

Antimicrobial Resistance Profile of Different Clinical Isolates against Rocephin

(Original Research Article)

Noor-alhooa Milood Al-awkally^{*1}, Maree Dokally Ali¹, Fathia Masoud Senossi², Nessren Mousa³, Rabeea mohamad Abd Al Hameed⁴, Salah Husayn Aqeelah⁵, Najla M El Hassi⁶ & Alreda Miloud Al-awkally⁷

¹Medical laboratory Department, Higher Institute of Science and Technology, Suluq, Libya

²Zoology Department, Collage of Art and Science, Benghazi University, Libya

³Statistics Department, Collage of Art and Science, Benghazi, University, Libya

⁴Ministry of health- Benghazi, Libya

⁵Alhaowari hospital, Benghazi, Libya

⁶Medical laboratory Department, Paediatric Hospital, Benghazi; Libya

⁷Ministry of health, Darna, Libya

Corresponding Author: Noor-alhooa Milood Al-awkally. Tel:+218917356491.

E.mail: noornooooor1973@gmail.com

Recieved on: 15 July 2021

Accepted on: 1 November 2021

Abstract

Antibiotic resistant microorganisms cause for an increase in illness and death as they increase the threat of unsuitable therapy. Therefore, documents on antibiotic resistance assistance describe the best probable treatment for different patients. Some bacteria can produce betalactamase enzymes such as in Gram negative bacteria. This enzyme can hydrolyze the betalactam ring of the ceftriaxone antibiotic so that the antibiotic cannot work. Hence, this study aimed to screen the antibiotic resistant profile rocephin drugs in Al saleem laboratory. Of 206 various samples were collected from patients during September through November 2020. The clinical samples such as wound swab, urine, sputum, and semen, blood, indwelling devices and fluids were collected from out patients, then; bacterial species were isolated and recognized as per the standard microbiological methods. Antimicrobial susceptibility tests were accomplished using rocephin antimicrobial disc by Kirby–Bauer disc diffusion method. Totally, 604 bacterial isolates were obtained from 280 (46.3%) female and 324 (53.6%) male patients. *Escherichia coli* spp (39.7%) and *Klebseilla* spp (15.9 %) were the predominant organisms isolated from specimens. About 295 (48.8%) of the total bacterial isolates were found to be resistant to ceftriaxone. But, 276 (45.7%) and 33 (5.5%) of the isolates remain susceptible and intermediate to

ceftriaxone, respectively.. Bacterial resistance towards ceftriaxone is rising to this drug. Moreover, these strains also showed multidrug resistance mainly against clinically used drug which could reduce therapy ineffective. So, in clinical use correct medications should be carefully chosen based on the data obtained from antibiotic susceptibility tests.

Keywords: Rocephin, *E. coli* spp, *Klebseilla* spp, urine, swab, Al saleem laboratory.

Introduction

Antimicrobial resistance is an increasing problem in the 21st century and known as the most serious threats to global public health.⁽¹⁾ The aminothiazol-cephalosporin ceftriaxone sodium (ceftriaxone) is a third-generation cephalosporin.

The product name for this drug is Rocephin (CRO) throughout the world. CRO is used for the treatment of many community-acquired infections and can be used as an intravenous or intramuscular injection; it has been used comprehensively because of its improved stability against traditional β -lactamases.

Ceftriaxone is a bactericidal agent that acts by inhibition of bacterial cell wall synthesis.^(2,3) Ceftriaxone has activity in the presence of some beta-lactamases, both penicillinases and cephalosporinases of Gram-negative and Gram-positive bacteria.⁽³⁾

Resistance to ceftriaxone is primarily through hydrolysis by beta-lactamase, alteration of penicillin-binding proteins (PBPs), and decreased permeability.⁽⁴⁾ Ceftriaxone has been shown to be active against Gram-negative bacteria and Gram-positive bacteria.^(5,6)

The aim of the study designated here was to define the bacteria responsible for community-acquired infections and determine their susceptibilities to rocephin.

Materials and Methods

Collection of Bacterial Strains

Out of the 604 patients included in this study. All gram-negative and gram positive bacteria isolated from clinical specimens during September through November 2020 by Al- saleem laboratory was included in the investigation.

The bacteria were identified in microbiology department according to standard procedures. Only samples yielding a significant number of a recognized pathogen were considered for the study.

Susceptibility Testing

Microbiology department performed disk susceptibility testing as follows. For gram positive bacteria and negative bacteria, Rocephin was tested on Mueller-Hinton agar at 37C°. 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.

