resistance patterns in these isolates.

**ACKNOWLEDGMENTS**

None.

**CONFLICTS OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

**FUNDING**

None.

**DATA AVAILABILITY**

All datasets generated or analyzed during this study are included in the manuscript.

**ETHICS STATEMENT**

The study was approved from the Universities Ethical Committee (ENREC).

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