Antibiotic sensitivity of common bacterial pathogens against levofloxacin

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Abstract
Levofloxacin is a member of the fluoroquinolone class of antibacterial agents with a safe and effective treatment. The aim of this study is to evaluate the susceptibility and resistance pattern of clinical isolates causing different types of infections to Levofloxacin. In vitro antibacterial activity and resistance patterns of levofloxacin antibiotic were studied using disk diffusion method. A total 485 strains isolated from various clinical specimens from September 2020 to November 2020 in Al Saleem Medical Laboratory. This is for isolation and identification of pathogenic gram negative and gram positive bacteria. The samples were involving urine, semen, swabs, High vaginal swab, Blood, Stool, CSF, Body Fluid and Sputum. The isolates were identified by biochemical tests as well as the sensitivity and resistant were tested by levofloxacin antibiotic. In present study, gram negative bacteria were prevalent, 75.6% gram positive bacteria 24.4%. In the four hundred eighty five clinical isolates comprising of Escherichia coli, Klebsiella spp, Staph Aureus, Enterobacter spp, Streptococcus pneumonia, Pseudomonas spp, Streptococcus pyogen, Citrobacter spp, proteus spp and Staph epidermidis were collected from different clinical specimens. The predominantly isolated bacteria were Escherichia coli, followed by Klebsiella spp, Staph Aureus and Enterobacter spp. The levofloxacin antibiotic has sensitive against most of the isolates, E Coli (31%), Klebsiella spp (14%), Staph Aureus (8.7), Enterobacter spp (6%), strep pneumonia (5%), Pseudomonas spp (3%), Strep pyogen (1%) and Citrobacter spp (0.6%). Levofloxacin exhibited statistically significantly high bactericidal activity against all strains of gram positive and gram negative bacteria. Levofloxacin was shown to be active against both Gram-positive and Gram-negative bacteria.

Keywords: Al Saleem Medical Laboratory, E coli, klebsiella spp, levofloxacin, urine

1. Introduction
Quinolones are group of synthetic broad spectrum antibiotics [1]. Levofloxacin is a member of the fluoroquinolone class of antibacterial agents with a safe and effective treatment on the World Health Organization's essential medicines and has microbiological activity against clinically related bacteria that cause nosocomial pneumonia, community-acquired pneumonia, genitourinary tract infections, respiratory and skin and skin structure [1-4]. Antibiotics are effective against microorganisms, but the reality is no antibiotic inhibits entirely microorganisms. Certain microorganisms are naturally resistant, while some acquire resistance either by altering permeability, or by producing enzyme that inactivates the antibiotics or by modifying target site or by plasmid mediated resistance [1-2,5]. Levofloxacin has been shown to be the more active component, with antibacterial activity 8-128 times that of D-oxofloxacin, and this difference in activity associates with differences in the DNA Gyrase inhibitory activities of the two compounds [6-7]. Norrby et al. [4] have reported that, levofloxacin is frequently used in clinical practice and it is less probable to select resistant strains. There are many common bacterial infections such as boils, folliculitis, eczema, cellulitis, caused by Pseudomonas aeruginosa and Staphylococcus Aureus. Nosocomial infection in hospital by Staphylococcus Aureus merits special attentiveness because of their prevalence and resistant to a number of antibiotics and chemotherapeutic agents. The aim of this study is to evaluate the susceptibility and resistance pattern of clinical isolates causing different types of infections to Levofloxacin.
2. Material and Methods

The work was carrying out to isolate the causative bacterial agents from various clinical samples. The clinical samples included in this study were collected and tested at a department of microbiology, the Al Saleem Medical Laboratory, Benghazi, Libya during a period of September 2020 to November 2020. The total 485 samples were collected in a sterile container and investigated. Clinical specimens were tested 3 months period at limited patient information, including Age and sex, health status and use of medicines or antibiotics.

The specimens comprising, sterile body fluids, joint fluid, pleural fluid, peritoneal fluid and swab sticks used for the collection of the samples were streaked directly on Chocolate, MacConkey agar and blood agar plate and incubated at 37°C for 24 hours [8-10]. Blood cultures were done in Brain Heart Infusion biphasic medium. Subcultures were done Macconkey agar and Blood agar and incubated at 37°C for 24 hours at the earliest visual detection of turbidity or blindly on days 1, 4 and 7 if the bottles did not show turbidity. Specimen was collected by Curet or surgical specimen and transport immediate and cultured on Blood agar Chocolate agar, MacConkey - aerobic and anaerobic blood agar for 35°C, Co2. Surgical specimens or aspirates were collected by Transport medium and cultured on Blood agar Chocolate agar, MacConkey - aerobic and anaerobic blood agar for 35°C, Co2. Wound (surface), eye Swab (small) for each eye or Corneal scraping, burn, Nasopharynx, throat swabs were taken from patients by Stuart’s, Amies and were cultured on Blood agar Chocolate agar and incubated at 37°C for 24 hours. Wound (deep) and Abscess drainage were collected by using of syringe or anaerobic swab kit and transport aspirate in the collecting syringe or Place aspirate into anaerobic transport container or vial or Collect pus onto swab and place directly into anaerobic transport. Genital specimen was collected by swab and transport immediate and cultured on Modified Thayer-Martin and Blood agar Chocolate agar, MacConkey - aerobic and anaerobic blood agar for 35°C, Co2. Collection of ear aspirates from otitis media or swab from of drainage and transport using of Transport medium, MacConkey agar 35°C, aerobic Blood agar, Chocolate (eye) 35°C, Co2.

