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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF KLEBSIELLA SPECIES IN CLINICAL SPECIMENS OF BOTH INDOOR AND OUTDOOR PATIENTS OF TERTIARY CARE HOSPITAL, RAWALPINDI

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Abstract

Klebsiella spp. are Gram negative, non-motile, usually capsulated rods. They are frequently found inthe large intestine but are also present in soil and water. These organisms are usually opportunistic pathogens that cause nosocomial infections, especially urinary tract and respiratory tract infections.Klebsiella species often produces beta-lactamases and are resistant to ampicillin. Cephalosporins andaminoglycosides are used to treat Klebsiella infections. Some Klebsiella strains show multiple drug resistance and are an important cause of hospital acquired infections. The aim of this study is to determine the prevalence and antimicrobial susceptibility patterns of Klebsiella species. Purely isolated colonies of *Klebsiella* spp. were obtained, biochemical tests and gram staining wereperformed for identification. Isolated colonies will be streaked on Mueller Hinton agar plate for antibiotic sensitivity testing. The Antibiotic susceptibility test will be determined according to the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria. An Antibiotic impregnated disk will be placed on MH agar and inoculated with the test bacterium. A clear zone or ring will be formed around an antibiotic disk after incubation if the agent inhibits bacterial growth. According toantibiogram results of 143 isolates of *K. pneumoniae,* the highest antibiotic resistance was related toamoxicillin-clavulanate (98%), cefexime (96.70%) and cefotaxime (97%) and 96.60% isolates weresusceptible to colistin. Isolates also show susceptibility to gentamicin (82%), amikacin (78.10%), tigecycline (75.50%), sulzone (69.20%), imipenem (56%). It also shows resistance to cotrimoxazole(89%), ceftriaxone (87.49%), ciprofloxacin (79%), ceftazidime (86.30%).

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INTRODUCTION

The tribe Klebsiella, which is a part of the Enterobacteriaceae family, includes the genus Klebsiella. The organisms are named for German microbiologist Edwin Klebs, who lived in the 19th century. (1)It is an opportunistic pathogen capable of causing a wide range of community-acquired and hospital acquired infections, such as urinary tract infections (UTIs), respiratory tract infections and infections of wounds and soft tissue. (3)

It has in recent years become one of the world's leading causes of nosocomial infections, with an increasing mortality rate, particularly in immunocompromised individuals, neonates and the elderly. It is also increasingly implicated in severe community-acquired infections such as pneumonia and meningitis. (4)

On their cell surfaces, members of the Klebsiella genus normally express two different types of antigens. The first is a capsular polysaccharide, and the second is a lipopolysaccharide (O antigens) (K antigen). These two antigens each add to pathogenicity. Klebsiella spp. are the leading cause of morbidity and mortality. For Klebsiella to be virulent, capsules are necessary. (5)

K. pneumoniae has emerged as a frequent source of hospital-acquired infections, including urinary tract infections (UTIs) and bloodstream infections (BSIs), where antibiotic-resistant strains are becoming more challenging to treat and are linked to a higher death rate. (12, 13)

Simply because of the increasing exposure to pathogens connected with healthcare with time, there does appear to be a positive association between the amount of time a patient must stay in the hospital and the probability of contacting K. pneumoniae infection.(14) One of the main causes of neonatal sepsis (NS) is Klebsiella pneumoniae. In developed countries, this microbe causes neonatal sepsis in 4-9% of cases, but it affects 16–28% of people in underdeveloped nations. (15, 16)

These microorganisms can be found in wood pulps, sawdust, and waters that receive these industrial effluents. Numerous isolates of K. pneumoniae and K. oxytoca has been isolated from untreated water samples collected from dams, seawater, sediment and intestinal contents of shrimps and freshwater fishes. As a result of its capacity to form capsules and subsequent biofilm, the organism can survive in water distribution system despite chlorination. (18)

Extended-spectrum -lactamase (ESBL)-producing organism infections are an issue on a global scale. Klebsiella pneumoniae, which produces ESBLs, are being linked to rising antibiotic resistance. (21) ESBLs are usually plasmid mediated. Since these plasmids are easily transmitted among different members of the Enterobacteriaceae, accumulation of resistance genes results in strains that contain multidrug resistant plasmids.

