American Journal of Sciences and Engineering Research
E-ISSN -2348 – 703X, Volume 7, Issue 2, 2024

Characterization and Antibiotic Susceptibility of Klebsiella Pneumonia Isolated from Clinical Samples

Rabia Akbar[1]*, Azeem Siddiqui[2], Nida Adeel[2], Syed Alamdar Abbas Zaidi[2], Muhammad Usama[3], Shameen Hashmi[4], Maryam aslam[5], Haris Latif[6], Humaira Maryam[7]

[1)] *Department of Pharmaceutical Sciences , University of Lahore, Lahore ,Pakistan.*
[2)] *Department of Biosciences, COMSATS University Islamabad, Pakistan.*
[3)] *Institute of Microbiology, Faculty of Veterinary Sciences, University of Veterinary and animal sciences, Lahore, Pakistan.*
[4)] *University College of Medicine & Dentistry*
[5)] *Department of diet and nutritional sciences,University of Lahore,Lahore,Pakistan*
[6)] *Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan*
[7)] *Environmental sciences*

ABSTRACT: *Klebsiella pneumonia is a gram negative Enterobacteriaceae k. pneumoniae is a ubiquitous in nature which is highly pathogenic, nonmotile, rod shaped, facultative anaerobic bacteria. Nosocomial infection and community acquired infections is caused by klebsiella species. Different types of diseases caused in human mainly include urinary tract infection, meningitis, septicaemia, and specially diseases caused in children. k. pneumoniae is highly resistance to antibiotics due to which they caused high morbidity and sometime death. To check biochemical identification and characterization of K. pneumoniae, different biochemical tests were performed such as gram staining, microscopy, oxidase test, catalase tests, indole tests and sugar fermentation test. For checking antibiotics susceptibility, Kirby Bauer disc diffusion method was performed on Mueller Hinton agar according to CLSL (2020) guideline. According to results k. pneumoniae strains were highly resistance to antibiotics Vancomycin (0mm). Ticarcillin (0mm), Doxycycline (9mm), Ceftriaxone (10mm), Cefotiam(11mm), Amoxicillin(12mm) and Polymyxin B (12mm). Antibiotics that were susceptible to K. pneumoniae were Chloramphenicol (21mm), Amikacin(22mm), Imipenem (26mm) and Tazobactam(28mm).*

Key words: *Antibiotics, biochemical, Infections, Klebsiella pneumonia*

I. INTRODUCTION

Introduction to klebsiella pneumonia

Klebsiella Pneumoniae was firstly described in 1885, and was named after Edwin klebs. German Pathologist (Paterson et al.,2004). Enterobacter, Klebsiella, Escherichia colporteur, Citrobacter, Salmonella, Serratia, and other different pathogens are members of Enterobacteriaceae. K. pneumonia is a Gram-negative Enterobacteriaceae and belongs to normal flora of the human intestine and mouth. K. pneumoniae is a nonmotile, rod shape, nitrate positive, mucoid colony forming, facultative anaerobic, encapsulated bacterium that can be found in the environment. After Escherichia coli, K. pneumoniae is the second most prevalent cause of gram_ negative bacteria (Sikarwar et al. 2011).

Occurrence

Klebsiella spp. Are found all over the world. k. pneumoniae has two frequent habitats: the environment, where they may be found in water surface, in sewage, and in soil, and also present on plants and the mammal's mucosal

surface like humans, horses, and pigs, which they invade (Bina et al., 2015). Klebsiella is particularly similar to Citrobacter and Enterobacter but not to E coli or Shigella, which are widespread in humans as compared to environment. As a saprophyte K. pneumoniae is found in the nasopharynx and the gastrointestinal tract in humans (Arivett et al., 2015).

Classification of Klebsiella Pneumoniae

Klebsiella pneumoniae clinical strains are categorized into two groups: the classical K. pneumoniae group, which includes MDR strains are typically found in the gastrointestinal (GI) tract of individuals with intestinal cancer. Hyper virulent K. pneumonia strains are new classical K. pneumoniae variations unlike classical K. pneumoniae viruses. The majority of hyper virulent strains (about 70%) are capsular K1 or K2 serotypes (Kislichkina et al.,2017). Four species of the genus Klebsiella include K. pneumoniae, K. oxytocin, K. rhinoscleromatis, and K. ozaenae, that were introduced as pathogens to human. K. granulomatis (called Calymmatobacterium granulomatis) also is a human pathogen (Peleg et al., 2010).

Diseases Caused by Klebsiella Pneumoniae

Klebsiella pneumoniae bacteremia causes significant morbidity and mortality (Tsay et al.,2004). K. granulomatis also is a human pathogen that cause a range of infections when people have bacteria in the digestive tract and spread to another part of the body includes skin, urinary tract infection, wound infection, liver abscesses, blood infection, and meningitis. Therefore, the common symptoms of these bacteria can cause pneumonia such as cough, chest pain, fever, and shortness of breath (Peleg et al.,2010).

Hyper virulent strains of K. pneumoniae can cause organ and life _ threatening infections in healthy people, that cause infections in a variety of locations, including meningitis, pyogenic liver abscesses, endophthalmitis, pneumoniae, and necrotizing fasciitis, all of which can lead to metastatic dissemination (Kislichkina et al.,2017).