Bacteria Identification

For the recognition and isolation of pathogenic bacteria, entirely the clinical specimens were collected by standard microbiological technique. Then, depending on the source of specimens, each sample was cultured onto Chocolate agar, MacConkey agar, Blood agar, Mannitol Salt agar, and CLED agar and then incubated aerobically at 37° C for 24 h.

Biochemical tests such as Triple sugar iron, urease test motility test, indole and citrate utilization (MIS); were used to recognize the clinical isolates. Thus, clinical strains of *Staphylococcus aureus*, *Escherichia Coli* spp, *Klebsiella* spp, *Proteus* species, *Citrobacter* spp, *Enterobacter* spp and streptococcus spp were isolated from the collected clinical samples.

Methodology: The data analyzed by SPSS programs.

Results

Distribution of Patients According to the Genders

Totally, 604 bacterial isolates were obtained from 280 (46.3%) female and 324 (53.6%) male patients.

Table 1: Distribution of patients according to the genders.

Gender	Number	Percent
Female	280	46.3%
Male	324	53.6%
Total	604	99.9%

Distribution of Clinical Specimens Collected from Patients

Totally, 604 bacterial isolates were obtained from urine cultures (75.3%), superficial swabs (10.8%), semen (7.9%), High vaginal swab (2.6%) indwelling devices (1.3%), Blood culture (1.2%), Fluid (0.7%) and sputum (0.2%) outpatients.

Table 2: Distribution of Clinical Specimens Collected from Patients.

Sample	Frequency	Percent	Valid Percent	Cumulative Percent
Blood	7	1.2	1.2	1.2
Fluids	4	.7	.7	1.8
HVS	16	2.6	2.6	4.5
Semen	48	7.9	7.9	12.4
Sputum	1	.2	.2	12.6
Swab	65	10.8	10.8	23.3
Urine	455	75.3	75.3	98.7
indwelling devices	8	1.3	1.3	100.0
Total	604	100.0	100.0	

Distribution of samples during the months

The most samples that were assembled in the study were the urine samples followed by samples of the swab and semen, where the highest assembly was found in October.

Table 3: Distribution of Samples during the Months

Sample	Date			Total
	9.2020	10.2020	11.2020	
Blood	0	5	2	7
	0.0%	.8%	.3%	1.2%
Fluid	0	2	2	4
	0.0%	.3%	.3%	.7%
HVS	3	7	6	16
	.5%	1.2%	1.0%	2.6%
Semen	8	22	18	48
	1.3%	3.6%	3.0%	7.9%
Sputum	0	1	0	1
	0.0%	.2%	0.0%	.2%
Swab	11	30	24	65
	1.8%	5.0%	4.0%	10.8%
Urine	83	193	179	455
	13.7%	32.0%	29.6%	75.3%
Wound	1	5	2	8
	.2%	.8%	.3%	1.3%
	106	265	233	604

Total	17.5%	43.9%	38.6%	100.0%
--------------	-------	-------	-------	--------

Distribution of Isolates in Clinical Specimens Collected from Patients

In the present study, *Escherichia coli* spp (39.7%) and *Klebsiella* spp (19.0 %) were the predominant organisms isolated from the study subjects.

The other bacterial isolates include *staph aureus* (11.8%), *streptococcus pneumonia* (11.6%), *Enterobacter* spp (7.0%), *pseudomonas* spp (6.5%), *streptococcus pyogen* (4.8%), *Proteus* spp (2.0%), *Citrobacter* spp (0.5%) and *Acinetobacter* spp and *diphtheria* spp (0.2%) equally as indicated in Table 2.

Table 4: Distributions of Isolates in Clinical Specimens Collected from Patients.

Bacteria	Frequency	%	Valid Percent	Cumulative Percent
<i>Acinetobacter</i> spp	1	.2	.2	.2
<i>Citrobacter</i> spp	3	.5	.5	.7
<i>Diphtheria</i> spp	1	.2	.2	.8
<i>E-coli</i> spp	240	39.7	39.7	40.6
<i>Enterobacter</i> spp	42	7.0	7.0	47.5
<i>Klebsiella</i> spp	96	15.9	15.9	63.4
<i>Proteus</i> spp	12	2.0	2.0	65.4
<i>Pseudomonas</i> spp	39	6.5	6.5	71.9
<i>Staph aureus</i>	71	11.8	11.8	83.6
<i>Streptococcus pneumonia</i>	70	11.6	11.6	95.2
<i>Streptococcus pyogen</i>	29	4.8	4.8	100.0
Total	604	100.0	100.0	

Resistance Ratio among Different Clinical Isolates against Rocephin

Organisms lying within the intermediate zones were not considered as sensitive pathogens, because they did not respond to normal therapy.