For this reason, ESBL-producing isolates are resistant to a variety of classes of antibiotics. Moreover, the emergence of these multiply resistant Klebsiella strains is unfortunately accompanied by a relatively high stability of the plasmids encoding ESBLs.

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Even years after the discontinuation of ceftazidime and other extended spectrum cephalosporins, continued colonization of patients by ESBL-producing Klebsiella strains has been observed. Risk factors for acquisition of these strains seem to be the length of stay in hospital and the performance of invasive procedures. (22)

Antibiotic resistance in K. pneumoniae has grown against various antibiotic families. The majority of these antibiotic resistance mechanisms (AMR) were acquired through horizontal gene transfer (HGT), which confers high level resistance to -lactam and quinolone antibiotics. Penicillin binding proteins (PBPs), which are specialized targets of -lactam antibiotics and enzymes that catalyze the formation of peptidoglycans, are one of the resistance strategies used by K. pneumoniae. PBPs' affinity for lactam antibiotics is reduced by structural changes, which leads to an increase in resistance to them. (23,24)

Additionally, alterations in outer membrane (OM) permeability play a crucial role in K. pneumoniae resistance (hydrophilic drugs). (25) Before it can bind to PBPs, OM is a barrier that must be overcome. Porins (OmpK35 and OmpK36), which the antibiotic molecules must employ, are either less in number or changed, reducing the membrane's permeability. (26,27) Quinolone resistance also results from altered permeability and the presence of efflux pumps. (28)

Previous studies have shown that K. pneumoniae strains that are resistant to a broad spectrum of antibiotics are rapidly expanding, especially when the bacteria are capable of forming biofilm. These bacteria can form a thick layer of extracellular biofilm which helps them attach to living and abiotic surfaces.

Treatment of infections caused by biofilm-forming K. pneumoniae strains is more difficult than other strains. The antibiotic resistance and bacterial tendency to biofilm formation may play a key role in the emergence of MDR-K. pneumoniae strains. Due to the antiphagocytic feature of biofilm, it is more challenging for the host immunity to eliminate this kind of bacterial pathogens.

The use of antibiotics in patients with bacterial infections can lead to bacterial elimination and accelerate the treatment process. Consequently, the increasing incidence of drug resistance causes complications in patients and higher medical costs. (29)

MATERIALS AND METHODS

A total of 143 samples were included which was calculated by the WHO sample size calculator. All these samples were taken from Microbiology laboratory of Tertiary Care Hospital, Rawalpindi. The ethical committee of university gave their approval to this study. Patient informed consent was obtained before any data was gathered. The Cross-sectional descriptive study is performed on this sample population. The Klebsiella species isolated from all clinical samples were included and clinical samples taken from all age groups were included in this study. Microorganisms other than Klebsiella were excluded.

