Ministry Of Higher Educations
And Scientific Research

University Of Al- Qadiysiah
College Of Pharmacy

Evaluation of Ceftriaxone effectiveness against E.coli

Researchers:

Hussein K. Gate

Hussein S. Hussein

Yassir J. Yassir

Supervised by:

Dr. Hussein A. Sahib

2017

بسم الله الرحمن الرحيم

((قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا))

صدق الله العظيم

سورة الكهف، ايه ١٠٩

List of Figures & Tables

Contents

Chapter One
Introduction

1- Introduction

The inexorable rise in multidrug-resistant Gram-negative bacteria has been widely reported. Multiple modes of resistance often present in a single strain of bacteria, and this may also be combined with an increase in virulence, both of which are leading to a significant increase in morbidity and mortality in patients. Against this background, the absolute number of new antibiotics licensed has declined especially for Gram-negative multidrug-resistant pathogens. [1]

Bacteria can be resistance to a specific antibiotic by many ways:

- Selective Pressure: In the presence of an antimicrobial, microbes are either killed or, if they carry resistance genes, survive. These survivors will replicate, and their progeny will quickly become the dominant type throughout the microbial population. [2]
- Societal Pressures: The use of antimicrobials, even when used appropriately, creates a selective pressure for resistant organisms. However, there are additional societal pressures that act to accelerate the increase of antimicrobial resistance. [2]
- Mutation: Most microbes reproduce by dividing every few hours, allowing them to evolve rapidly and adapt quickly to new environmental conditions. During replication, mutations arise and some of these mutations may help an individual microbe survive exposure to an antimicrobial. [2]
- Gene Transfer: Microbes also may get genes from each other, including genes that make the microbe drug resistant.[3]
- Inappropriate Use: Selection of resistant microorganisms is exacerbated by inappropriate use of antimicrobials. Sometimes healthcare providers will prescribe antimicrobials inappropriately, wishing to placate an insistent patient who has a viral infection or an as-yet undiagnosed condition. [3]

- Inadequate Diagnostics: More often, healthcare providers must use incomplete or imperfect information to diagnose an infection and thus prescribe an antimicrobial just-in-case or prescribe a broad-spectrum antimicrobial when a specific antibiotic might be better. These situations contribute to selective pressure and accelerate antimicrobial resistance. [3]
- Hospital Use: Critically ill patients are more susceptible to infections and, thus, often require the aid of antimicrobials. However, the heavier use of antimicrobials in these patients can worsen the problem by selecting for antimicrobial-resistant microorganisms. The extensive use of antimicrobials and close contact among sick patients creates a fertile environment for the spread of antimicrobial-resistant germs. [4]
- Agricultural Use: Scientists also believe that the practice of adding antibiotics to agricultural feed promotes drug resistance. More than half of the antibiotics produced in the United States are used for agricultural purposes. However, there is still much debate about whether drug-resistant microbes in animals pose a significant public health burden. [5]

Mechanisms of resistance

- The inactivation or modification of the antibiotic. [6][7]
- An alteration in the target site of the antibiotic that reduces its binding capacity. [6][7]
- The modification of metabolic pathways to circumvent the antibiotic effect. [6][8]
- The reduced intracellular antibiotic accumulation by decreasing permeability and/or increasing active efflux of the antibiotic. [6][8]

1-1 Ceftriaxone

Ceftriaxone is third generation cephalosporin, sold under the trade name **Rocephin**® is an antibiotic useful for the treatment of a number of bacterial infections. This includes pneumonia, ear infections, skin infections, urinary tract infections, gonorrhea, pelvic inflammatory disease, sepsis, bone and joint infections, intra-abdominal infections, and meningitis. [9][10]

1-1-1 Spectrum of activity: Like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive bacteria and expanded Gram-negative coverage compared to second-generation agents. Include *Staphylococcus aureus, Streptococcus pneumonia, Streptococcus spp., Haemophilus influenzae, Moraxella catarrhalis, Neisseria meningitides, Neisseria gonorrhoeae, Enterobacteriaceae, E.coli* [11] [12]

1-1-2 Available forms: ceftriaxone available as vial for administration via the intramuscular or the intravenous routes. [10][9]

1-1-3 Specific populations:

- **Pregnancy**, Ceftriaxone is pregnancy category B. It has not been observed to cause birth defects in animal studies, but a lack of well-controlled studies done in pregnant women exists. [13]
- **Breastfeeding**, Low concentrations of ceftriaxone are excreted in breast milk that is not expected to cause adverse effects in breastfed infants. The manufacturer recommends that caution be exercised when administering ceftriaxone to women who breastfeed. [13]
- **Newborns**, Hyperbilirubinemic neonates are contraindicated for the use of ceftriaxone. It can compete with bilirubin and displace it from binding to albumin, increasing the risk of bilirubin encephalopathy. [14]
- **Elderly**, according to the package insert, clinical studies did not show differences in efficacy and safety of ceftriaxone in geriatrics compared to younger patients but "greater sensitivity of some older individuals cannot be ruled out. [14]