Rocephin was highly effective against *E. coli* spp and least sensitive against *streptococcus pneumonia* and *klebsiella* spp. Sensitivity patterns of ciprofloxacin against different pathogens are summarized in Table 5

Table 5: Resistance Ratio among Different Clinical Isolates against Rocephin.

Bacteria	CRO			Total
	I	R	S	
<i>Acinetobacter spp</i>	0	0	1	1
	0.0%	0.0%	.2%	.2%
<i>Citrobacter spp</i>	0	3	0	3
	0.0%	.5%	0.0%	.5%
<i>Diphtheria</i>	0	1	0	1
	0.0%	.2%	0.0%	.2%
<i>E-coli spp</i>	8	112	120	240
	1.3%	18.5%	19.9%	39.7%
<i>Enterobacter spp</i>	0	18	24	42
	0.0%	3.0%	4.0%	7.0%
<i>Klebsiella spp</i>	12	38	46	96
	2.0%	6.3%	7.6%	15.9%
<i>Proteus spp</i>	2	7	3	12
	.3%	1.2%	.5%	2.0%
<i>Pseudomonas spp</i>	3	12	24	39
	.5%	2.0%	4.0%	6.5%
<i>Staph aureus</i>	8	52	11	71
	1.3%	8.6%	1.8%	11.8%
<i>Streptococcus pneumonia</i>	0	39	31	70
	0.0%	6.5%	5.1%	11.6%
<i>treptococcus pyogen</i>	0	13	16	29
	0.0%	2.2%	2.6%	4.8%
Total	33	295	276	604
	5.5%	48.8%	45.7%	100.0%

Not: I- Intermediate; R- Resistance; S-Susceptibility.

Distribution of Pathogenic Bacteria during the Months

E. coli spp and *klebseilla* spp were more isolates from samples and the highest isolation was recorded in November.

Table 6: Distribution of Pathogenic Bacteria during the Months.

Bacteria	Date			Total
	9.20	10.20	11.20	
<i>Acinetobacter spp</i>	0	1	0	1
	0.0%	.2%	0.0%	.2%
<i>Citrobacter spp</i>	0	3	0	3
	0.0%	.5%	0.0%	.5%
<i>diphtheria spp</i>	0	1	0	1
	0.0%	.2%	0.0%	.2%
<i>E-coli spp</i>	37	93	110	240
	6.1%	15.4%	18.2%	39.7%
<i>Enterobacter spp</i>	5	28	9	42
	.8%	4.6%	1.5%	7.0%
<i>Klebsiella spp</i>	23	33	40	96
	3.8%	5.5%	6.6%	15.9%
<i>Proteus spp</i>	2	6	4	12
	.3%	1.0%	.7%	2.0%
<i>pseudomonas spp</i>	1	14	24	39
	.2%	2.3%	4.0%	6.5%
<i>staph aureus</i>	21	34	16	71
	3.5%	5.6%	2.6%	11.8%
<i>streptococcus pneumonia</i>	4	38	28	70
	.7%	6.3%	4.6%	11.6%
<i>streptococcus pyogen</i>	13	14	2	29
	2.2%	2.3%	.3%	4.8%
Total	106	265	233	604
	17.5%	43.9%	38.6%	100.0%

Resistance Profiles of Clinical Isolates to Rocephin

All the isolates were tested for susceptibility against selected third-generation cephalosporins (ceftriaxone).

Out of 604 bacterial isolates, 295 (48.8%) were found to be resistant to ceftriaxone. But, 276 (45.7%) and 33 (5.5%) of the isolates remain susceptible and intermediate to ceftriaxone, respectively.

Table 7: Resistance Profiles of Clinical Isolates to Rocephin

CRO	Frequency	Percent	Valid Percent	Cumulative Percent
Intermediate	33	5.5	5.5	5.5
Resistance	295	48.8	48.8	54.3
Sensitive	276	45.7	45.7	100.0
Total	604	100.0	100.0	

Comparison between Bacterial Growth and Susceptibility Patterns

Total of 604 isolates, 295 (48.8%) gave resistance to rocephin, while 276 (45.6%) recorded a high sensitive and 33 (5.4%) were intermediated.

Table 8: Comparison between Bacterial Growth and Susceptibility Patterns.

CRO	Bacterial number	%
I	33	5.464
R	295	48.841
S	276	45.695
Total	604	100.000

Susceptibility Patterns during the Three Months

Various species of gram positive and gram negative bacteria have showed a high sensitivity to rocephin in October followed by November.

Table 9: Susceptibility Patterns during the Three Months.