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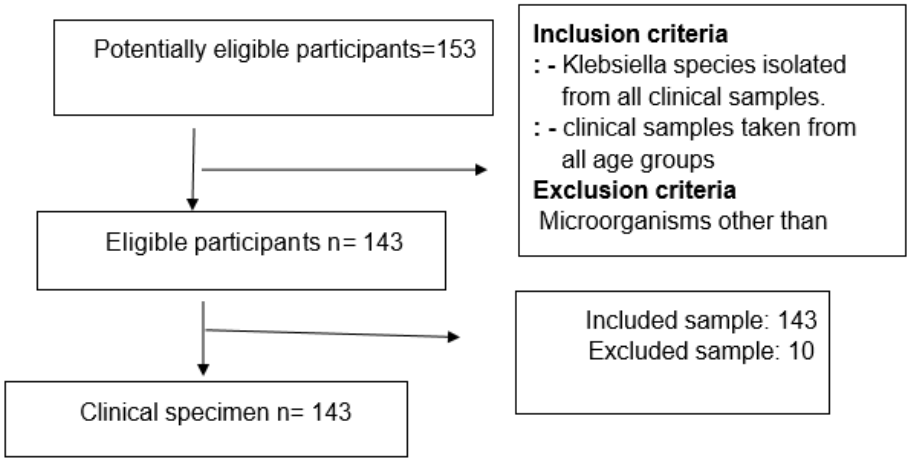
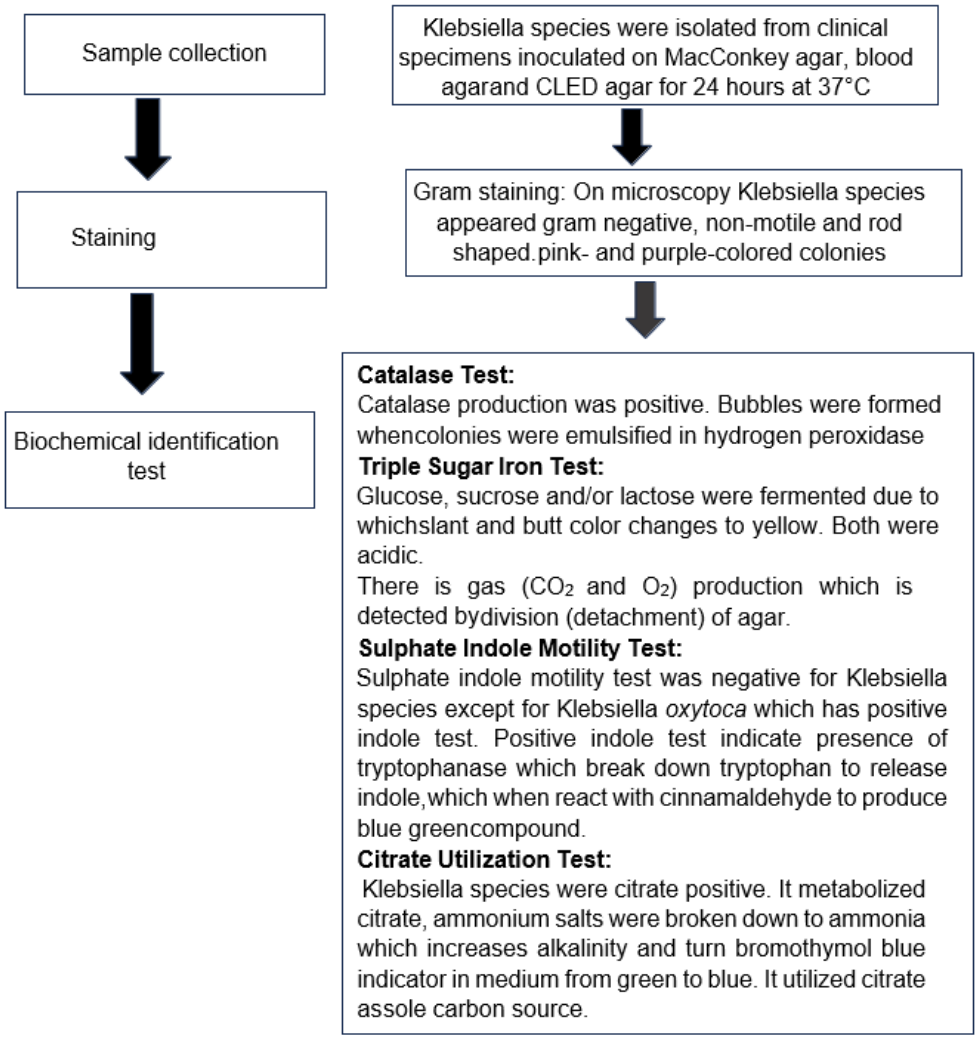


METHODOLOGY



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Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing performedafter the isolation of Klebsiella species by Disk Diffusion method using Kirby-Bauer technique. The organism was inoculated on MH agar plates by sterile swabs and then discs were placed on media andpressed gently followed by overnight incubation at 37°C. The antibiotics included amoxicillin- clavulanic acid (30 µg), amikacin (30 µg), gentamycin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), cefepime (30 µg), ciprofloxacin (5 µg), trimethoprim- sulfamethoxazole (cotrimoxazole) (25 µg), piperacillin-tazobactam (110 µg), cefoparazone-sulbactam(105 µg), tigecycline (TGC), colistin (10 µg), Fosfomycin, nitrofurantoin (300 µg). The zone of inhibitions of each antibiotic was recorded in millimeter (mm) and corresponds to CLSI standard valueof respective antibiotic.

