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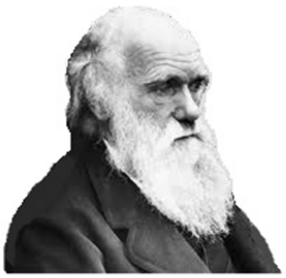

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Evaluating Cephalosporin Resistance in Pathogenic Bacteria Isolated Clinically

Falah Hasan Obayes AL-Khikani*, Bashar Jawad Kadim, Aalae Salman Ayit, Mustafa Hamza Abidalali

Department of Microbiology, Al-Shomali General Hospital, Babil, Iraq

*E-mail address: falahgh38@gmail.com

ABSTRACT

Third-generation cephalosporins are a class of b-lactam antibiotics that are often used for the treatment of human infections caused by Gram-negative and gram-positive bacteria. This study aimed to detect the pattern of bacterial antibiotic sensitivity to the third generation of cephalosporins that assist doctors with appropriate empirical therapy. For study purposes, various bacteria were isolated clinically from urine, high vaginal swab (cervical swab), ear and wound samples during the period from November 2019 to March 2020 at Al-Shomali general hospital, Babil, Iraq and a private laboratory in Babil city. A total of 154 patients were involved in this study, samples were processed at the hospital laboratory during this period, diagnosis and antibiotic sensitivity test having been done by routine bacteriological diagnosis, as well as by VITEK 2 system. Three common third-generation cephalosporins; cefotaxime, Cefotazidime, and ceftriaxone were evaluated. Out of these 154 samples, 46 (30%) have bacterial growth. Specimens with bacterial growth were taken from urine, cervical swab, ear discharge and wound infection, the counts being 24, 8, 8, and 6 respectively. All 46 isolated bacteria were 100% resistant to cefotaxime and Cefotazidime, while 36 (78%) were resistant to ceftriaxone. The prevalence of bacterial isolation in different specimens showed a high predominance of *Enterococcus* spp 16 (35%) from the total samples. The current study revealed that the increasing burden of bacterial resistance to third-generation cephalosporins, especially to cefotaxime and Cefotazidime, may due to misuse and inappropriate high administration of these drugs. This should be countered by early detection of development third-generation resistance in patients by restricted clinical monitoring and through judicious use of antibiotics. Of note, the highest rate of resistance was observed in age groups less than 15 years old.

***Keywords*:** Cephalosporins, antibiotic resistance, ceftriaxone, third-generation cephalosporins, cefotaxime, antimicrobial agents

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1. INTRODUCTION

The third-generation cephalosporins are broad-spectrum antibiotics that possess activity against both gram-negative and gram-positive bacteria. However, these drugs are more effective against gram-negative organisms and bacteria that are resistant to the first and second-generation cephalosporins [1]. Over the past decade, resistance predominance in hospital-acquired infections has risen dramatically; Infections caused by resistant organisms are thought to result in prolonged hospitalization, and higher morbidity and mortality.

Third-generation cephalosporin use began with cefotaxime being released to market 30 years ago, and resistance in bacterial species emerged a few years later due to selective pressure exerted by these new cephalosporins [2]. At present, antibiotic resistance is considered a global health emergency and extended-spectrum beta-lactamase-producing bacteria that can resist third-generation cephalosporins are on the rise and increasing with time [1].

Cephalosporins are bactericidal; acting by disrupting the synthesis of the cell wall layers, and while they have the same mode of action as other β-lactam antibiotics like penicillin, they are less susceptible to β-lactamases.

During the past fifteen years, dissemination and developing of β-lactam resistance in nosocomial gram-negative bacteria and *Pseudomonas aeruginosa* became a serious problem globally, especially the increasing resistance to third and fourth generation cephalosporins [2]. As with most beta-lactam antibiotics, third-generation cephalosporins are generally well tolerated and characteristically have a low toxicity profile. However, some toxicity profiles may be particularly severe. For instance, reports exist of coagulopathies leading to bleeding with the use of third-generation cephalosporins [1]. All told, recorded adverse effects of third-generation cephalosporin adminitration are few in number in comparison to the fetal adverse effects of some antibiotics [3, 4].

