Klebsiella spp. Isolate Distribution from Different Infections in Najaf in 2021

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ABSTRACT

The genus. Klebsiella is a gram negative bacteria belonging to the family of. Enterobacteria. These bacteria's natural habitat is the human and animal intestinal tract, but they were the causative agent a range of respiratory and urinary tract the infections, bacteremia, and burn and wound smears so are typical bacteria that may colonize both people and animals and cause both of them to get seriously ill. Due to their high the level of adaptation to environmental and synthetic circumstances and their capacity that to acquire antimicrobial and metal. Biocide resistance determinants,bacteria Klebsiella organisms typically exhibit by resistance to various antibiotics.

The current study includeincludeed eighty (80) Klebsiella spp. Various types of samples were collected during 10. 2021, from hospital laboratories of Al-Sadr in Holy-Najaf, Iraq. A statistical analysis of antibiotic resistance in bacteria revealed the most effective antibiotics, are Tetracyclines, Ertapenem, Amikacin, imipenem, and Gentamycin this analysis reveal different percentages.

The study also showed that females have a higher infections rate (66.3%) higher than infection rates of males (33.7%). The study conducted on various samples. However, the majority are urine samples 57.5%.

Finally, current study found that the infection rate increased during the summer more than other seasons.

Keywords:

1.Introdaction

1.1. *Klebsiella spp*

The genus. Klebsiella is a gram negative bacteria belonging to the family of. Enterobacteria.. Named after by German microbiologist Edwin Klebs. Klebsiella spp is non-motile, rod shaped organisms. The non motile Klebsiella spp and is shaped like a straight rod and measures 0.6 to 6 μm in length and 0.3 to 1μ m in diameter. It has a noticeable so polysaccharide the outer layer capsule (1).

Due to its high the resistance property, this outer layer appears to give resistance against a number of host defensive systems and accounts for the bacteria's bigger it appearance on Gram stain so. (2)

These bacteria typically it live in the gastrointestinal tracts of humans and other the animals. Burned skin and wounds have been used to isolate these germs. They are also taking into account the causes of skin inflammation and wound inflammation.these Additionally, bacteremia is brought on by respiratory and urinary tract infections it(3)

For the majority of the bacterial species, the mineral iron is an important and nutrient. As a cofactor for electron transport chain and several other it enzymes, it plays a crucial part (4) Common in the human microbiota,and Klebsiella spp. Are a major source of nosocomial opportunistic infections. Klebsiella oxytoca is an emerging pathogen, and multidrug resistant (M.D.R) variants of Klebsiella pneumoniae are more so frequently and causing that serious infections (5) Klebsiella spp characteristically grow as large mucoid colonies on MacConkey agar (6)

1.2 Klebsiella Classification

According to recently classification Klebsiella spps involve five types(Bethesda, MD 2019):

Klebsiella pneumonia
Klebsiella oxytoca
Klebsiella aerogenes.
Klebsiella granulomatis
Klebsiella grimontii

1.3. Epidemiology

The Numerous Klebsiella species can be found in soil and water, and other it surfaces in nature (7) Numerous the bacteria called Klebsiella spp. Can live on both humans and animals.that moreover occasionally causing both serious so illnesses Because of their remarkable adaptability to environmental and synthetic settings and their capacity to acquire antimicrobial, metal, and biocide and resistance genes present(8)The mostly of infections are nosocomial (hospital acquired. Around 55% of the all Klebsiella infections) it despite the fact that they are common pathogens that for community acquired pneumonias and bacteremiass (9) Klebsiella spp contamination of people,the food, and ecosystems. Variery foods can become are contaminated that by Klebsiella species, which can leads to illness and foods degradation. It Since Klebsiella species are extensively spread in the nature and in the gastrointestinal tracts so of a variety of animals (10), the source of contamination is not always obvious. Even more than other intestinal bacteria, Klebsiella are can well adapted to live in the environment. (11)

1.4. Pathogenicity

The People and the environment surrounding both contain and spread Klebsiella spp. The Klebsiella genera are well known Etiology pathogens that cause serious illnesses. They A lot of result in respiratory infections and other respiratory illnesses. Between 1% and 24% of hospital acquired Klebsiella cases and 2% to 6% of all community earned pneumonia cases are caused by Klebsiella.