RESULTS

According to this research K. pneumoniae is prevalent in females that is 55%. According to antibiogram results of 143 isolates of K. pneumoniae, the highest antibiotic resistance was related to augmentin (amoxicillin/clavulanic acid) (98%) and 96.60% isolates were susceptible to colistin. All the data is tabulated and analyzed using Statistical Package for Social Sciences (SPSS Version 25). Frequency and percentage were calculated for categorical data.

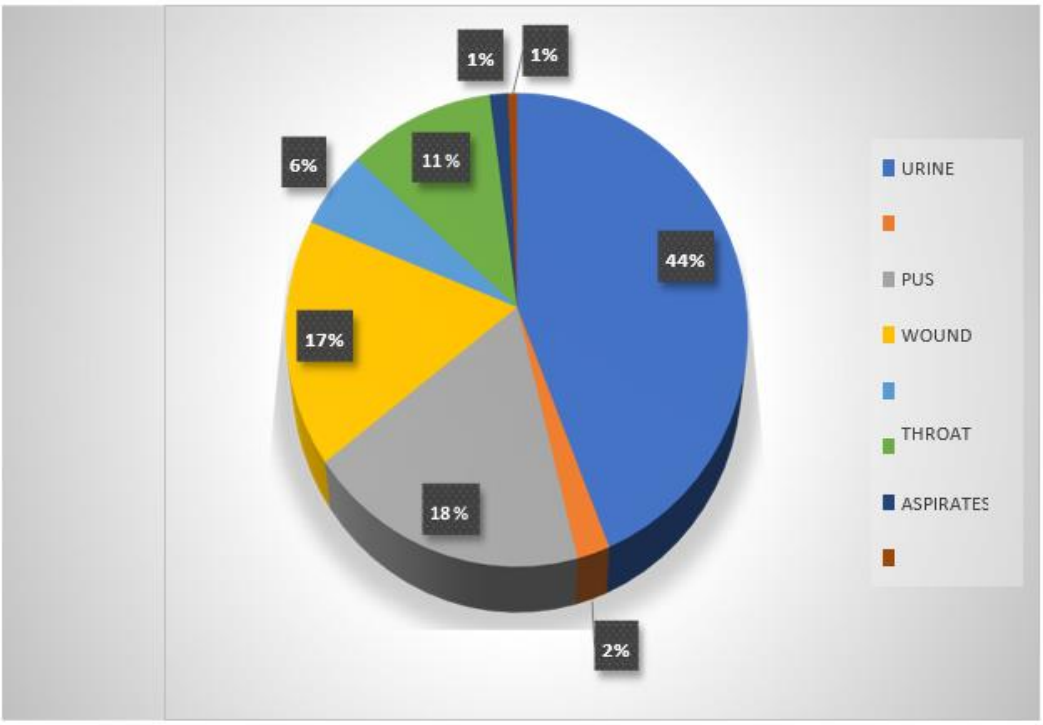

Figure 1: This above figure shows the source of isolates included in this work. Out of 143 samples

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K. pneumoniae clinical isolates, 8 (5.6%) were collected from throat culture, 63 (44.1%) from urine, 3 (2.1%) from blood culture, 26 (18.2%) from pus culture, 24 (16.8%) from wound, 16 (11.2%) from aspirates/ body fluids, 16 (11.2%) from tip (ETT, CVP, Folley's catheter) culture and1 (0.7%) from bile sample.