1-1-4 Mechanism of action:

CTR is bactericidal and have the same mode of action as other β-lactam antibiotics (such as penicillins), but is less susceptible to β-lactamases. CTR disrupts the synthesis of the peptidoglycan layer forming the bacterial cell wall. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases, known as penicillin-binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. Beta-lactam antibiotics mimic the D-Ala-D-Ala site, thereby irreversibly inhibiting PBP crosslinking of peptidoglycan. [15][16]

1-2 *E.coli*

Escherichia coli (*E. coli*) is the type species of the genus (*Escherichia*) and in turn, Escherichia is the type genus of the family Enterobacteriaceae. It belongs to a group of bacteria informally known as coliforms that are found in the gastrointestinal tract of warm-blooded animals. A gram-negative, rod-shaped bacterium lives in aerobic and anaerobic conditions. It is able to survive outside the body for a limited amount of time, *E. coli* stains gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, *E. coli* picks up the color of the counterstain safranin and stains pink. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that *E. coli* is not damage by penicillin.[21]

1-2-1 Pathogenic E.coli

E. coli can be grouped as normal flora type which is cohabiting with warm-blooded animals and, pathotypes which classified into six types:[17][18][19][20]

- ***Enterotoxigenic E. coli*** (ETEC): causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle. ETEC uses fimbrial adhesins (projections from the bacterial cell surface) to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins. ETEC strains are noninvasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in children in the developing world, as well as the most common cause of traveler's diarrhea.[17][18]
- ***Enteropathogenic E. coli*** (EPEC): causative agent of diarrhea in humans, rabbits, dogs, cats. Like ETEC, EPEC also causes diarrhea, but they use an adhesin known as intimin to bind host intestinal cells.[18][19]

- ***Enteroinvasive E. coli*** (EIEC): found only in humans. EIEC infection causes a syndrome that is identical to shigellosis, with profuse diarrhea and high fever.[17][18]
- ***Enterohemorrhagic E. coli*** (EHEC): found in humans, cattle, and goats. The most infamous member of this pathotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It's called shiga toxin producing strain.[19][20]
- ***Enteroaggregative E. coli*** (EAEC): found only in humans. So named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAEC are noninvasive.[21][20]
- ***Adherent-Invasive E. coli*** (AIEC): found in humans. AIEC are able to invade intestinal epithelial cells and replicate intracellularly. It is likely that AIEC are able to proliferate more effectively in hosts with defective innate immunity. [23] [22]

1-2-2 The risks of resistant *E.coli*

E.coli has flexible DNA which permit to:

- **Mutations**

  E.coli has 0.005 mutations per genome per generation, so it can produce 2 mutations spontaneously, in addition to the adaptive mutations which produces. [24][25]

- **Plasmid exchange**

  E. coli possess the ability to transfer and receive plasmid via bacterial conjugation or transduction, which allows genetic material to spread horizontally through an existing population. The process of transduction, which uses the bacterial virus called a bacteriophage, is where the spread of the gene encoding for the Shiga toxin from the *Shigella* bacteria to *E. coli* helped produce *E. coli* O157:H7, the Shiga toxin producing strain of *E. coli*. [26]

  At 2016, scientists have discovered a gene in *E. coli* that makes it resistant to a class of "last-resort" antibiotics known as polymyxins [28][27]. *E.coli* can also transfer resistance to other epidemic pathogens such as K. pneumoniae and Pseudomonas aeruginosa. [28][29]

1-2-3 Mechanism of resistance:

1- Express single genes that encode efficient drug modifying enzymes.[8]
2- Membrane impermeability, and drug efflux [8][15]

Chapter Two
Methodology

2.1 Methodology

2.1.2 Study population and design

This study was performed in four medical centers, Al-Hussain teaching hospital, General Hospitals Al Rumatha, General Hospitals Al Khedher, Maternity & Children teaching Hospital at Al Muthanna Governorate.

Patients who had a clinical culture positive for Ceftriaxone-resistant *E. coli* between August 2016 and February 2017 were prospectively identified and included in this observation and its participated 317 samples. Age range from one month to 50 years and grouped as pediatric and child (one month – 15 years) and adult (more than 15) [29]. Classification according to sex was 223 females and 94 males. Criteria for inclusion were the following:

1- Patient diagnosed with pathogenic *E.Coli*

2- Cultures included (Urine, Stool and Wound)

3- Sensitivity test for ceftriaxone has been made

Excluded patients as following:

1- Patient with normal flora *E.Coli*

2- Cultures (seminal – H.V.S)

3- Sensitivity test without ceftriaxone test

Medical records were reviewed for collection of clinical data, including age, sex, relationship, place (city – rural), animal rare, in/out patient, culture sample, ceftriaxone and other antibiotics sensitivity, other bacteria and ceftriaxone sensitivity test result.