As stated by (12) Klebsiella spps are frequently cause inflammation in alcoholic patients greater than nonalcoholic patients. As well as Arumugam Kamaladevi and his colleagues found that the Klebsiella spp Chiefly target the immunocompromised individuals who are hospitalized (13)The circulation, lungs, and urinary tract are just a few of the physiological systems that Klebsiella can invade. The location of the infection will affect the signs and symptoms of a Klebsiella infection. (14) Klebsiella pneumoniae has the capacity to colonization the gastrointestinal tract, the nasopharynx, so and the skin. It can also that cause a number of infection syndromes, such as pneumonia,it skin and soft tissue infections, intraabdominal infections, and urinary tract infections, in both the general public and in healthcare settings. (15)

1.5. Virulence factors

As an antibiotic, Klebsiella is differentiated by its ability to manufacture -lactamase in response to -lactams, as well as by the presence of a lipopolysaccharide and a capsule that is phagocytosis-resistant. The development of biofilm by these bacteria, which causes chronic infections mostly due to their resistance to phagocytosis and death as a result of humoral and cellular immunity, is a significant role (16) These bacteria are creating mucoid colonies after primary isolation during the diagnostic characterization of these bacteria because the capsule of the lipopolysaccharide type is present(17)
Lipopolysaccharides are embedded in the bacterial membrane of Klebsiella. Three parts make up (LPS) structurally: a lipid O antigen, an oligosaccharide core that serves as the structure's anchor in the bacterial membrane, and a terminal side chain. The fatty A series of enzymes expressed by the lpx gene cluster manufacture a hydrophobic moiety that is located in the outer leaflet of the outer membrane and is a component of LPS. (18)
Although lipid A changes brought on by infection can seriously impair immune cells' ability to recognize Klebsiella (19) The organism's polysaccharide capsule, which enables the bacteria to resist opsonophagocytic and serum death by the host organism, is its most crucial virulence factor. Without a capsule, Klebsiella species are typically less pathogenic. Lipopolysaccharides, which cover a gram-negative bacteria's outer surface, are a second virulence factor. The induction of an inflammatory cascade in the host organism in response to the presence of lipopolysaccharides is a significant factor in the sequela in sepsis and septic shock. Fimbriae, a different virulence factor(20)

1.6. Klebsiella and antibiotics

Numerous antibiotics are frequently ineffective against Klebsiella species. The primary source of the resistance genes, according to current evidence, is plasmids (21)Klebsiella pneumoniae is primarily responsible for hospital acquired Klebsiella pneumonia, which affects patients during the early stages of mechanical ventilation. Due to the production of plasmid mediated extended spectrum beta-lactamases, Klebsiella spp. Can develop resistance to 3rd and 4th generation cephalosporins despite being intrinsically resistant to penicillin (E. S. B. L. s). These plasmids usually contain enzymes that alter aminoglycosides. Due to the production of constitutiv. AmpC β lactamase, Enterobacter. Are intrinsically resistant to ampicillin, amoxicillin clavulanate, and cefoxitin. (22) Antibiotic resistance developed as a result of overuse, which is to blame for the current issues facing the entire world. This is a representation of a number of microbes, including Kelpsialla, which can create defenses against certain drugs more quickly. On the other hand, it is quite challenging to identify antibiotic alternatives. The majority of the genetic or acquired alterations that generate bacterial resistance to several antibiotics are acquired modifications that result from the acquisition of resistant genes. These genes, which produce a number of different enzymes, degrade the antibiotics and stop them from killing the bacteria. Referred to as carbapenemase, which Enterobacteriaceae generate and which boosts Klebsiella species' resistance (23).
Co trimoxazole and cephalosporin, and fluoroquinolone sensitivity in Klebsiella pneumonia isolates has been shown to be moderate it (24)

2.1. Materials:

2.1.1. Devices and instrument (macfaddin 2000)

No	Name
1	Oven
2	Water Bath
3	Loop
4	Benzene Burner
5	Sensitive Balance
6	Autoclave
7	Refrigerator
8	Plane Tubes
9	Petri Dishes Plates
10	Millipore Filters
11	Slides
12	Pipettes
13	Compound Light Microscope
14	Distillator
15	pH-Meter
16	Centrifuge
17	Incubator

2.1.2. Culture media

- Methyl Red- Vogus Proskaur Media
- Urea agar bas
- Nutrient Broth
- Blood Agar Base
- Agar Nutrient
- Muller-Hinton Agar
- Simmon's Citrate Agar
- MacConkey`s Agar

2.1.3. Reagents and stain

- Gram stain
- Methyl red reagent
- Oxidase reagent
- Catalase reagent
- Voges-Proskauer reagent
- Macfrland Solution

2.2.Mothed :

Study design:

The current study included eighty (80) specimens Klebsiella spp The samples were collected from Laboratories in hospital alsadr in Holy-Najaf / Iraq during the period 2021.