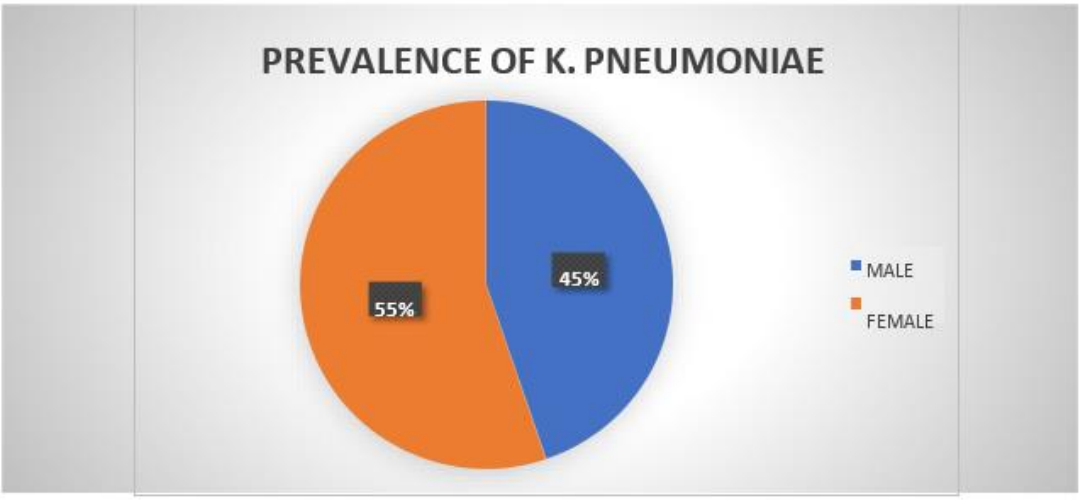

Figure 2: shows prevalence of K. pneumoniae in male and female patients. About 64 (44.8%) K. pneumoniae strains were obtained from male and 79 (55.2%) from female patients. Among all the clinical isolates 54 (37.8%) were taken from outpatients and 89 (62.2%) from inpatients specimens

Table 1: The frequency of antimicrobial susceptibility of different antibiotics are shown in above table

ANTIBIOTICS	SENSITIVE	RESISTANT
COLISTIN	96.60%	3.40%
TIGECYCYCLINE	75.50%	16.10%
AMIKACIN	78.10%	21.90%
GENTAMICIN	82%	18%
SULZONE	69.20%	30.8%
TAZOCIN	63.20%	36.80%
CEFTRIAXONE	12.51%	87.49%
CEFTAZIDIME	13.7%	86.30%
CEFEPIME	13.34%	86.66%
CEFOTAXIME	3%	97%
CEFEXIME	3.30%	96.70%
AUGMENTIN	2%	98%
CIPROFLOXACIN	21%	79%
IMIPENEM	56%	44%
COTRIMOXAZOLE	11%	89%

1. According to antibiogram results of 143 isolates of *K. pneumoniae*, the highest antibiotic resistance was related to augmentin (amoxicillin/clavulanic acid) (98%) and 96.60% isolates were susceptible to colistin. After augmentin, the most resistance was to cefotaxime (97%) and cefexime (96.70%).

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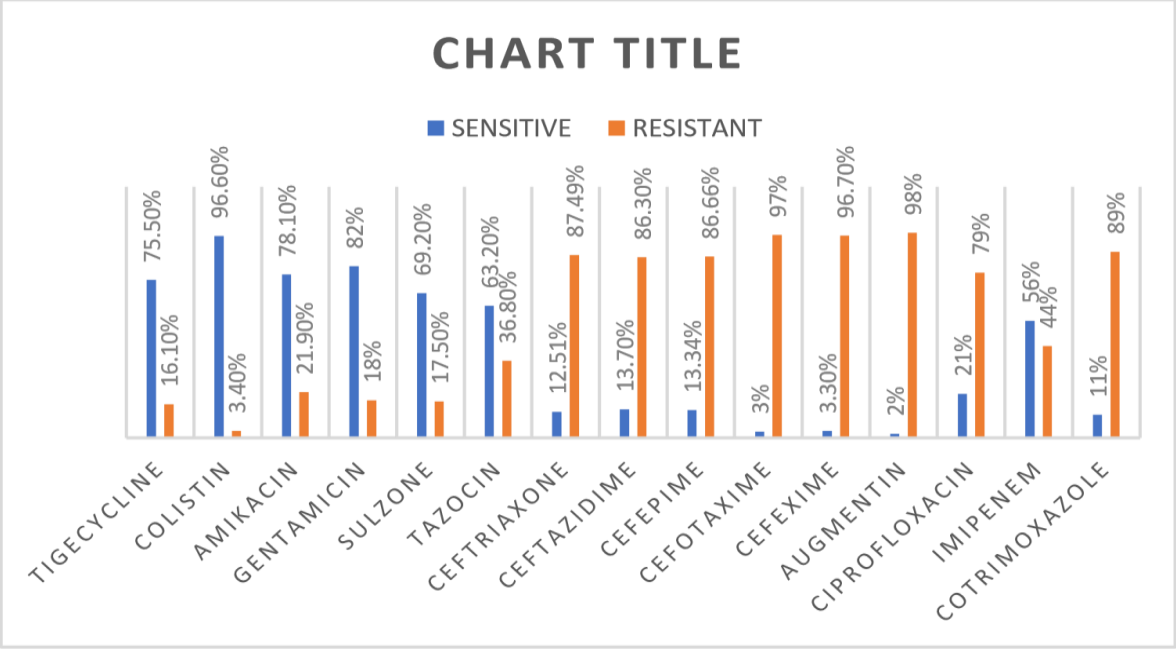