2.2 Microbiological study

Patients were selected according to clinical diagnosis, and the sensitivity test was made to the samples.

Sensitivity test:

1- The sample is drawn or collected from the patient, and labeled.
2- Take a sterile swab and dip it into the broth culture of organism
3- Take a sterile blood and Mackonkey agar
4- Use the swab with the test organism to streak Blood and Maconkey agar
5- Incubate for 24h, under 37° C
6- After incubation gram positive bacteria grow on Maconkey agar only, gram negative grow on both agars
7- To detect *E.coli*, do oxidase test:
    - Strip of Whatman's No. 1 filter paper are soaked in a freshly prepared 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride.
    - Paper laid in a petri dish and moistened with distilled water.
    - The colony to be tested is picked up with a platinum loop and smeared over the moist area.
    - *E.coli* gives negative reaction by absence of purple colouration or by colouration later than 60 seconds.

2.3 Susceptibility test:

1- Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.
2- Take a sterile swab and dip it into the broth culture of organism.
3- Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.
4- Take a sterile Mueller-Hinton agar (MHA) plate or a nutrient agar (NA) plate.
5- Use the swab with the test organism to streak a MHA plate or a NA plate for a lawn of growth.
6- After the streaking is complete, allow the plate to dry for 5 minutes.
7- Antibiotic discs can be placed on the surface of the agar using sterilized forceps.
8- Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.
9- Carefully invert the inoculated plates and incubate for 24 hours at 37° C.
10- After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.
11- Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.
12- Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested antibiotic.

MIC	Sensitive	Intermediate	Resistance
30 µg	≤19	20-22	≥23

2.4 Statistical Analysis

The results were analyzed using the SPSS version 24.0 for Windows software (SPSS Inc., Chicago, IL, USA). The categorical variables were compared by Fisher exact tests or Pearson chi-square tests, as appropriate, and the continuous variables were compared using Student t test or the Mann-Whitney U test. All tests of significance were two-tailed; $p \leq 0.05$ was considered to indicate significance. Logistic regression analysis was performed to identify risk factors for Ceftriaxone resistance.

Chapter Three
Results

3.1 Results

The data was collected for 400 persons, of which 258 (64.5%) were resistant and 142 (35.5%) were sensitive (Figure 1). Samples were included are urine with frequency of 358 (89.5%), stool 16 (4%) and wound 26 (6.5%) to show which sample is more resistance. Urine sample was 242 (67.6%) resistance and 116 (32.4) sensitive. Urine samples were the most resistance with percentage of 60.5% of total followed by wound 2.3% and stool 1.8%, respectively. (table 1)