Samples collection:

It contained a variety of samples (including urine, sputum, and a swab from which a sputum sample was separated for Gram staining). Gram-negative, short, plump bacilli are what Klebsiellae look like. Typically, a capsule that looks to be a clear area surrounds them.

Culture preparation:

The Cultures need to be collected from potential sites (wounds, peripheral or central intravenous access sites, urinary catheters, and respiratory support equipment). It is possible to isolate Klebsiellae it from wounds, pleural fluid, blood, and urine.

Biochemical tests:

Because Klebsiellae are microaerophilic, they can flourish either in the presence of oxygen or without it. They do not have any unique cultural requirements. Since most species can only use citrate and glucose as carbon sources, they can thrive on the majority of common media.

Although Klebsiella oxytoca and some strains of Klebsiella pneumoniae are outliers, Klebsiellae are lactose fermenting, urease-positive, and indolenegative organisms. Depending on the sickness, sputum culture analysis, blood culture analysis, or midstream urine analysis can be used to detect Klebsiellae, which do not create hydrogen sulfide and give positive findings on both Voges and methyl red tests..

In the Gram stained smears, the gram negative rods may be encapsulated and non sporing, which suggests Klebsiella.

Material is injected into the blood agar and Mac Conkey agar media, and an aerobic incubation process is then carried out. Mucoid, non hemolytic colonies on blood agar MacConkey Agar: pink-colored, mucoid colonies it that are lactose fermenting so Pure cultures are determined by biochemical responses and colony morphology. It after being obtained by choosing colonies from aerobically incubated plates.

3:Results

3.1. Antibiotics used and effect

After using 30 different antibiotics on *Klebsiella*, a survey was conducted on 80 samples of infected patients over the course of a year, and it was discovered that the resistance and of *Klebsiella* to the antibiotics used, where it was more sensitive to *Klebsiella* Tigecycline in proportions (100 %) without any resistance, Ertapenem (94.2 %). Gentamycin (85%), Amikacin (89%) and Imipenem (85%). (74 %). Meropenem (65.3 %) *Klebsiella* also demonstrated higher resistance to some antibiotics, such as ceftriaxone (80%), ceftazidime (72%), as shown in the table (3-1) and figure (3-1) details for each antibiotic used.

3.2. Infection in sex according types Specimens

Statistics were collected for 80 infected males and females, as well as for various samples such as urine, swab, sputum, seminal fluid, pus, and abscess. The highest number of injuries were found in female urine (82.6 %) and male urine (17.4 %), while male swab samples had a percentage of (60 %) and female swab samples had a percentage of (60 %) (40 %) Whereas the total ratio of males (33.7%) and females (66.3%) is shown in the table (3-2) and figure (3-2) below for all samples.

Table (3-1) Antibiotic sensitivity and resistance of the antibiotics used in the study.

No	Antibiotics	Abbreviations	S No.	%	R No	%
1	Amikacin	Ak,AN	67	89	8	11
2	Ampicillin	Am,Amp	4	12.5	28	87.5
3	Aztreonam	ATM,AT	9	23.7	29	76.3
4	Cefepime	FEP	7	27	19	73
5	Cefazolion	CZ	8	18	36	82
6	Cefotaxime	CTX	3	33	6	67
7	Cefoxitin	Fox,CX	21	51	20	49
8	Ceftazidime	CAZ	19	28	49	72
9	Ceftriaxone	CRo	8	19.5	33	80.5
10	Cephalothin	CR,CEP	1	33	2	67
11	Chloramphenicol	C	1	25	3	75
12	Ciprofloxacin	Cip	31	69	14	31
13	Ertapenem	ETP	16	94.2	1	5.8
14	Gentamicin	GM	26	74.3	9	25.7
15	Impienem	Imp,imi	30	85.7	5	14.3
16	Levofloxacin	Lev	22	61	14	39
17	Minocyline	Mino,MNo	13	72	5	28
18	Tetracycline	Tc,Te	9	47	10	53
19	Ticarcillinclavalanicacij	Tcc	17	68	8	32
20	Ampicillinsulbactam	SAM	7	46.6	8	53.4
21	Piperacillin-Tazobacam	Tzp	29	60.4	19	39.6
22	Cefoperazone	Cfp	6	26	17	74
23	Colistin	Cs	0	0	1	100
24	Fucijic Acid	FA	1	50	1	50
25	Nitrofurantion	FT,Nit	4	20	16	80
26	Piperacillin	Pi,pip,pp	11	31.5	24	68.5
27	Tigecyline	TGc,Tige	30	100	0	0
28	Tobramycin	To,Tm,Top	24	60	16	40
29	Meropenem	MeM	32	65.3	17	34.7
30	Trimethoprimsulfamethoxaxole	SXT	26	54	22	46