CRO	Date			Total
	9.20	10.20	11.20	
I	6	17	10	33
	1.0%	2.8%	1.7%	5.5%
R	64	120	111	295
	10.6%	19.9%	18.4%	48.8%
S	36	128	112	276
	6.0%	21.2%	18.5%	45.7%
Total	106	265	233	604
	17.5%	43.9%	38.6%	100.0%

Discussion

The standards for understanding of the results considered the fact that, consistent with the CLSI (2015).⁽¹⁰⁾ The “intermediate” category, term used in this study, is designed to the connection between antibiotic and bacterial samples, for which the response degrees in blood and tissue levels may be lower than those obtainable by susceptible samples.⁽¹⁰⁾

The prevalent use of broad spectrum antibiotics has led to the occurrence of antibiotic resistant strains of bacterial group; including *E. coli*. spp.⁽⁹⁾ High degrees of resistance have been mostly detected in bacteria that source common health problems. In the present study large number of the isolated bacteria strains were resistant to ceftriaxone drugs which are in agreement with 2014 WHO reports.⁽¹⁾ Totally, 604 bacterial isolates were obtained from 280 (46.3%) female and 324 (53.6%) male patients.

The majority of these isolates were *Escherichia coli* spp which is a gram-negative bacterium. Similarly, other research finding reported that *Escherichia coli* spp exhibited the highest isolates from clinical specimens.⁽⁸⁾ For *E. coli* spp, 50% of isolates were resistant to ceftriaxone. This could be owing to the high degree of adaptive alteration. Resistant organisms transmission their resistant genes either to their progeny by replication or by conjugation where the plasmids carrying the resistant gene are exchanged between the adjacent organisms.^(19,20)

This uropathogen is the major extended spectrum beta-lactamase (ESBL) producer, severely limiting the therapeutic management in cases of urinary tract infections⁽¹¹⁾ Hence, isolates of these strains have relatively high abilities of developed resistance developing resistance.⁽¹²⁾ Additionally, most of *Escherichia coli* spp isolated from the entire specimen were found to be resistant to the action of Rocephin in the current study. One of the reasons of the resistance mechanism to beta-lactam antibiotics such as Rocephin is because this bacteria produce betalactamase enzymes such as in Gram negative bacteria *E. coli* spp which synthesize the enzyme beta-lactamase AmpC. This enzyme can hydrolyze the betalactam ring of the ceftriaxone antibiotic so that the antibiotic cannot work.⁽¹⁶⁾ It was also discovered that the percentage of resistance to Rocephin amplified meaningfully for *Escherichia coli* spp infections since 2004.⁽¹³⁾ Also, other research discovery reported that *Escherichia coli* spp showed the maximum resistance to Rocephin.^(14,15) *Klebseilla* spp were more resistant to Rocephin in this study. Similarly, other research finding reported that *Klebseilla* spp exhibited the highest resistance to Rocephin.⁽⁸⁾ The majority of *streptococcus pneumoniae* were more resistant to Rocephin in this study. However, it is in line with other study showed in different areas which reported the resistant of the strains towards the rocephin. *Staphylococcus aureus* strains were found to be more resistance to ceftriaxone and rocephin which is inconsistent with previous study in which most of the strains were susceptible.⁽⁸⁾ similarly, in vitro antimicrobial study in in Karachi, Pakistan revealed that most of the isolated *staph aureus* were resistant to it.⁽¹⁷⁾ *Proteus* spp tested isolates

were resistant to rocephin. Similarly, in vitro antimicrobial study in Senegal revealed that most of the isolated Enterobacteriaceae strains were resistant to rocephin.⁽¹⁸⁾

Recommendations

Our results recommend ceftriaxone as the drug of optimal for the treatment of patients. Antimicrobial stewardship programs are highly needed to screen and control antimicrobial intake, which in turn could assistance in stoppage the increasing disaster of antimicrobial resistance.

Conclusion

Rocephin is becoming a first-line another procedure for the treatment of gram negative and gram positive Bactria.

Acknowledgements

I thanks to the staff of Microbiology department for providing patients isolates.

Conflicts of Interests

Authors declare that they have no conflicts of interest.