The information derived from this study would be helpful in establishing empiric therapy guidelines to prevent the emergence of further resistance and to contribute data to larger and more extensive surveillance programs.

2. MATERIAL AND METHODS

The study protocol has been approved by the Ethical Committee in the Babil Health Directorate on October 20, 2019. Verbal approval was also gained from the patients before obtaining the sample. Health safety precautions were taken during sampling. This work was done according to the Ethics Committee of the Iraqi Ministry of Health and was performed under agreement with all national regulations.

A total number of 154 patients were entered into the study, and various bacteria were isolated clinically from urine, high vaginal swab, ear, and wound samples during the period from November 2019 to March 2020 at Al-Shomalli general hospital, Babil, Iraq and a private laboratory in Babil city.

UTI patients suffering from clear symptoms of UTI as a complaint of frequent urge to urinate and painful, non-repetitive midstream urine discharge were included in the study.

Other samples taken as ear and wound infection were associated with pus and discharges, while high vaginal swab samples were taken by a specialist doctor from the cervical area. All these specimens were sent to our main laboratory by clinicians. Sampling for direct examination

and basic bacterial-cultured methods were done immediately, while direct inoculation on agar media was also performed immediately.

The bacterial diagnosis was identified by VITEK 2 (Biomerieux). Further identification was done as per routine laboratory protocols. A questionnaire survey was also completed by the enrolled. The antimicrobial susceptibility investigation was done according to Kirby–Bauer's disc diffusion method [7]. Three most common cephalosporins were tested, Ceftriaxone (30 mg), Ceftazidime (30 mg), Cefotaxime (30 mg).

3. RESULTS

Out of 154 samples clinically collected, 114 (74%), 20 (13%), 12 (8%), and 8 (5%) were from urine, cervical swab, wound infection and ear, respectively (Figure 1).