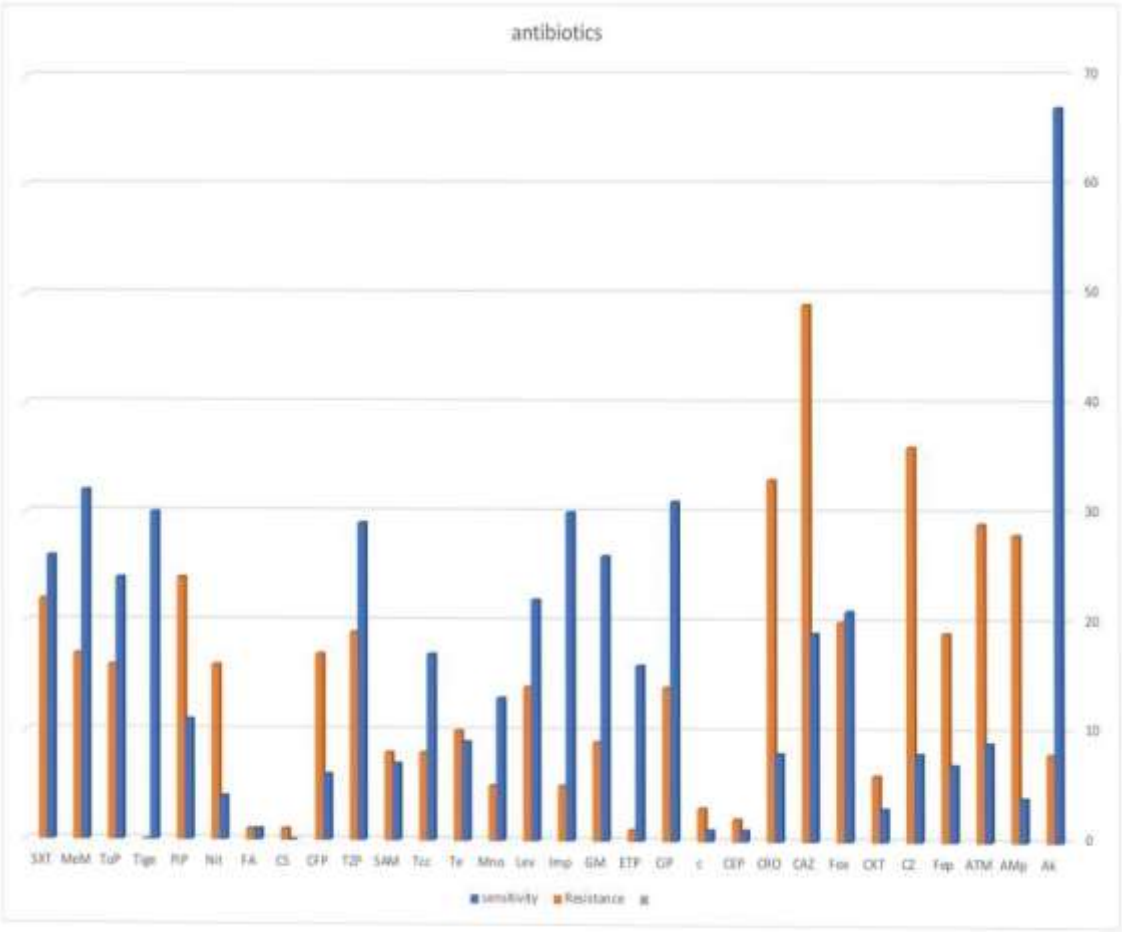

Figure (3-1): Antibiotic sensitivity and resistance of the antibiotics used in the study

	Total	*Male*		*Female*	
		No	**%**	**No**	**%**
Sample					
Urine	46	8	17.4%	38	82.6%
Swab	20	12	60%	8	40%
Sputum	9	5	55%	4	45%
Seminal fluid	2	2	100%	0	0%
Pus	1	0	0%	1	100%
Abcesc	1	0	0%	1	100%
Ascitic fluid	1	0	0%	1	100%
Total	80	27	33.7%	53	66.3%