Figure 3

Figure 3: Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*. The prevalence of antimicrobial susceptibility pattern to tigecycline, amikacin, gentamicin sulzone, tazocin, ceftriaxone,cefepime, ceftazidime, ciprofloxacin, imipenem and cotrimoxazole are shown in figure 3. Different antibiotic susceptibility pattern in various clinical specimens, for example out of 63 urine samples 73.2% were resistant to nitrofurantoin and 61.5% resistant to Fosfomycin, was observed. In addition to colistin,82% isolates show susceptibility to gentamicin, 78.10% isolates to amikacin, 75.50% to tigecycline, sulzone (69.20%), tazocin (63.20%) and 56% to imipenem.

DISCUSSION

Klebsiella has emerged as a potential virulent pathogen over the years. Previously, a common cause of community acquired pneumonia, urinary tract infections, bacteremia and neonatal septicemia, hypervirulent forms with a potential for metastatic spread has occurred. These hypervirulent forms areprimarily the cause of amoebic liver abscess, meningitis and metastatic endophthalmitis. An insight of different virulence factors and their mechanism of interference with the host immune mechanism responsible for disease causation would enable to curb their harmful effects.

The *K. pneumoniae* isolates used in this study came from wide range of samples (figure1). The main sample sources were urine (44.1%), pus (18.2%), wound culture (16.8%), tips culture (11.2%), throatculture (5.6%), blood culture (2.1%), fluids/aspirates (1.45) and from bile (0.7%). In this study 96.60%isolates were susceptible to colistin and the highest antibiotic resistance was related to augmentin (98%), cefotaxime (97%), and cefexime (96.70%). The results of this study were consistent with thatconducted at two major

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Hamadan hospitals, west of Iran from September 2018 to march 2019. A totalof 83 *K. pneumoniae* isolates were collected from different clinical specimens. The highest antibioticresistance was related to cefotaxime (92%) and all isolates were susceptible to colistin. This study alsoshow that clinical isolates obtained from inpatient department are more prevalent as compared to thatof outpatient department which is consistent with our present study. Another study was conducted at Bolan Medical Complex, Quetta, Balochistan. Clinical samples (n=107) of urine and sputum were collected and processed for *K. pneumoniae* isolation using selectiveculture media. All the isolates (100%) were resistant to amoxicillin, cefixime, amoxicillin-clavulanic acid, cefotaxime, and ceftriaxone followed by ciprofloxacin (76.2%), sulphamethoxazol (66.7%), norfloxacin (42.9%).

A similar study was conducted at department of Microbiology, Dhaka Medical University, Dhaka, Bangladesh. Among 316 samples that yielded culture positivity, *K. pneumoniae* were identified as second most common organism. Most of the isolates were resistant to sulphamethoxazole- trimethoprim (90.67%) and ceftriaxone (90.67%) followed by cefotaxime (89.33%) and ceftazidime (89.33%). The most sensitive antibiotic for the isolates was tigecycline.