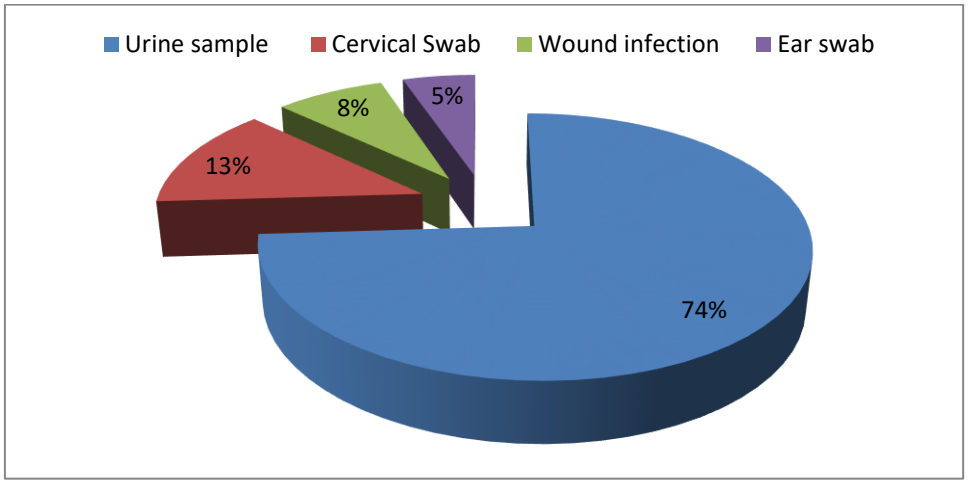

Figure 1. Distribution of samples included in this study

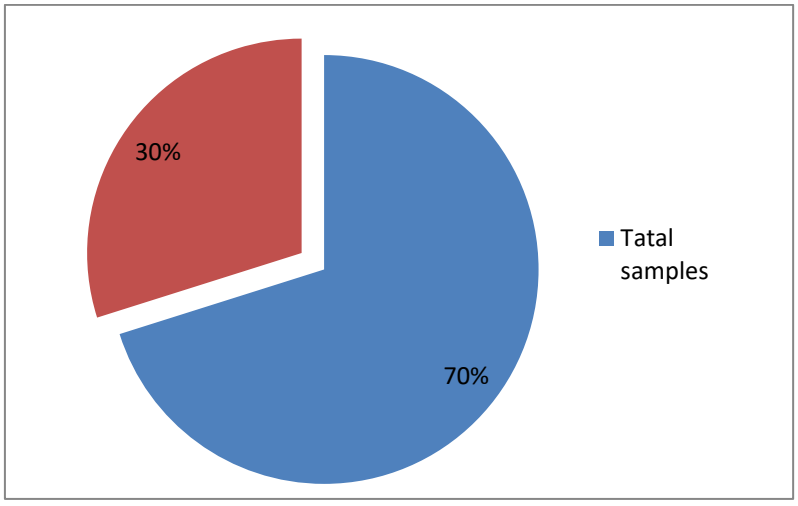

Figure 2. Growth rate of all clinical isolated bacteria

From 154 specimens, just 46 (30%) samples showed bacterial growth on artificial media, being 24, 8, 8, and 6 clear growth from urine, cervical, ear and wound samples, respectively (Figure 2 and Figure 3).

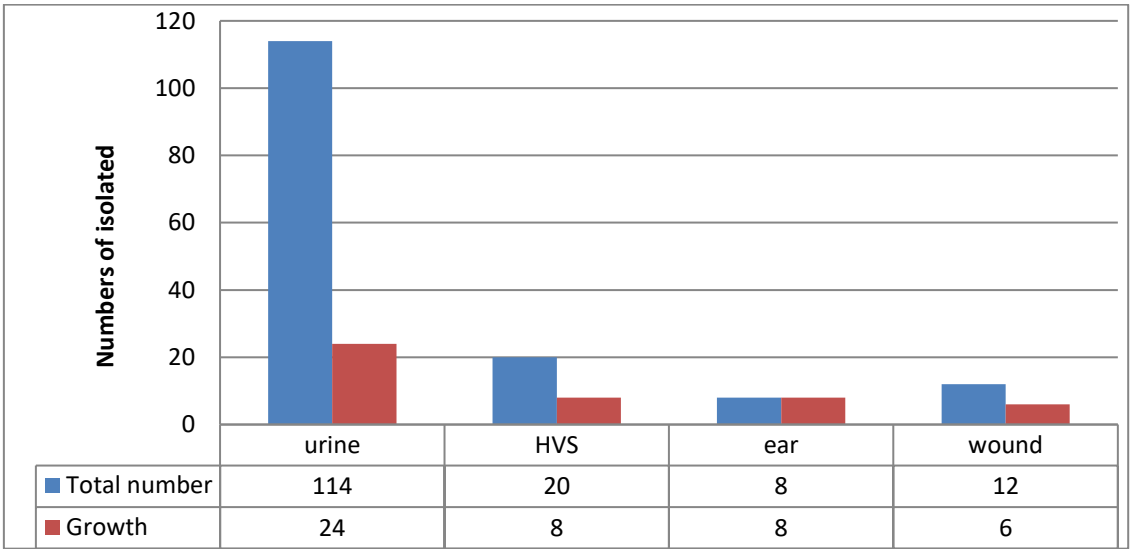

Figure 3. Numbers of growth samples

The bacterial isolates in urine included 12 *Enterococcus spp* and 6 *E. coli*, while *Staph aureus*, *Staph saprophyticus*, and *Klebsiella oxytoca* were 2 for each one. Of those showing bacterial infection, 91% (N = 22) of all patients with UTI were females, while males were represented by just 9% (N = 2) (Figure 4).