Table (3-2):Distribution of different samples according to sex

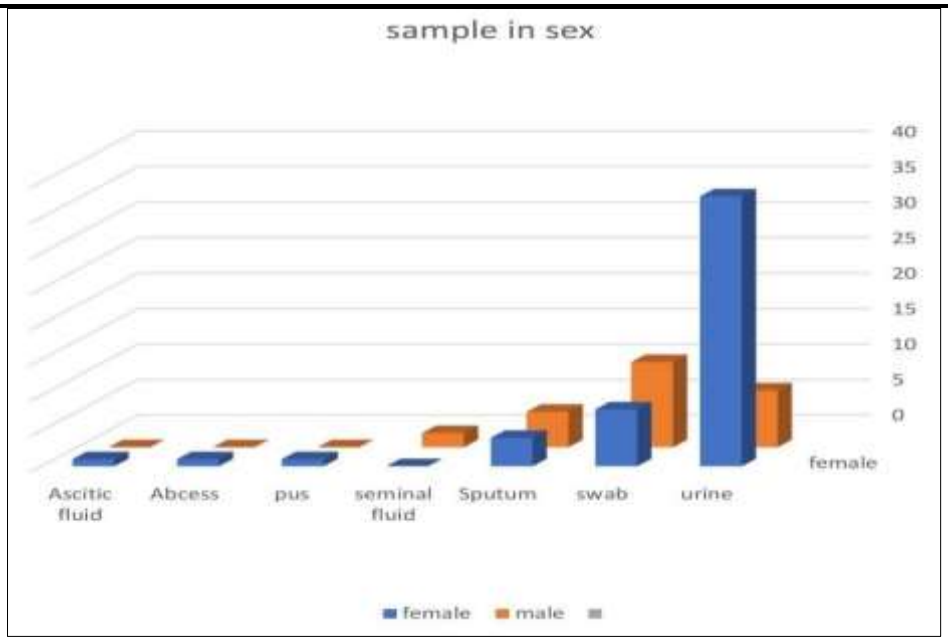

Figure (3-2) Distribution of different samples according to sex

3.3. Number and Types specimens

The samples taken from 80 patients were 7 different types: urine, swab, sputum, seminal fluid, Pus and Abscess. And Ascitic fluid Where the most frequently used samples were urine (57.5%) As shown in the table
(3_3) And in the figure below (3_3)

3.4. Rate Infection every Month

The number of infections in each month during the year for 80 infected and per sample, where the largest part of urine. Samples were in terms of the number of injuries among the months, the highest number of injuries per month was for 8 and 9 As shown in the table (3_4) In the figure below (3_4)

Table (3_3) number and types specimens

Sample	Number sample	Percentage %
1-Urine	46	57..5%
2-Swap	20	25%
3-Sputum	9	11.25%
4-seminal fluid	2	2.5%
5-Pus	1	1.5%
6-Abcess	1	1.5%
7-Ascitic fluid	1	1.5%
Total	80	100%