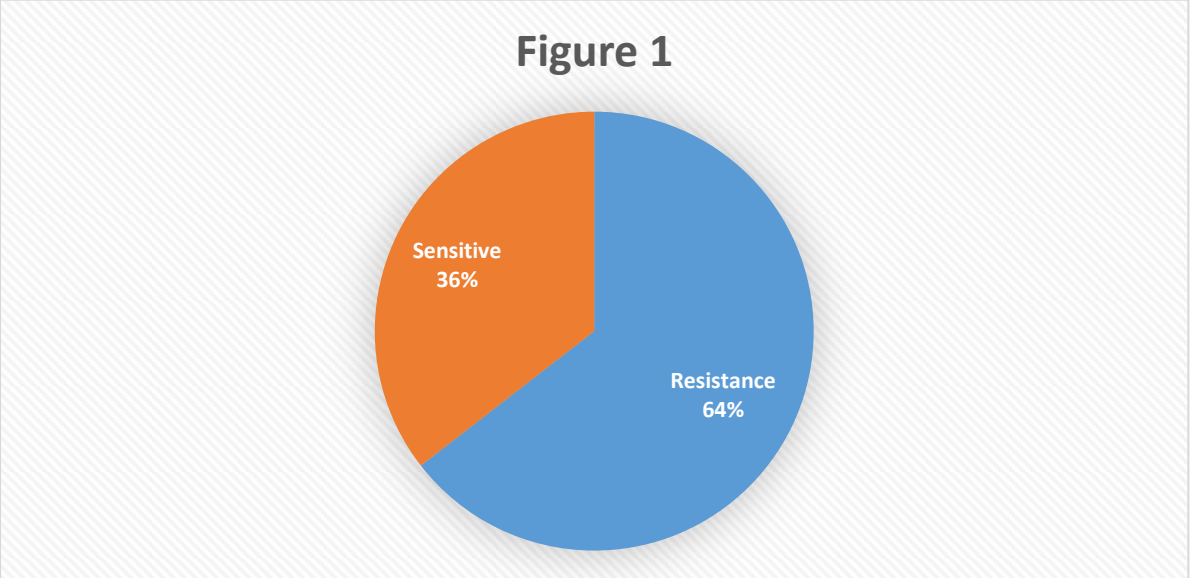

Figure 1: percentages of Sensitivity test

Sample		R	S	Total
Stool	Frequency	7	9	16
	%s of sample	43.8%	56.3%	100.0%
	% of total	1.8%	2.3%	4.0%
Urine	Frequency	242	116	358
	% of sample	67.6%	32.4%	100.0%
	% of total	60.5%	29.0%	89.5%
wound	Frequency	9	17	26
	% of sample	34.6%	65.4%	100.0%
	% of total	2.3%	4.3%	6.5%

Table 1: (Percentages of resistance and sensitivity in the same sample and the ratio to total.)

Data included 287 (71.8%) females, among them 190 (66.2%) were resistant and 97(33.8%) were sensitive, for males 113 (28.2%) resistant was found in 68 (60.2%) and sensitivity was 45 (39.8%) (figure 2). according to T-test independent sample test (P = 0.258) which is not significant and show no relationship between Gender and resistance.

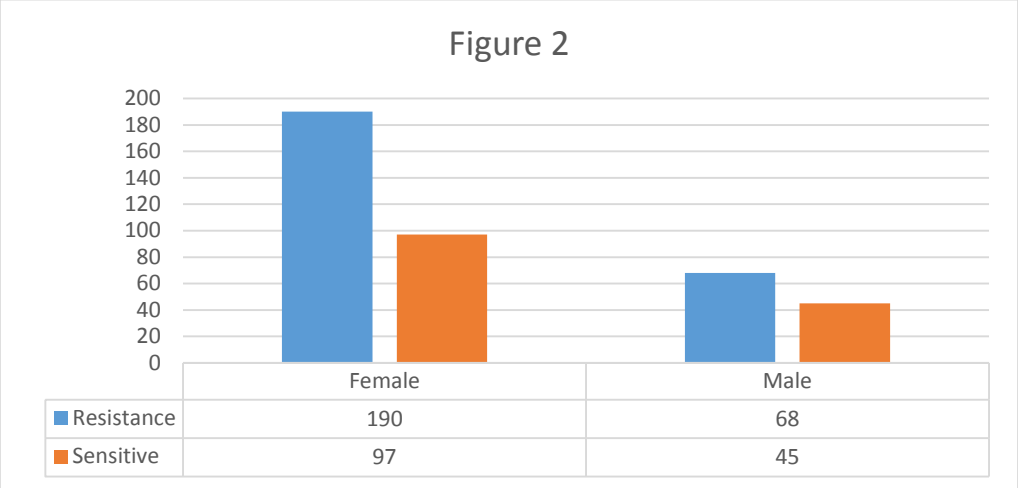

Figure 2: Relation Between Gender and sensitivity

Age was divided into (child and adult), based on Ceftriaxone doses which in most use from one month to 15 years and from 15 and above. Adults were 312 (78%) among them 214(68.6%) resistant and 98(31.4%) were sensitive, for children 44(50%) resistant and the same for sensitive (figure 3), for these resulted (P=0.001) which show relationship between age and resistance,

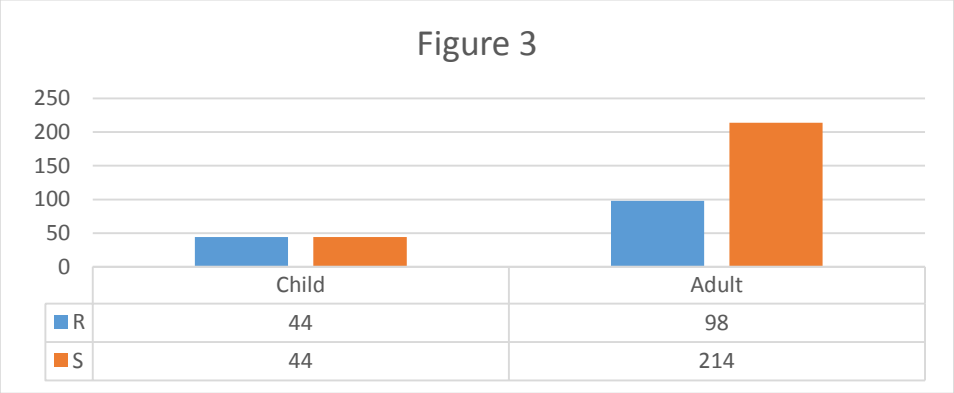

Figure 3: Relation Between Age and sensitivity

Figure 4 shows the relationship of ceftriaxone resistance and marital status, children was excluded and the data calculated for 312 persons, in which divided into married and single. Married people were 217 (69.6%) of which 161(74.2%) resistant and 56(25.8%) sensitive, singles 95(30.4%) of which 53(55.8%) and 42(44.2%) were resistant and sensitive, respectively. Which shows significance at level 0.05 (P=0.001).