The isolated pure colonies were identified by colony morphology characteristics and gram staining. Depending upon their colony morphology and their gram staining characters, they were separated as Gram Positive COCCI or Gram negative bacilli [10-12]. Pure strains were subjected to all biochemical tests like oxidase, catalase, coagulase, urease, Inddol test, citrate test, IMViC Triple sugar iron etc. were used to characterize the isolates [13].

Biochemical identification tests to identify Gram negative bacteria. For this purpose, samples were inoculated in Triple Sugar Iron media (TSI), Citrate media and kept in an incubator for 18hrs at 37°C. Staphylococcus spp were further tested for the production of free coagulase enzyme using tube coagulase test.

The samples were processed for culture and sensitivity testing in the department of microbiology. McFarland Standards is used in the antimicrobial susceptibility testing procedure where the bacterial suspension is compared to Standard McFarland, prior to swab on Muller Hinton agar. It is a part of quality control to check and adjust the densities of bacterial suspension that can be used for identification and susceptibility proceeds. However, used concentration for the antimicrobial susceptibility testing and the culture media performance testing is done by 0.5 McFarland standards in the microbiological laboratory. Levofloxacin soaked disk (30µg) were sited with sterile forceps on the Muller Hinton agar surface. The plates were then incubated at 37°C for 24 hours and observed for diameter of zone of inhibition. Results were interpreted as per clinical and laboratory standards institute (CSLI) guidelines [14].

2.1 Methodology: The data analyzed by SPSS programs.

3. Results

3.1 Distribution of types of clinical specimens

Since urine samples received were very much higher in number than other samples. In addition to semen (5.8%), swabs (4.7%), High vaginal swab (2.3%), Blood culture (0.6%), Stool (0.2%) CSF, Body Fluid and Sputum (0.2%) equally.

### Table 1: Distribution of types of clinical specimens

| Sample      | Frequency | Percent | Valid Percent | Cumulative Percent |
|-------------|-----------|---------|---------------|--------------------|
| Blood       | 3         | .6      | 6             | 6                  |
| HVS         | 11        | 2.3     | 2.3           | 2.9                |
| CSF         | 1         | .2      | .2            | .3                 |
| Body fluid  | 1         | .2      | .2            | .3                 |
| Semen       | 28        | 5.8     | 5.8           | 9.1                |
| Sputum      | 1         | .2      | .2            | .3                 |
| Stool       | 2         | .4      | .4            | .7                 |
| swab        | 23        | 4.7     | 4.7           | 14.4               |
| Urine       | 415       | 85.6    | 85.6          | 100.0              |
| Total       | 485       | 100.0   | 100.0         |                    |

**3.2 Distribution of isolates in clinical specimens collected from patients**

From table 2 it is clear that the Escherichia coli (41%) is most predominant (37.5%) in urine infections followed by Klebsiella spp (16.1%), staph Aureus (12.8%) and Enterobacter spp (11.8%).

### Table 2: Distribution of isolates in clinical specimens collected from patients

| Bacteria      | Frequency | Percent | Valid Percent | Cumulative Percent |
|---------------|-----------|---------|---------------|--------------------|
| Citrobacter spp | 7        | 16.1    | 16.1          | 16.1               |
| E. coli       | 199       | 41.0    | 41.0          | 41.9               |
| Enterobacter spp | 57      | 11.8    | 11.8          | 53.6               |
| Klebsiella spp | 78       | 16.1    | 16.1          | 69.7               |
3.3 Distribution of pathogenic bacteria in variable medical diagnosis
A total of 367 Gram-negative and 118 Gram-positive isolates from the urine (85.6%), semen (5.8%), swab (4.7%), HVS (2.3%), blood (1%), stool (2%) and sputum (0.2%) were tested.