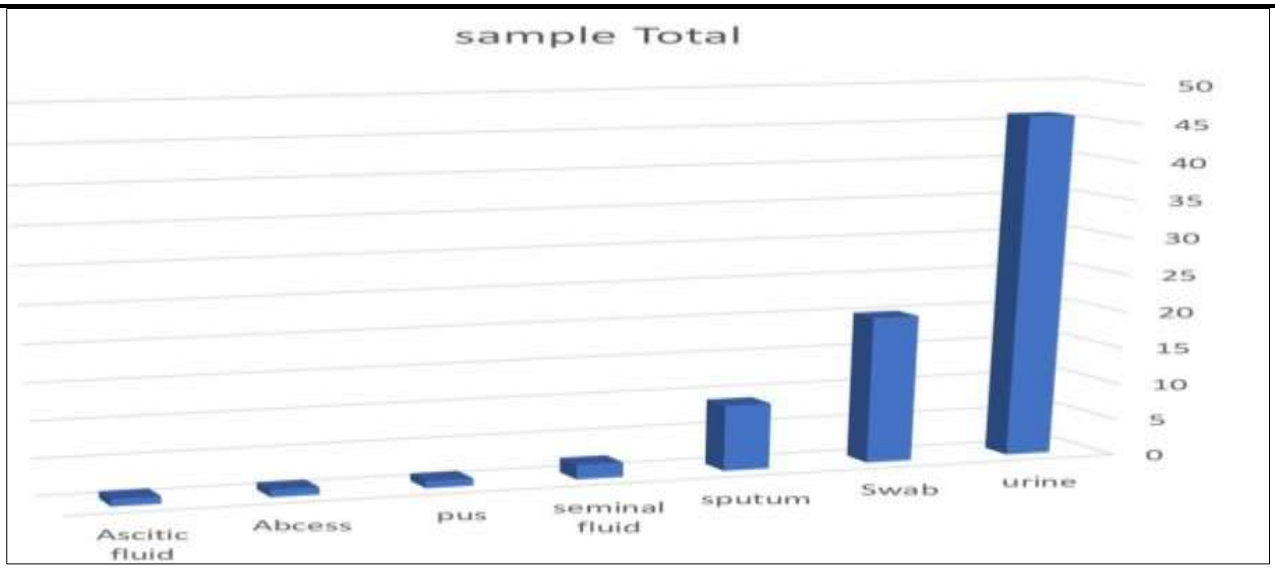

Figure (3_3) number and types specimens

Sample	Total Sample	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Act	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Urine	46	8	17.4	4	8.7	1	2.2			5	10.9	5	10.9	2	4.3	8	17.4	9	19.5		
Swap	20	1	5	4	20	1	5	1	5	2	10	1	5	3	15	2	10	2	10	1	5
Suprum	9					1	11.1			2	22.2	2	22.2			2	22.2	1	11.1	1	11
Seminal Fluid	2	1	50	1	50																
Pus	1							1	100												
Abcess	1					1	100														
Ascitic fluid	1									1	100										
Total	80	10	12.5	9	11.25	5	6.25	2	2.25	8	10	9	11.25	4	5	13	16.25	12	15	2	2.5