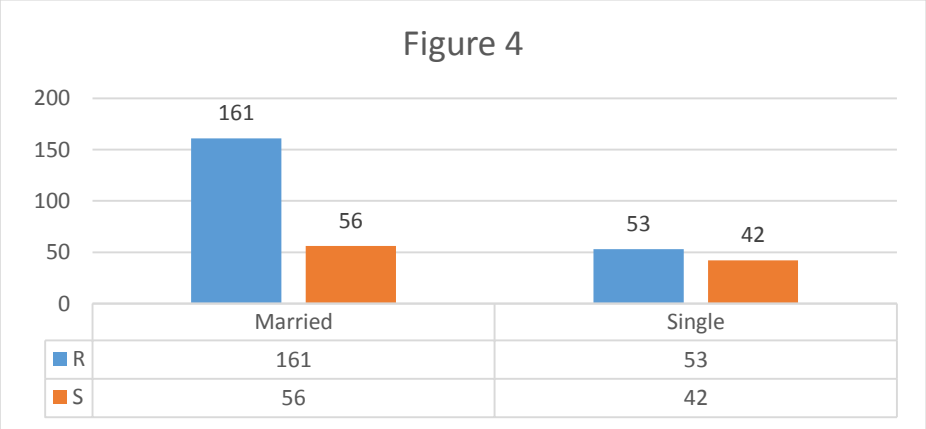

Figure 4: Relation Between Marital status

Living place another factor affects the resistance, so it divided into (rural and urban), rural 253(63.2%), of which 183(72.3%) were resistance and 70(27.7%) were sensitive. Another hand urban147(36.8%), resistant were in 75(51%) case, and 72(49%) were sensitive (figure 5). There is a statistical significance with (P=0.000).

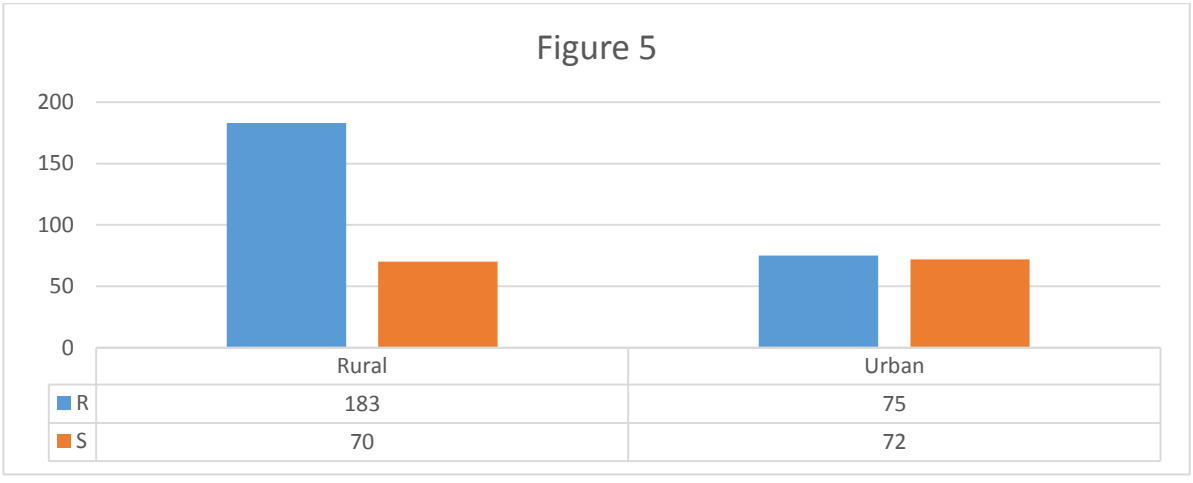

Figure 5: Relation of Place and Sensitivity

Place linked to animal rearing, so the collected data divided into (rearing, Not), Cases rearing animals 97(24.3%), resistance were found in 72(74.2%) resistant and 25(25.8%) were sensitive. In cases whom not rear animals 303(75.8%) resistant was in 186(61.4%) and sensitivity was in 117(38.6), the calculated statistics gives (P= 0.021)