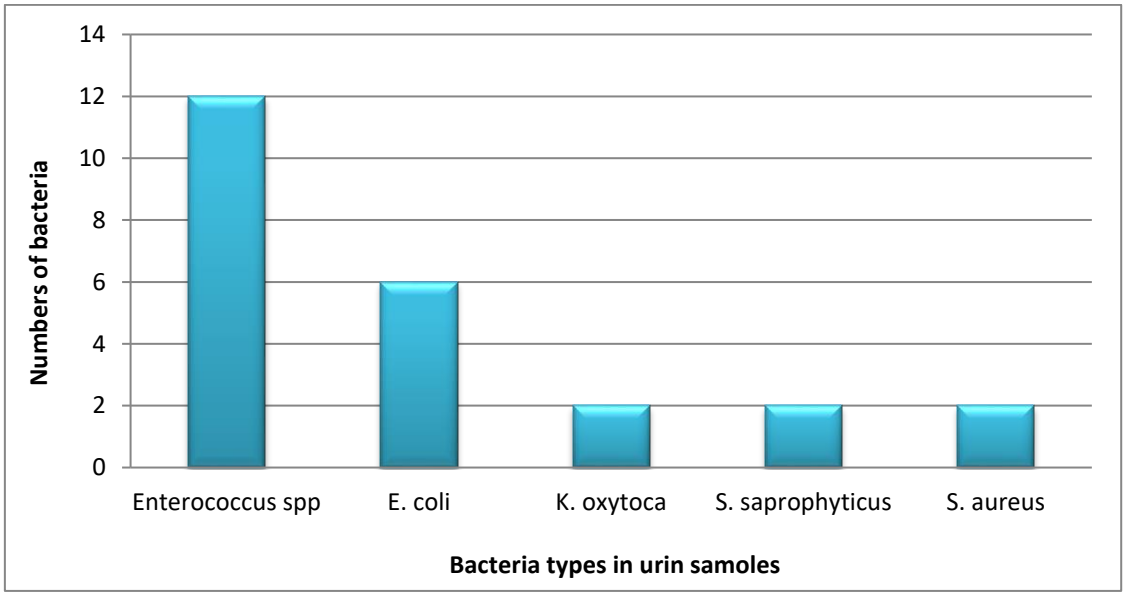

Figure 4. Numbers of isolated bacteria in urine samples.

Bacterial types in ear discharge included two *Proteus mirabilis*, 4 *Staph aureus*, one *P. aeruginosa*, and one mix growth of *Staph aureus* plus *P. aeruginosa*. In cervical samples, 4 *Enterococcus* spp, 2 *E. coli*, and 2 *staph aureus* were found. In wound, 3 *P. aeruginosa*, 2 *Proteus* spp, and one mix growth *P. aeruginosa* plus *E. coli* were noted (Table 1).

Table 1. Bacteria distribution among specimens.

	Ear swab	**Cervical swab**	**Wound infection**	**Urine samples**	**Total**
P. aeroginosa	1	-	3	-	4
S. aereus	4	2	-	2	8
E. coli	-	2	-	6	8
Proteus spp	2	-	2	-	4
Enterococcus spp	-	4	-	12	16
K. oxytoca	-	-	-	2	2
S. saprophyticus	-	-	-	2	2
P. aeroginosa plus S. aureus	1	-	-	-	1
S. aereus plus E. coli	-	-	1	-	1
Total	8	8	6	24	46

Age of involved persons in the current study ranged from 2 to 63 years for all specimens. Distribution of specimens according to age group showed that the 16-30 years of age group is the most common infected groups for urine, cervical and wound specimens. However, half of the patients with ear discharge were in the age group 0-15 years (Table 2).

Table 2. Age group distribution among specimens.

	0-15	**16-30**	**31-45**	**>45**	**Total**
Urine	6	14	-	4	24
Cervical	-	6	2	-	8
Ear	4	2	2	-	8
Wound	-	4	2	-	6
Total	10	26	6	4	46

Table 3 reveals the distribution of isolated bacteria according to age groups of involved individuals. Herein it is evident that *Enterococcus* spp is most prevalent in the age group 16-30 years, while *P. aeruginosa* and *S. aureus* are most predominant in the 0-15 years category (Table 3).