Table (3_4) rate Infection in months

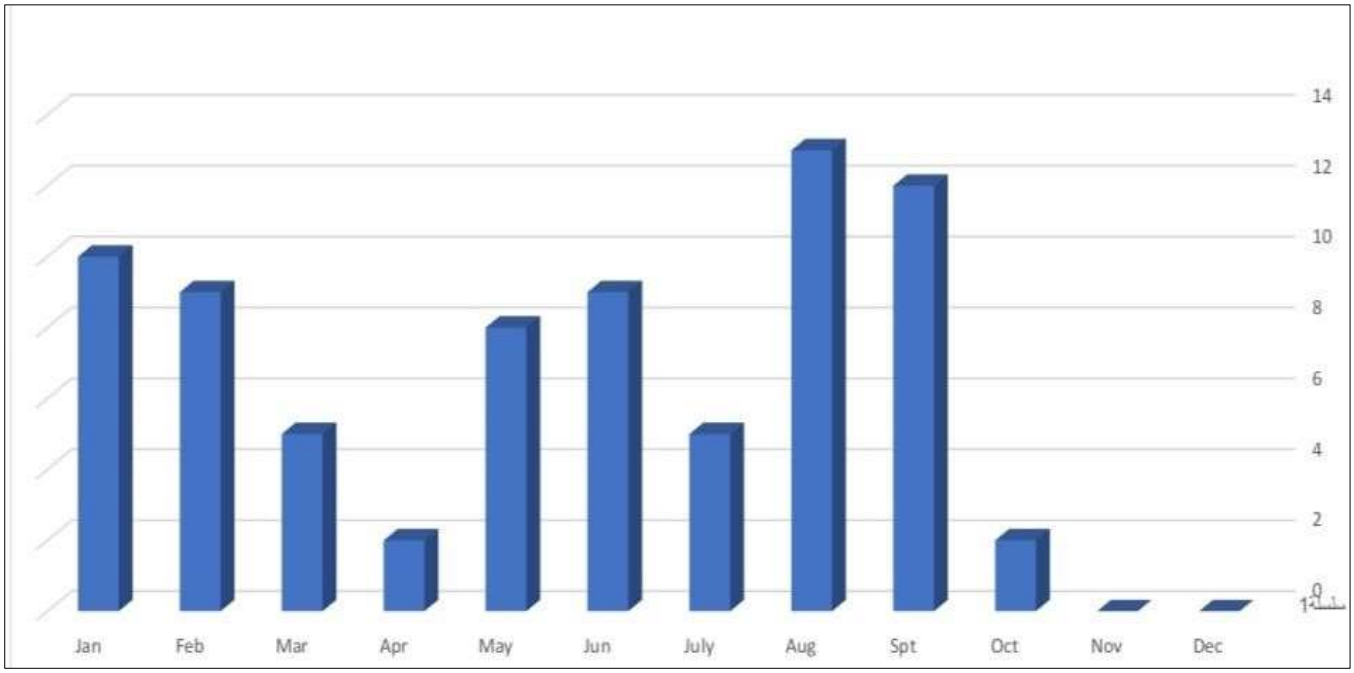

Figure (3_4) rate Infection in months

4: Discussion

4.1. Antibiotics

The results revealed the high efficacy of the antibiotics Tigecycline ،Ertapenem, Amikacin, imipenem, Meropenem, Gentamycin, Ceftazidime. As other studies revealed, tigecycline were the most effective antibiotics. The overall prevalence of multidrug (25) The Klebsiella Oxacillin (97%),and cefoxitin (71.8%), and ceftriaxone (43.4%), cefotaxime (52%), and ceftazidime (31.9%) it were all ineffective against the Klebsiella species. Additionally, they demonstrated that resistance to the carbapenems , such as imipenem (29%), and ertapenem (85%), and meropenem (87%). The isolated Klebsiella species did, however, exhibit susceptibility to the antibiotics gentamicin (83%), amikacin (79%),and ciprofloxacin (38.9%), and ofloxacin (76%). From18 isolates were present (26).Ampicillin (86.9%), ceftriaxone (61.8%), and cefepime (68.8%) also had substantial resistance rates. The isolates were susceptible to ertapenem (94.2%) and imipenem it (88%), which had the highest susceptibility rates. Conclusions: The findings showed that the antibiotic susceptibility pattern of Klebsiella isolates from various clinical specimens varied, with ampicillin showing the highest resistance.it The most effective antibiotics against the isolates were ertapenem and imipenem (27).

4.2. Infections in sex

The findings indicated that females (64. 2%) and had a larger percentage of infections than males (31.5%).According to other the studies, the ratio of male to female patients was 1:4 with females being more prevalent overall (28).Additionally,so more female patients' isolates (60%) than male patients' present (37%) isolates were examined it (29)

4.3. Type Specimens

The results showed that urine (54.7%) and swab (22%) sample were so the most positive. Also studied was it. All of the isolate sample sources had different levels of antibiotic resistance, with urine samples having the highest rates for all antibiotics. Additionally, the positive rate in urine samples was 23.1%, which was significantly higher than the positive rate in sputum samples (17.2%) from case (30)

4.4. Rate infections in Seasons

The findings indicated a different percentage rise in the rate of illnesses over the summer. According to a study, summertime is when infections are most common in North America, Europe so, the Middle East, Australia, and Asia. Temperature rise and (BSI) rates were shown to be correlated (31)

Conclusion

In conclusion, theresults. of the study. showed:

1.Among the most effective antibiotics Tigecycline, Ertapenem, Amikacin imipenem, where the largest antibiotics have the ability to reduce the incidence of resistance, so attention should be paid to the appropriate doses of the antibiotics whose effectiveness appeared.

2.The rate of infection in females is more than in males.

3.The infection rate is more widespread in the summer.

Recommendations

1.Based on the results that have been reached, several recommendations can be put forward, including:

2.Consider Tetracyclines, Ertapenem Highly effective against bacteria.

3.Also, do not overuse antibiotics, as they make bacteria more resistant.

4.As well as enhancing the prevention of bacterial infection resulting from their Locations

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References

1. Mandell D. (2009). Enterobacteriaceae. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Churchill Livingstone, An Imprint of Elsevier **.**
2. Arumugam Kamaladevi, Shanmugaraj Gowrishankar, Krishnaswamy Balamurugan Handbook of Foodborne Diseases, 361-367,2018
3. Rebekah M Martin, Michael A Bachman Frontiers in cellular and infection microbiology 8, 4, 2018
4. Wieland, C. W., Lieshou, M. H. P., Hoogendijk, A. J., and vander Poll, T. (2011). Host defence during Klebsiella pneumonia relies on haematopoietic expressed Toll-like receptors 4 and 2. J Eur Respir.37: 848-857.
5. Warren P Herridge, Preetha Shibu, Jessica O'Shea, Thomas C Brook, Lesley Hoyles Journal of medical microbiology 69 (2), 176, 2020
6. Randall G. Fisher, in Feigin and Cherry's Textbook of Pediatric Infectious Diseases (Sixth Edition), 2009
7. Martin, R. M., and Bachman, M. A. (2018). Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Front. Cell. Infect. Microbiol. 8:4. Doi: 10.3389/fcimb.2018.00004
8. Andre Hammerl Science of The Total Environment 804, 150000, 2022
9. Janda, J. M., & Abbott, S. L. (2006). The Genera Klebsiella and Raoultella. The Enterobacteria (2nd ed., pp. 115-129). Washington, USA: ASM Press.
10. N. Gundogan, in Encyclopedia of Food Microbiology (Second Edition), 2014
11. S. Cooney, … S Fanning, in Encyclopedia of Food Safety, 2014
12. Kamaladevi, Shanmugaraj Gowrishankar, Krishnaswamy Balamurugan Handbook of Foodborne Diseases, 361-367,2018
13. Arumugam Kamaladevi, Shanmugaraj Gowrishankar, Krishnaswamy Balamurugan Handbook of Foodborne Diseases, 361-367,2018
14. Quereshi, Shahab. Klebsiella Infections. Medscape. December, 2018
15. 15.Melot, B., Colot, J. & Guerrier, G. Bacteremic communityacquired infections due to Klebsiella pneumoniae: clinical and microbiological presentation in New Caledonia, 2008–2013. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases 41, 29–31, /j.ijid.2015.10.013 (2015) .
16. Saade Abdalkareem Jasim, Sumaya Ayad Abdulrazzaq, Raed Obaid Saleh Systematic Reviews in Pharmacy 11 (6), 2020