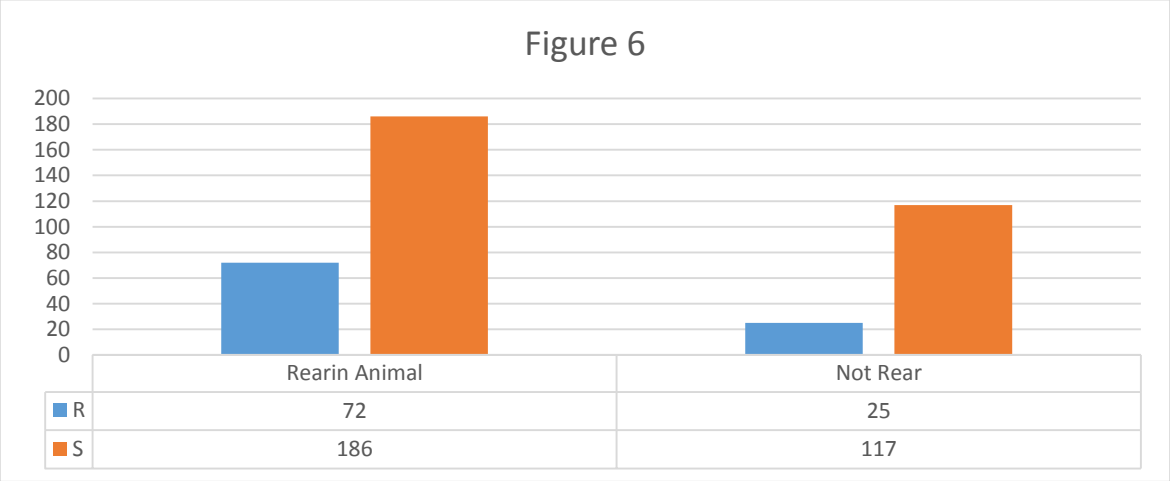

Figure 6: Relation of Animal and sensitivity

Grouping data according to in/out patient, results was patient out of hospital were 351(87.8%) of which 220(62.7%) resistant, and 131(37.3%) sensitive, the in hospital patient frequency 49(12.3%) of which 38(77.6%) resistance, and 11(22.4%) sensitive (Figure 7). The Chi-square test shows (P=0.042) which is significance at level 0.05. comparing to data collected on 2008-2009 (P=0.001).

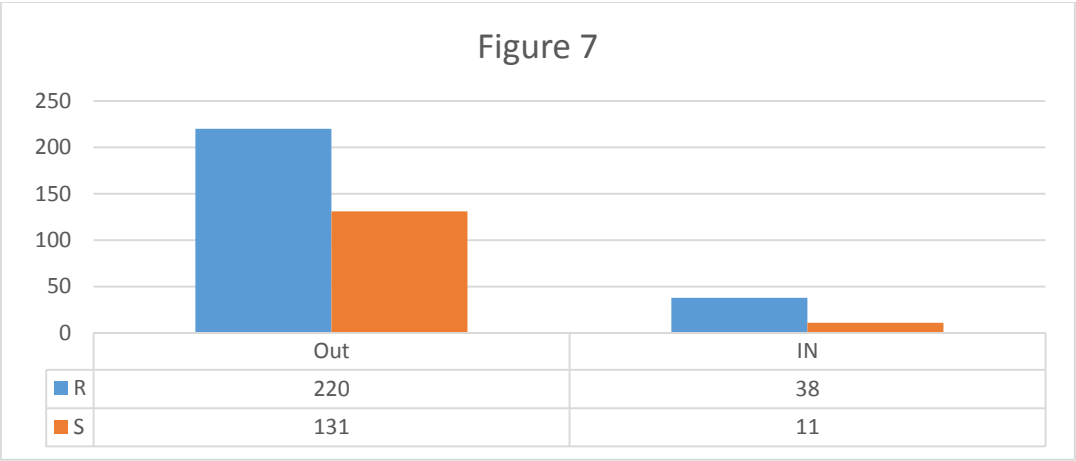

Figure 7: Relation Between In/Out and sensitivity

Previous exposure to antibiotic shows likelihood ratio with (P=0.001).

- Resistance to ceftriaxone raised during research time at Al-Hussain teaching hospital, General Hospitals Al Khedher, especially from October 2016 to February 2017. (Figure 9).
- The Maternity & Children teaching Hospital show descending in resistance.
- General Hospitals Al Rumatiha show partially constant resistance