Table 3. Age groups and bacteria distribution.

	0-15	16-30	31-45	> 45	Total
P. aeroginosa	2		2	-	4
S. aereus	4	4	-	-	8
E. coli	-	4	2	2	8
Proteus spp	-	4	-	-	4
Enterococcus spp	3	10	1	2	16
K. oxytoca	-	2	-	-	2
S. saprophyticus	-	2	-	-	2
P. aeroginosa plus S. aureus	1	-	-	-	1
S. aereus plus E. coli	-	-	1	-	1
Total	10	24	6	4	46

Cephalosporin resistance was very high among all isolates, reaching 100% (N = 46) resistance to cefotaxime and ceftazidime, while resistance to ceftriaxone was at 78% (N = 36) from the total clinical isolated bacteria (Figure 5).

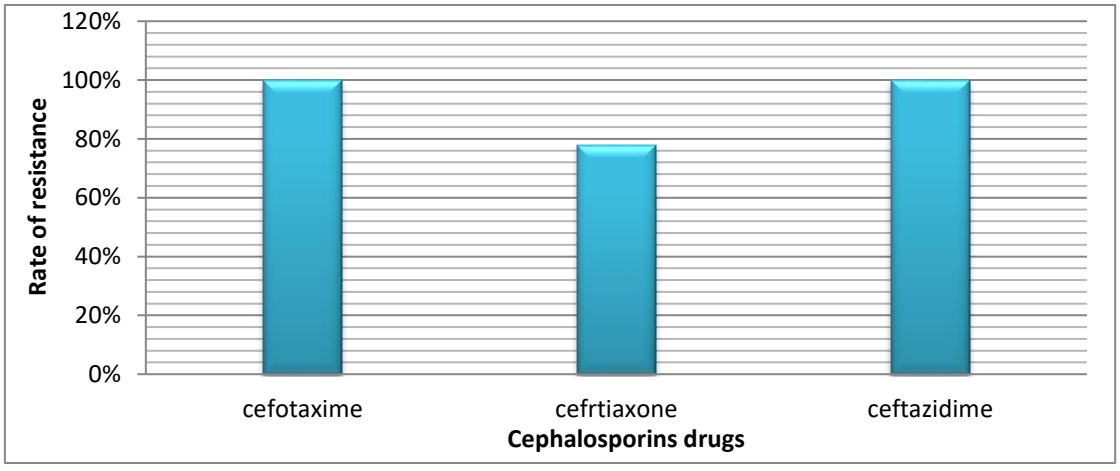

Figure 5. Rate of cephalosporins resistance profile in all samples

Figure 6 displays the prevalence of bacterial isolation in different specimens. What is notable is the high prevalence of *Enterococcus* spp in 16 (35%) samples, followed by both *E. coli* & *S aureus* in 8 (17.5%) specimens each, respectively (Figure 7).