This study also highlights greater prevalence of *K. pneumoniae* in females (55.2%) than males (44.8%). A similar study conducted on 130 clinical isolates of *K. pneumoniae* obtained from various specimens in Duhok City, Iraq during January 2017-February 2019 which show that the isolates weremore predominant in females (n=99) compared to males (n=31). The resistance rate of *K. pneumoniae* varied among different isolate clinical sample sources. Overall high resistance rates were reported for ampicillin (96.9%), ceftriaxone (65.8%) and cefepime (60.8%). However, imipenem showed highest susceptibility rate against the isolates.

In June 2015, a carbapenem resistant K. pneumoniae was isolated from the tracheal secretion and an endotracheal tube of a 32-year-old female patient at the intensive care unit at the tertiary hospital, Pakistan Institute of Medical sciences, Islamabad. Isolates were found to be multi-drug resistant and sensitive only to Tigecycline and Colistin.

A retrospective study focused on drug susceptibility of *K. pneumoniae* isolated from intensive care unit (ICU) patients with bloodstream infection in Shanghai, China showed that out of 78 *K. pneumoniae* isolates, the highest resistance is related to amoxicillin-clavulanate (93.5%), aztreonam (93.5%) and ciprofloxacin (92.3%). In contrast, relatively low resistance to colistin (11.5%) and tigecycline (23%).

CONCLUSION

The results of this study show that most isolated species of Klebsiella is *Klebsiella pneumoniae* among isolates obtained from various clinical specimens at tertiary care hospital, Rawalpindi. *K. pneumoniae,* the cause of urinary tract infection and pneumonia, is becoming resistant to various antibiotics with most resistance to amoxicillin-clavulanate, cefotaxime and cefexime due to βlactamase production. Colistin is the most effective drug against Klebsiella according to our study with some susceptibility to

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amikacin, gentamicin, imipenem, sulzone and tazocin. The prevalence of *K. pneumoniae* among females is greater as compared to males and most prevalent in admitted patients than outpatient's specimens.

Our results suggested that there is high antibiotics resistance among *K. pneumoniae* isolates towards commonly prescribed antibiotics, which is an alarming situation for developing countries like Pakistan. Continuous monitoring and strict antimicrobial policy will have a great impact on reducing antimicrobial resistance towards antibiotics and development of proper treatment options against *K. pneumoniae* infections.

References

1) Introduction to Klebsiella https://emedicine.medscape.com/article/219907-overview.
2) John E. Bennett MD, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 2020
3) Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews. 1998 Oct 1; 11(4):589-603.
4) Shon AS, Bajwa RP, Russo TA. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence. 2013 Feb 15; 4(2):107-18.
5) Randall G. Fisher, in Feigin and Cherry's Textbook of Pediatric Infectious Diseases (Sixth Edition), 2009
6) Cryz Jr SJ, Fürer F, Germanier R. Experimental Klebsiella pneumoniae burn wound sepsis: role of capsular polysaccharide. Infection and immunity. 1984 Jan; 43(1):440-1.
7) Cryz Jr SJ, Fürer E, Germanier R. Safety and immunogenicity of Klebsiella pneumoniae K1 capsular polysaccharide vaccine in humans. Journal of Infectious Diseases. 1985 Apr 1; 151(4):665-71.
8) Podschun R, Penner I, Ullmann U. Interaction of Klebsiella capsule type 7 with human polymorphonuclear leucocytes. Microbial pathogenesis. 1992 Nov 1; 13(5):371-9.
9) Podschun R, Ullmann U. Klebsiella capsular type K7 in relation to toxicity, susceptibility to phagocytosis and resistance to serum. Journal of medical microbiology. 1992 Apr 1; 36(4):250-4.
10) Yokochi T, Nakashima I, Kato N. Effect of capsular polysaccharide of Klebsiella pneumoniae on the differentiation and functional capacity of macrophages cultured in vitro. Microbiology and immunology. 1977 Oct 20; 21(10):601-10.
11) Ofek I, Doyle RJ. Bacterial adhesion to cells and tissues. Springer Science & Business Media; 2012 Dec 6.
12) Liu P, Li X, Luo M, Xu X, Su K, Chen S, Qing Y, Li Y, Qiu J. Risk factors for carbapenem-resistant Klebsiella pneumoniae infection: a meta-analysis. Microbial drug resistance. 2018 Mar 1; 24(2):190-8.
13) Nouvenne A, Ticinesi A, Lauretani F, Maggio M, Lippi G, Guida L, Morelli I, Ridolo E, Borghi L, Meschi T. Comorbidities and disease severity as risk factors for carbapenem-resistant Klebsiella pneumoniae colonization: report of an experience in an internal medicine unit. PloS one. 2014 Oct 15; 9(10): e110001.
14) Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Risk factors for acquisition of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae in North-Indian hospitals. Saudi Journal of Biological Sciences. 2015 Jan 1; 22(1):37-41.