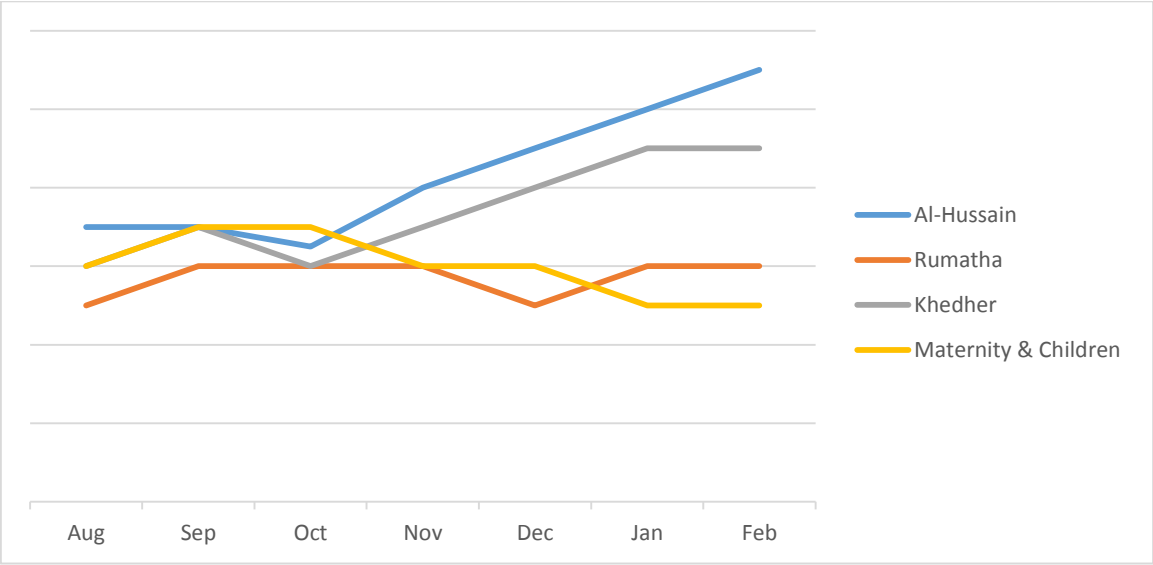

Figure 9: Hospitals resistance through research period

Chapter Four

Discussion

Conclusion

Recommendation

4.1 Discussion

Data included 400 case, resistance was 258(64%), this because of miss and over use of antibiotic and administration without culture. *E.coli* the most resistance bacteria because of high rate of mutation and abundant causing (UTI, GTI, wound infection), the samples show 89%, 6.5%, 4.0%, of resistance in urine, wound and stool, respectively.

Resistance doesn't show any dependency on gender, calculated P-value at level of 0.05 was 0.258 but the rate of infection is more in female than male with ratio of 2.5. In compare to 2003-2008 for 3rd generation cephalosporin *E. coli* resistance (P=0.39). [30]

Kiffer et al. [30] conducted a study comparable to ours, in terms of patient's population (both males and females of any age), number of isolates (35 782), and selected age groups. They also found (1) a lower percentage of *E. coli* isolation in patients younger than 13 years (69.0%). as compared to the age group 13–60 years (79.7%); (2) a higher difference in *E. coli* rates of isolation, between males and females.

The rate of infection high in females, especially in urine due to urinary and productive system design and exposure to bacteria. Females are more prone to UTIs than males because, in females, the urethra is much shorter and closer to the anus. As a woman's estrogen levels decrease with menopause, her risk of urinary tract infections increases due to the loss of protective vaginal flora.[31] Additionally, vaginal atrophy that can sometimes occur after menopause is associated with recurrent urinary tract infections.[32]

Age is related to resistance, pediatric and children show less resistance than adults, with significance calculated P= 0.001 in compare to the data from 2003-2008 (P=0.39). [30]

The resistance in adults linked to previous exposure to antibiotics or traveling and contacting to catch resistance so the previous exposure to antibiotic (all AB not ceftriaxone especially) show significance p-value = 0.001 this may be due *E.coli* adaptation against previous B-lactam ring antibiotics.

Samples show more resistance (69.6%) in married people especially in sexually active people, in contrast single and advanced age show less resistance. Which show a significance p- value = 0.001 this may be due transmit of resistance bacteria among couples at unprotected sex.[33]

Living place is a risk factor of resistance which show significance p-value = 0.021, rural places show more resistance (63.2%), and urban (36.8%), and this is same to previous data collected at 2003-2008 with (P= 0.029)[30], this may due to unhealthy environment and irrigation water source and it may because of transition of resistance from animals because E.coli is a normal flora in both, people whom rearing animals show more resistance (74.2%).[34]

In hospital patients shows more resistance (62.7%) which show a significance p-value = 0.04, this because hospital environment that has abundant bacteria that transport by contact to contaminated materials.

4.2 Conclusion

Ceftriaxone resistance raising with time, gender was an independent risk factor. Despite the rate of infection in females more than males, but the resistance is the same in both, so the gender is not affects the resistance.

Age, married, place, rearing animals, in/out patient and previous exposure to antibiotic were a dependent risk factors. Which, shows a relationship to resistance.

4.3 Recommendations

1- Don’t prescribe ceftriaxone without susceptibility test.
2- Use antibiotic combination to overcome resistance.
3- Do antibiotic cycling or rotation every 2-3 years
4- Stop using broad spectrum antibiotic as soon as susceptibility result appears.
5- Taking care of hospitals sanitation.
6- Advise the patient to complete the antibiotic course even after being well.
7- Tell the patient don’t share his treatment even same symptoms appears.