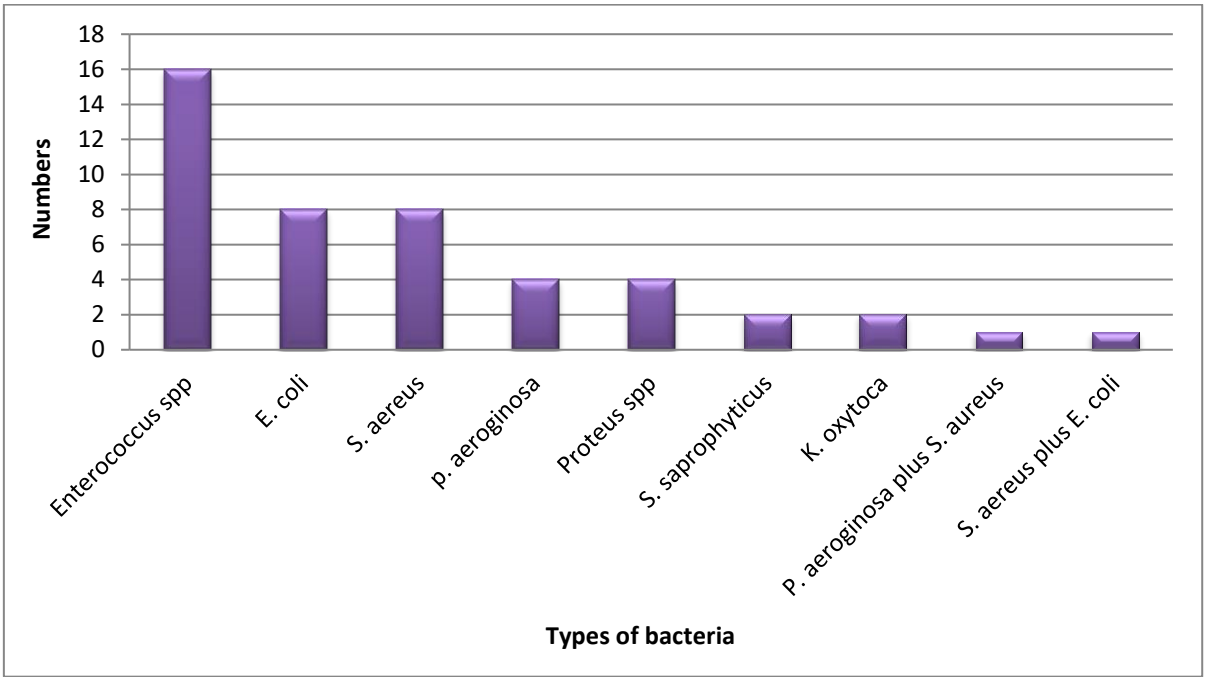

Figure 6. Numbers of bacterial isolation in all specimens

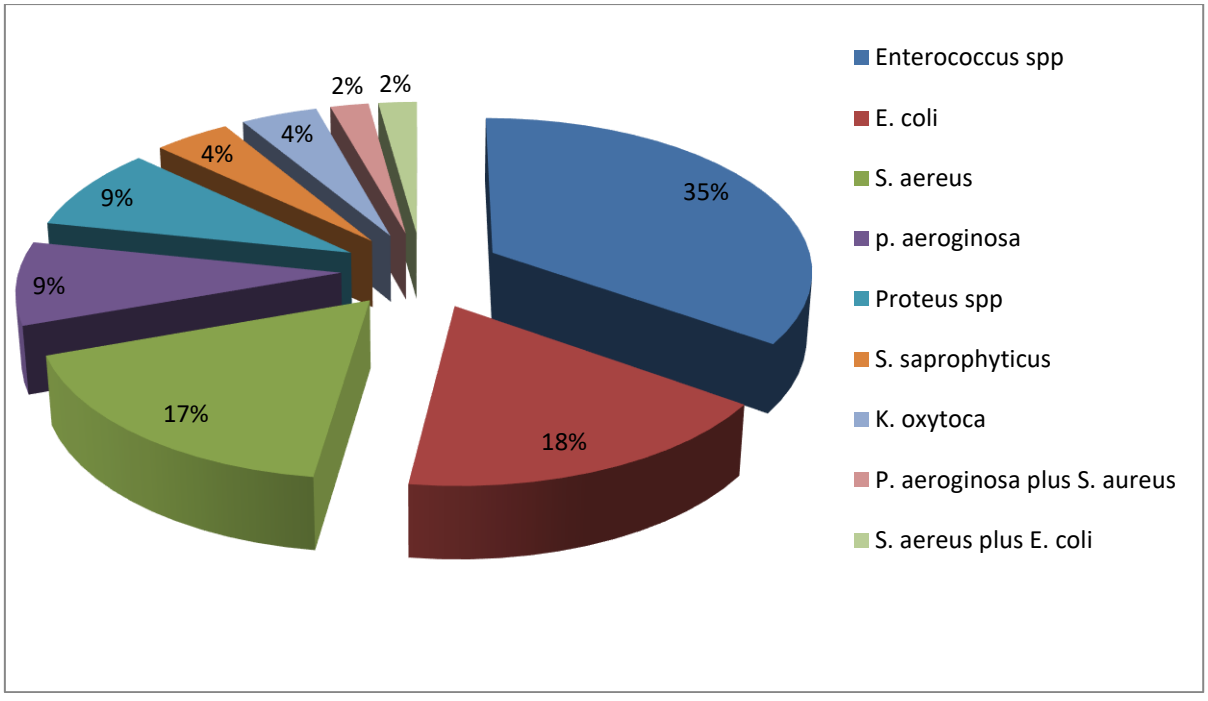

Figure 7. Percentage of isolated bacteria in all specimens

Figure 8 shows the distribution of ceftriaxone resistance among the different age groups. Herein, 100% resistance was seen in the age group 0-15 years, followed by resistance rated as 83% in the age group 16-30 years, while resistance in the age group 31-45 years was 66%. Cefotaxime and ceftazidime saw resistance at 100% for all isolated samples and across the different age groups.

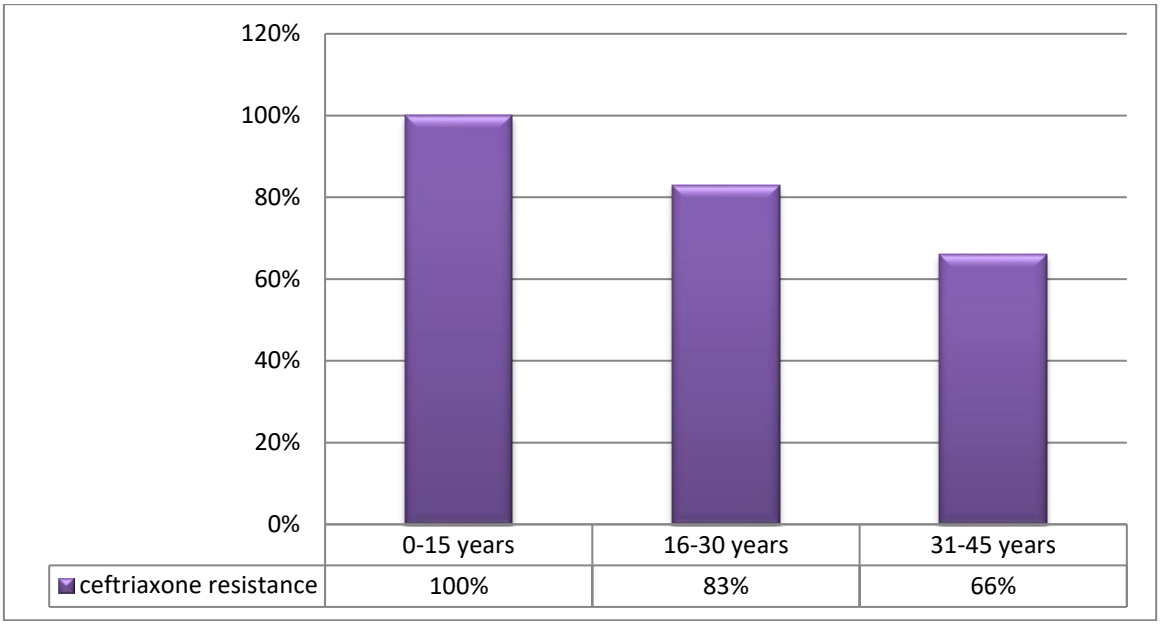

Figure 8. Ceftriaxone resistance according to age group

4. DISCUSSION

Antibiotic-resistant bacteria are becoming a crucial world-wide problem, and are being implicated in increasing morbidity among hospitalized patients. Infections with resistant species result in prolonged stays in hospitals and compromised immune systems. This fact, along with exposure to multiple antibiotics, are main factors that increase the risks for uncontrollable infections and multidrug resistance. The emergence of antibiotic resistance in various bacteria from different specimens is associated with significant adverse outcomes.