References

1. World Health Organization, Essential Drugs Monitor: Antimicrobial Drug Resistance: A Global Treat, World Health Organization, Geneva, Switzerland, 2000.
2. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement, CLSI document M100-S23. CLSI document M100-S23, Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2013.
3. Harriet M. eftriaxone An Update of its Use in the Management of Community-Acquired and Nosocomial Infections. *Drugs*. 2002; 62(7):1041–89
4. Fluit AC, Jones ME, Schmitz F-J, et al. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates n Europe from the SENTRY Antimicrobial Surveillance Program, 1997 and 1998. *Clin Infect Dis*. 2000; 30:454–60.
5. Walkty A, Adam HJ, Laverdière M, et al. The Canadian Antimicrobial Resistance Alliance (CARA). In vitro activity of ceftobiprole against frequently encountered aerobic and facultative Gram-positive and Gram-negative bacterial pathogens: results of the CANWARD 2007–9 study. *Diagn Microbiol Infect Dis*. 2011 Mar; 69 (3):348–55.

6. Thornsberry C, Jones ME, Hickey ML, et al. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in the United States, 1997–8. *J Antimicrob Chemother.* 1999; 44:749–59.
 7. Ogidi CO, Oyetayo VO (2013). Antibiotic sensitivity of microorganisms. *J Res Sci* 9: 209-216
 8. Fanta Gashe , 1 Eshetu Mulisa,1 Mekidim Mekonnen,2 and Gemechu Zeleke 1.(2018). Antimicrobial Resistance Profile of Different Clinical Isolates against Third-Generation Cephalosporins. *Hindawi Journal of Pharmaceutics* Volume 2018, Article ID 5070742, 7 pages <https://doi.org/10.1155/2018/5070742>
 9. Patrícia Luciana de Oliveira; Caroline S. Paula; Lisandra D. Rocha; Guilherme B. Collares; Roger T. Franco; Carolina P. Silva; Luiz M. Farias; Francisco José Penna; Edilberto N. Mendes; Teresa Cristina A. Ferrari; Paula P. Magalhães. Antimicrobial susceptibility profile of enterotoxigenic and enteropathogenic *Escherichia coli* isolates obtained from fecal specimens of children with acute diarrhea. *J Bras Patol Med Lab*, v. 53, n. 2, p. 115-118, April 2017
 10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA, 2015.
 11. J. A. Karlowsky, M. E. Jones, D. C. Draghi, C. Tornsberry, D. F. Sahm, and G. A. Volturo, “Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 3, article 7, 2004.
 12. C. K. M. Wong, K. Kung, P. L. W. Au-Doung et al., “Antibiotic resistance rates and physician antibiotic prescription patterns of uncomplicated urinary tract infections in southern Chinese primary care,” *PLoS ONE*, vol. 12, no. 5, 2017.
 13. A. Asensio, T. Alvarez-Espejo, J. Fernandez-Crehuet et al., “Trends in yearly prevalence of third-generation cephalosporin and fluoroquinolone resistant *Enterobacteriaceae* infections and antimicrobial use in Spanish hospitals, Spain, 1999 to 2010,” *Eurosurveillance*, vol. 16, no. 40, 2011.
 14. R. Polse, S. Yousif, and M. Assaf, “Prevalence and antimicrobial susceptibility patterns of uropathogenic *E. coli* among people in Zakho, Iraq,” *International Journal of Research in Medical Sciences*, vol. 4, no. 4, pp. 1219–1223, 2016.
 15. S. Sabir, A. A. Anjum, T. Ijaz, M. A. Ali, M. U. R. Khan, and M. Nawaz, “Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital,” *Pakistan Journal of Medical Sciences*, vol. 30, no. 2, pp. 389–392, 2014.
- [27] S. Eshetie, F. Tarekegn, G

16. Istiantoro Y, Gan V. Penisilin, sefalosporin dan antibiotik betalaktam lainnya. Dalam: Farmakologi dan terapi edisi 5. Jakarta: Departemen Farmakologi dan Terapeutik Fakultas Kedokteran Universitas Indonesia, 2007.
17. H. M. Shoaib, S. N. Baqir, D. Sheikh, and H. K. Hashmi, "Cephalosporin resistance and β -lactamase production in clinical isolates of staphylococcus aureus in Karachi," Pakistan Journal of Pharmaceutical Sciences, vol. 14, no. 2, pp. 23–32, 2001
18. S. Breurec, C. Bouchiat, J.-M. Sire et al., "High third-generation cephalosporin resistant Enterobacteriaceae prevalence rate among neonatal infections in Dakar, Senegal," BMC Infectious Diseases, vol. 16, article 587, 2016.
19. World Health Organization, Essential Drugs Monitor: Antimicrobial Drug Resistance: A Global Treat, World Health Organization, Geneva, Switzerland, 2000
20. P. Baral, S. Neupane, B. P. Marasini, K. R. Ghimire, B. Lekhak, and B. Shrestha, "High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal," BMC Research Notes, vol. 5, article 38, 2012.