Table 3: Distribution of pathogenic bacteria in variable medical diagnosis

| Bacteria                          | Blood | HVS | Semen | Swab | Stool | Urine | Total |
|----------------------------------|-------|-----|-------|------|-------|-------|-------|
| Citrobacter spp                  | 0     | 0   | 0     | 0    | 0     | 4     | 4     |
| E. coli                          | 0.0%  | 0.0%| 0.0%  | 0.0% | 0.0%  | 0.8%  | 0.8%  |
| Enterobacter spp                 | 1     | 0   | 6     | 0    | 1     | 46    | 57    |
| Klebsiella spp                   | 3     | 1   | 3     | 1    | 3     | 67    | 78    |
| proteus spp                      | 0     | 0   | 0     | 0    | 0     | 3     | 3     |
| Pseudomonas spp                  | 2     | 2   | 0     | 0    | 0.0%  | 0.6%  | 0.6%  |
| Staph albus                      | 0     | 0   | 1     | 0    | 7     | 54    | 62    |
| Staph epidermidis                | 0     | 0   | 0     | 0    | 1     | 0     | 1     |
| Strep Penumonia                  | 0     | 4   | 5     | 0    | 1     | 37    | 47    |
| Strep pyogen                     | 0     | 1   | 2     | 0    | 0     | 5     | 8     |
| Total                            | 5     | 11  | 28    | 1    | 2     | 415   | 485   |

3.4 Distribution of sample during the months
Majority of specimens 196 (40%) were cultured during September, followed by 172 (35%) in October, while the lowest specimens 117 (24%) was cultured during December.

Table 4: Distribution of sample during the months

| Years    | Sample | Percent | Valid Percent | Cumulative Percent |
|----------|--------|---------|---------------|--------------------|
| 9.2020   | 196    | 40.4    | 40.4          | 40.4               |
| 10.2020  | 172    | 35.5    | 35.5          | 75.9               |
| 11.2020  | 117    | 24.1    | 24.1          | 100.0              |
| Total    | 485    | 100.0   | 100.0         |                    |

3.5 Distribution of different sample during the months
In this following study, the majority of samples were collected in September, followed by October (35%).

Table 5: Distribution of different sample during the months

| Sample                | 9.2020 | 10.2020 | 11.2020 | Total |
|-----------------------|--------|---------|---------|-------|
| Blood                 | 2      | 1       | 0       | 3     |
| High Vaginal Swab     | 2      | 4       | 5       | 11    |
| CSF                   | 0      | 1       | 0       | 1     |
| Body fluid            | 0      | 1       | 0       | 1     |
| Semen                 | 11     | 9       | 8       | 28    |
| Sputum                | 0      | 1       | 0       | 1     |
| Stool                 | 0      | 2       | 0       | 2     |
3.6 Distribution of isolates during the months

In the presented study, majority of isolates were isolated during September. *E. coli* 73 (15.31%) isolates, followed by *Klebsiella* spp 91 (18.52%) and *staph aureus* 72 (14.98%) isolates, 44 (70.9%) and *E. coli* but this activity is not 100% resistant to Levofloxacin. While 125 (25.8%) were found to be resistant to it. 125 (70.9%) were found to be resistant to Levofloxacin. Out of 485 bacterial isolates, 73 (15.31%) isolates, followed by *Klebsiella* spp 39 (8%) and *staph aureus* 27 (5.6%) were considered to be the most isolates who have been isolated this month.

### Table 6: Distribution of isolates during the months

| Bacteria                  | Month       | Total     |
|---------------------------|-------------|-----------|
|                           | 3.90        | 10.20     | 11.20     |
| *Citrobacter* spp         | 3           | 1         | 0         |
| *E. coli*                 | 73          | 69        | 57        |
| *Enterobacter* spp        | 24          | 18        | 15        |
| *Klebsiella* spp          | 39          | 21        | 18        |
| *Proteus* spp             | 2           | 1         | 0         |
| *Pseudomonas* spp         | 5           | 12        | 9         |
| *Staph aureus*            | 27          | 23        | 12        |
| *Staph epidermidis*       | 1           | 0         | 0         |
| *Strep pneumonia*         | 14          | 27        | 6         |
| *Strep pyogen*            | 8           | 0         | 0         |
| Total                     | 196         | 172       | 117       |

3.7 Sensitivity of gram positive and gram negative organism groups to Levofloxacin

All the bacterial isolates were tested for susceptibility against Levofloxacin. Out of 485 bacterial isolates, 44 (70.9%) and 16 (3.3%) of the isolates remain susceptible and intermediate to Levofloxacin respectively. 125 (70.9%) were found to be resistant to Levofloxacin. While 125 (25.8%) were found to be resistant to it.