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15) Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatric Clinics. 2013 Apr 1; 60(2):367-89.

16) Verma P, Berwal PK, Nagaraj N, Swami S, Jivaji P, Narayan S. Neonatal sepsis: epidemiology, clinical spectrum, recent antimicrobial agents and their antibiotic susceptibility pattern. Int J Contemp Pediatr. 2015 Jul; 2(3):176-80.

17) N. Gundogan, in Encyclopedia of Food Microbiology (Second Edition), 2014

18) Randall G. Fisher, in Feigin and Cherry's Textbook of Pediatric Infectious Diseases (Sixth Edition), 2009

19) Vali *et al:* Klebsiella oxytoca with reduced sensitivity to chlorhexidine isolated from a diabetic foot ulcer 20. Mechanism of resistance and bacterial susceptibility in extended spectrum beta lactamases phenotype Klebsiella pneumoniae and Klebsiella oxytoca isolated between 2000 and 2010 in Japan. J Med Microbial (2015)

20) Eliopoulos GM, Bush K. New β-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clinical Infectious Diseases. 2001 Apr 1; 32(7):1085-9.

21) Hibbert-Rogers LC, Heritage J, Gascoyne-Binzi DM, Hawkey PM, Todd N, Lewis IJ, Bailey C. Molecular epidemiology of ceftazidime resistant Enterobacteriaceae from patients on a paediatric oncology ward.

22) Journal of Antimicrobial Chemotherapy. 1995 Jul 1; 36(1):65-82. Chong Y, Yakushiji H, Ito Y, Kamimura

23) Kaczanowska, M.; Rydén-Aulin, M. Ribosome biogenesis and the translation process in Escherichia coli. Microbiol. Mol. Biol. Rev. 2007, 71, 477–494, doi:10.1128/MMBR.00013- 07.

24) Villa, L.; Feudi, C.; Fortini, D.; Garcia-Fernandez, A.; Carattoli, A. Genomics of KPC- producing Klebsiella pneumoniae sequence type 512 clone highlights the role of RamR and ribosomal S10 protein mutations in conferring tigecycline resistance. Antimicrob. Agents Chemother. 2014, 58, 1707–1712, doi:10.1128/AAC.01803-13.

25) Dutta, S.; Kawamura, Y.; Ezaki, T.; Balakrish Nair, G.; Iida, K.I.; Yoshida, S.I. Alteration in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV in quinolone- resistant Shigella dysenteriae serotype 1 clinical isolates from Kolkata, India. Antimicrob. Agents Chemother. 2005, 49, 1660–1661, doi:10.1128/AAC.49.4.1660-1661.2005.

26) Ruiz, J.; Pons, M.; Gomes, C. Transferable mechanisms of quinolone resistance. Int. J. Antimicrob. Agents 2012, 40, 196–203, doi: 10.1016/j.ijantimicag.2012.02.011.

27) Horan TD, Culver D, Jarvis W, Emori G, Banerjee S, Martone W, Thornsberry C. Pathogens causing nosocomial infections preliminary data from the national nosocomial infectionssurveillance system. Antimicrobic Newsletter. 1988 Sep 1; 5(9):65-7.

28) Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiology and Molecular Biology Reviews. 2014 Sep; 78(3):510-43.

29) Zheng JX, Lin ZW, Chen C, Chen Z, Lin FJ, Wu Y, Yang SY, Sun X, Yao WM, Li DY, Yu ZJ.Biofilm formation in Klebsiella pneumoniae bacteremia strains was found to be associated withCC23 and the presence of wcaG. Frontiers in cellular and infection microbiology. 2018 Feb 23; 8:21.