References

1-	Introduction to Antibiotic Resistance, Handbook of experimental pharmacology 211(211):1-12 · October 2012
2-	Frontiers in Clinical Drug Research: Anti-Infectives page 273
3-	Mellon M, Benbrook C, Benbrook KL. Hogging it: Estimates of antimicrobial abuse in livestock. Cambridge (MA): Union of Concerned Scientists; 2001.
4-	National instituent of Allergy and Infectious diseases, The problem of Antimicrobial Resistance, April 2006
5-	A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential; Timothy F. Landers, RN, CNP, PhD,a Bevin Cohen, MPH,b Thomas E. Wittum, MS, PhD,c and Elaine L. Larson, RN, PhD, FAAN, CICb
6-	McManus MC. Mechanisms of bacterial resistance to antimicrobial agents. Am. J. Health Syst. Pharm. 54, 1420–1433 (1997).
7-	Medical Microbiology. 4th edition., Chapter 11Antimicrobial Chemotherapy, Harold C. Neu and Thomas D. Gootz.
8-	Mechanisms of Antibiotic Resistance, Jose M. Munita and Cesar A. Arias 2016 Oct 1.
9-	http://www.medscape.com/viewarticle/756378_2
10-	Ceftriaxone Sodium Monograph for Professionals - Drugs.com". www.drugs.com. Retrieved 2016-08-27.
11-	Antianaerobic Antimicrobials: Spectrum and Susceptibility Testing Itzhak Brook, Hannah M. Wexler, and Ellie J. Goldsteinc,
12-	The Merck Manual of Medical Information. Mark H. Beers et al., eds. 2nd Home Edition. Whitehouse Station, NJ: Merck; 2003.
13-	"Ceftriaxone Pregnancy and Breastfeeding Warnings". www.drugs.com. Retrieved 27 August 2016.
14-	Shrimali, JD; Patel, HV; Gumber, MR; Kute, VB; Shah, PR; Vanikar, AV; Trivedi, HL (Nov 2013). "Ceftriaxone induced immune hemolytic anemia with disseminated intravascular coagulation.". Indian Journal of Critical Care Medicine. 17 (6): 394–5. doi:10.4103/0972-5229.123465. PMC 3902580
15-	How antibiotics kill bacteria: from targets to networks, Michael A Kohanski, Daniel J Dwyer, and James J Collins, 2010 DEC 1
16-	Ruppe E, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Ann Intensive Care. 2015 Dec;5(1):61.
17-	Diarrheagenic Escherichia coli, James P. Nataro* and James B. Kaper, 1998 JAN, 142-201
18-	"World Health Organization. Enterotoxigenic "Escherichia coli" (ETEC)". Who.int. 2010-12-08. Retrieved 2011-06-05.
19-	Rendón, M. A.; et al. (2007). "Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization". PNAS. 104 (25): 10637–10642.
20-	Martinez-Medina M, Garcia-Gil LJ (2014). "Escherichia coli in chronic inflammatory bowel diseases: An update on adherent invasive Escherichia coli pathogenicity."
21-	Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley. pp. 444–454.
22-	Guarner F, Malagelada J. Gut flora in health and disease. Lancet. 2003;360:512–519.

23-	Salyers AA, Whitt DD. Bacterial pathogenesis: a molecular approach. Washington DC: ASM Press; 2002.
24-	Adaptive Mutation in Escherichia coli, Patricia L. Foster, 2004 Aug; 186(15): 4846–4852.
25-	Rates of Spontaneous Mutation; John W. Drake,* Brian Charlesworth,† Deborah Charlesworth† and James F. Crow‡
26-	Lateral genetic transfer and the construction of genetic exchange communities, Elizabeth Skippington, Mark A. Ragan
27-	http://www.nature.com/news/antibiotic-resistance-the-last-resort-1.13426
28-	Origins and Evolution of Antibiotic Resistance Julian Davies and Dorothy Davies, 2010 SEP, 74(3): 417-433
29-	https://www.drugs.com/dosage/ceftriaxone.html
30-	Third-generation cephalosporin resistance of community-onset Escherichia coli and Klebsiella pneumoniae bacteremia in a secondary hospital. Shinwon Lee et al. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3932395/
31-	Dielubanza, EJ; Schaeffer, AJ (January 2011). "Urinary tract infections in women.". The Medical clinics of North America. 95 (1): 27–41.
32-	Goldstein, I; Dicks, B; Kim, NN; Hartzell, R (December 2013). "Multidisciplinary overview of vaginal atrophy and associated genitourinary symptoms in postmenopausal women.". Sexual medicine. 1 (2):
33-	Transmission of uropathogens between sex partners. Foxman B, Zhang L, Tallman P, Andree BC, Geiger AM, Koopman JS, Gillespie BW, Palin KA, Sobel JD, Rode CK, et al.
34-	Characterization of bacterial pathogens in rural and urban irrigation water. Aijuka M, Charimba G, Hugo CJ, Buys EM. 2015 Mar;13(1):103-17