### Table 7: Sensitivity of gram positive and gram negative organism groups to Levofloxacin

| Levofloxacin | Frequency | Percent | Valid Percent | Cumulative Percent |
|--------------|-----------|---------|---------------|--------------------|
| Intermediate (I) 14-16mm | 16 | 3.3 | 3.3 | 3.3 |
| Resistant (R) ≤13mm | 125 | 25.8 | 25.8 | 29.1 |
| Sensitive (S) ≥ 17mm | 344 | 70.9 | 70.9 | 100.0 |
| Total | 485 | 100.0 | 100.0 |

3.8 Sensitivity of gram positive and gram negative organism groups to Levofloxacin

The levofloxacin antibiotic has sensitive against all the isolates, *E. coli* (31%), *Klebsiella* spp (14%), *staph Aureus* (8.7), *Enterobacter* spp (6%), *strep pneumonia* (5%), *Pseudomonas* spp (3%), *Strept pyogen* (1%) and *Citrobacter* spp (0.6%). Levofloxacin has antibacterial activity against Escherichia coli but this activity is not 100%
3.9 Resistance and sensitivity profile of clinical isolates to Levofloxacin during the months

More susceptible of various bacteria to the levofloxacin (31%) was recorded in September, while the higher resistant rates (11%) were observed in October.

Table 9: Resistance and sensitivity profile of clinical isolates to Levofloxacin during the months

| Levofloxacin | Date       | Total |
|--------------|------------|-------|
|              | 9.2020     | 10.2020 | 11.2020 |       |
| Intermediate | 5          | 5       | 6       | 16    |
|              | 1.0%       | 1.0%    | 1.2%    | 3.3%  |
| Resistant    | 40         | 54      | 31      | 125   |
|              | 8.2%       | 11.1%   | 6.4%    | 25.8% |
| Sensitive    | 151        | 113     | 80      | 544   |
|              | 31.1%      | 23.3%   | 16.3%   | 70.9% |
| Total        | 196        | 172     | 117     | 485   |
|              | 40.4%      | 35.5%   | 24.1%   | 100.0%|

4. Discussion

Of the 485 samples collected with positive bacterial growth, only 10 isolates were obtained. During the present study, antimicrobial agent Levofloxacin was used against 10 clinical isolates of *E. Klebsella coli*, staph aureus, *Enterobacter* spp, *Strep pneumonia*, *Pseudomonas* spp, *Strep pyogen*, *Citrobacter* spp, *proteus* spp and *Staph epidermidis*. This is corroborating the work done by Joseph et al. [19] on Antimicrobial susceptibility pattern of *Escherichia Coli* Isolates from Clinical Sources. In our present study, 75.6% of infections were caused by gram negative bacteria and 24.3% by gram positive bacteria. The dissimilar majority of gram positive bacteria have been observed in previous studies conducted in the India, USA and Pakistan [16-18]. Our study has shown *E. coli* as a predominant bacterial isolate which is in line with previous studies done in Jamma University Specialized Teaching Hospital and Referral Hospital [19-20]. In contrast to the above observation, some studies in India [11-21] reported *Staph aureus* to be the most common cause of a wide range of infections, ranging from mild skin infections to wound infections and bacteremia. This study revealed that clinical isolates collected from Al saleem laboratory were susceptible to Levofloxacin. Some strains of *E. coli* and *Klebsella* spp were resistant to levofloxacin. However; my results showed *Klebsella* spp, *Staph aureus* and *Pseudomonas* spp were highly sensitive to levofloxacin [13]. This observation corroborates with other study reporting the same antimicrobial susceptibility activity of these isolates to levofloxacin [25]. *Enterobacter* spp and acinetobacter spp were demonstrated high degree of sensitive to Levofloxacin drugs. Based on our findings, levofloxacin regimens demonstrated better activity against *Strep pyogen*, *strep pneumonia* and *staph Aureus*. This observation corroborates with other studies reporting antimicrobial susceptibility patterns of Gram positive bacteria isolated from clinical sample. And contrast with other studies were showed the higher rates of antimicrobial resistance reported among these bacterial pathogens due to extensive use, and haphazard of existing antibiotics in human therapy has resulted in bacteria rapidly increasing resistance to these agents [18-21]. The percentage of the isolates susceptible to levofloxacin for *E. coli* (31%), *Klebsella* spp (14%), *staph aureus* (8.7), *Enterobacter* spp (6%), *strep pneumonia* (5%), *Pseudomonas* spp (3%), *Strep pyogen* (1%) and *Citrobacter* spp (0.6%).

5. Conclusion

Levofloxacin is a broad-spectrum antibiotic and one of the new fluoroquinolones to which the most common clinical isolates in Benghazi are susceptible. Levofloxacin is well endured, and is associated with lowest side effects. Bacteria show different effect on levofloxacin antibiotics, most of them are sensitive; some are sensitive and moderately sensitive to levofloxacin.

6. References

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