In the current study, 12 *Enterococcus* spp and 6 *E. coli*, 2 *Staph aureus*, 2 *Staph saprophyticus* and 2 *Klebsiella oxytoca* bacterial isolates were found in the urine samples. *Enterococcus* spp was the most prevalent bacterial pathogen in UTI, this finding, however, is in disagreement with other studies that found *E. coli* to be the most common prevalent pathogen isolated from UTI - for example, [5] noted *E. coli* prevalence at 42,2 %, and [6] noted *E. coli* prevalence.

This study showed 91% (N = 22) of all patients with UTI were females, while males were represented by just 9% (N = 2). This finding agrees with many studies [7, 8].

Because cephalosporins can penetrate various tissues, this study evaluated third-generation cephalosporins sensitively of clinical bacteria isolated from different parts of the body. Third-generation cephalosporins can cross into genitourinary tract infections, bone and joint infections, blood-brain barrier, and skin and soft tissue infections [1]. Ceftriaxone revealed

78% resistance and it is used less than other antibiotics in this study. This level of resistance maybe due to Ceftriaxone having high protein binding capacity and the longest-half life of antibiotics in this generation and that it is administrable as a once-daily dose. Pharmacokinetically, some third-generation compounds are poorly absorbed in the gastrointestinal tract and are administered only intramuscularly or intravenously. These include ceftriaxone, ceftazidime and cefotaxime. [1]

Some studies also showed increased resistance to cephalosporins, analysis of 31 strains of *P. aeruginosa* showed high resistance to ceftazidime, Mutation-dependent overproduction of intrinsic -lactamase is considered the main cause of resistance [9]. Another study revealed in 2019 that resistance to ceftriaxone was at 86%, cefotaxime at 80%, and ceftazidime at 93% to *Klebsiella pneumonia* [10].

In this work, *K. oxytoca* was resistant to cephalosporin as Ceftriaxone, Cefotazidime, and Cefotaxime, and this agrees with [11]. Almost all isolates of *Klebsiella* species were initially considered to be susceptible to cephalosporin; studies over the last two decades have shown variable susceptibility to this antibiotic class, this resistance is mediated by plasmid-mediated extended-spectrum beta-lactamases (ESBLs) [12-14]. This high rate of cephalosporin resistance that reaches 100% resistance may due to prolonged inappropriate administration of these drugs by doctors, and also little personal education on drug self-administration that is presented by an incomplete full course of antibiotics [1].

5. CONCLUSIONS

Worryingly, the incidence of human infections caused by third-generation cephalosporin-resistant bacteria is increasing worldwide. This is considered a serious public health challenge to patients and to clinicians, so we need to direct more attention to antimicrobial resistance monitoring and surveillance. This must be fundamental for creating and developing effective antimicrobial resistance control strategies and for accurate antibiotic prescriptions in clinical settings. Regular monitoring of the judicious use of antibiotics assists in conserving the effectiveness of sensitive antibiotics and in preventing the emergence of further resistance, as resistance to multiple antibiotics limits the therapeutic options for infections. In this search, all 46 clinical isolated bacteria were 100% (N = 46) resistant to cefotaxime and Cefotazidime, while they were 78% (N = 36) resistant to ceftriaxone. *Enterococcus* spp was the most prevalent bacterial pathogen in UTI. Resistance to third-generation cephalosporins in age groups less than 15 years old is especially notable. The prevalence of bacterial isolation in different specimens showed the high predominance of *Enterococcus* spp in 16 (35%) samples, followed by both *E. coli* at 8 (17.5%) and *S. aureus* also at 8 (17.5%).

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