17. .Aher T, Roy A and Kumar P. (2012). Molecular detection of virulence genes associated with pathogenicity of Klebsiella spp. Isolated from the respiratory tract of apparently healthy as well as sick goats, J. Veterinary Med., 67(4), 249-252
18. C.R. Raetz, Z. Guan, B.O. Ingram, D.A. Six, F. Song, et al.Discovery of new biosynthetic pathways: the lipid A story J Lipid Res, 50 (Suppl) (2009), pp. S103-
19. E. Llobet, V. Martínez-Moliner, D. Moranta, K.M. Dahlström, V. Regueiro, et al. Deciphering tissue-induced Klebsiella pneumoniae lipid A structure Proc Natl Acad Sci U S A, 112 (46) (2015), pp. E6369-6378,
20. Rønning TG, Aas CG, Støen R, Bergh K, Afset JE, Holte MS, Radtke A. Investigation of an outbreak caused by antibioticsusceptible Klebsiella oxytoca in a neonatal intensive care unit in Norway. Acta Paediatr. 2019 Jan;108(1):76-82 .
21. Hudson, Corey; Bent, Zachary; Meagher, Robert; Williams, Kelly (June 6, 2014). "Resistance Determinants and Mobile Genetic
22. Elements of an NDM-1-Encoding Klebsiella pneumoniae Strain". PLOS ONE. 9 (6): e99209. Bibcode:2014PloSO…999209H. Doi:10.1371/journal
23. .AC EJC Goldstein, A Torres Journal of Antimicrobial Chemotherapy 58 (5), 916-929, 2006
24. Lauren R Biehle, Jessica M David J Thompson, Rachel L Filipek, J Nicholas O'Donnell, Todd M Lasco, Monica V Mahoney, Elizabeth B Hirsch PloS one 10 (11), e0143845, 2015
25. Redgrave LS, Sutton SB, Webber MA, Piddock LJV .Fluoroquinoloneresistance:mechanism impact on bacteria, and role in evolutionarysuccess. Cell Press. 2014;22(8):438–45
26. Deepa Karki, Binod Dhungel, Srijana Bhandari, Anil Kunwar, Prabhu Raj Joshi, Basudha Shrestha, Komal Raj Rijal, PrakashGhimire, Megha Raj Banjara Gut pathogens 13 (1), 1-16, 2021
27. Ejikeugwu Chika, Duru Carissa, Eluu Stanley, Oguejiofor Benigna, Ezeador Chika, Ogene Lilian, Iroha Ifeanyichukwu Global Journal of Pharmacy & Pharmaceutical Sciences 2 (3), 54-58, 2017
28. Ibrahim Abdulqader Naqid, Nawfal Rasheed Hussein, Amer A Balatay, Kurdistan Abdullah J Kermanshah Univ Med Sci 24, 2020
29. Pooja Shakya, Dhiraj Shrestha, Elina Maharjan, Vijay K Sharma, Rabin Paudyal The open microbiology journal 11, 23, 2017
30. Muhammad Sohail, Mohsin Khurshid, Hafiz Ghulam Murtaza Saleem, Hasnain Javed, Abdul Arif Khan Jundishapur journal of microbiology 8 (7), 2015
31. Guogang Li, Sheng Zhao, Sipei Wang, Yingqian Sun, Yangxiao Zhou, Xinling Pan Annals of clinical microbiology and antimicrobials 18 (1), 1-7, 2019
32. H Richet Clinical microbiology and infection 18 (10), 934-940, 2012….. P