Identification

Identification and differentiation of Klebsiella pneumoniae species are usually followed by their biochemical reactions (Sikarwar et al., 2011). k. pneumoniae have been identified as the third leading cause of hospital acquired infections in the United States behind Clostridium difficile and Staphylococcus aureus, K. pneumoniae are also a leading cause of ventilator _associated pneumoniae (VAP) among patients in intensive care units (Martin et al., 2018). This species received the name for its ability to cause sever pneumoniae. Although K. pneumoniae is more usually associated with nosocomial infections, food has been described as a probable vector of transmission. Raw meat, raw vegetable, fruit juice, and ready _to eat foods have all been shown to contain K. pneumoniae (Paterson et al.,2004).

Resistance

Antibiotic_ resistance forms of K. pneumoniae are on the rise. Antibiotics overuse has resulted in widespread bacterial resistance in k. pneumoniae is now well understood (Sikarwar et al.,2011). By Alexendar Fleming in 1929 as a gram-negative bacteria K. pneumonia discovered resistance to β-lactam antibiotic that cause hydrolysis of beta-lactam ring in antibiotics due to produce β-lactamase. Extended spectrum-β-lactamase (ESBL) can hydrolyse oxyimino cephalosporin rending third-generation cephalosporins (Sahly et al.,2004).

Due to pathogen's high intrinsic antibiotic resistance, K. pneumoniae infections are difficult to cure. Because of the presence of β-lactamase producing genes in chromosomal genome, K. pneumoniae is innately resistant to ampicillin (Comandatore et al.,2013). k. Pneumonia is a strong candidate for dissemination and horizontal gene transfer across Gram - negative species because of its extensive ecological range and capacity to carry multidrug resistance genes (Khadri et al.,2007).

Antibiotic Susceptibility for Klebsiella pneumonia

Antibiotic susceptibility for K. pneumoniae was tested using the Kirby Bauer disc diffusion method on stranded media Mueller_ Hinton agar according to the recommendations which were provided by the clinical and Laboratory Standards Institute (CLSI) 2020. The following used antibiotics agents were tested Vancomycin (VA), Amoxicillin + Clavulanate (AMC), ceftriaxone (CRO), Meropenem (MEM), Imipenem (IPM) , Chloramphenicol (C) , Doxycycline (DO), Amikacin(AK) , Piperacillin (PRL) ,Ticarcillin (TIC) , Polymyxin B (PB), Cefepime (FEP) , Tazobactam piperacillin (TZP) , Ciprofloxacin(CIP) , Ampicillin (AMP) . The results of the antibiotics susceptibility tests were interpreted according to the guidelines provided by the CLSI (Yu et al.,2006) .

Aim and Objectives

The aim of the present research is to isolate the pathogenic Klebsiella pneumoniae strains from clinical samples such as urine, puss and swab collected from different hospital of Peshawar.

The main objective of the present research project include;

1. To isolate the pathogenic K. pneumoniae strains from clinical patients.
2. To determine biochemical identification and characterization of K. pneumoniae.
3. To find out drug resistance against K. pneumoniae.

II. LITERATURE REVIEW

In 2020 Chong et al. (2020) collected 305 samples from 9 markets in Nakhon Si Thammarat, Southern Thailand, and the prevalence, antimicrobial susceptibility, and molecular genetic characteristics of extended-spectrum lactamase (ESBL)-producing K. pneumoniae isolates from 10 varieties of raw vegetables were investigated. ESBL-producing K. pneumoniae isolates were detected in 14 of the 305 samples collected from seven different vegetable kinds. K pneumoniae isolates were resistant to both -lactam carbapenem drugs and non-lactam aminoglycosides. The most resistant ESBL producers were to -lactam antibiotics, such as ampicillin and cephalosporins cefotaxime.

Yang et al., (2019) Studied 137 strains of K. pneumoniae that were isolated from cows, pigs, sheep and chickens at animal hospitals in Henan Province, China, from March to December 2017. Furthermore, 52 nosocomial K. pneumoniae strains were discovered from a Xinxiang Medical University teaching hospital. Biochemical tests were done for all isolated bacteria and were identified using staining, antimicrobial susceptibility testing was done using the disc diffusion method. The resistance rates against 15 antibiotics of the 189 K. pneumoniae isolates ranged from 11.6% to 77.8%. The nosocomial bacteria were more resistant to the 15 drugs than the animal-source strains. The nosocomial strains had the highest resistance to AMP (80.8%), followed by CIP (75.0%), TCY, and KAN (69.2%), and the lowest resistance to AZM and MEM (both 69.2%).

Lin et al., (2012) investigated seroepidemiology of K. pneumoniae isolates from the intestinal tract of healthy Chinese in Asian countries. Stool specimens from healthy adult Chinese residents of Taiwan, Japan, Hong Kong, China, Thailand, Malaysia, Singapore, and Vietnam were collected from August 2004 to August 2010 for analysis. The intestine was one of the major reservoirs of K. pneumoniae. During the study period, the isolation rate was highest in Malaysia. The isolation rate was lowest in Japan.

In January 2013, Rodriguez-Zulueta et al., (2013), isolated 39 Klebsiella spp. from community-acquired infections at a Mexican hospital based on clinical symptoms samples obtained from all outpatients were regarded as an infection. All isolates were subjected to a molecular test utilising multiplex-PCR amplification. The Mueller-Hinton (MH) disc diffusion method was used to evaluate the minimal inhibitory concentration (MIC) of ampicillin, amikacin, cefotaxime, nalidixic acid, and ciprofloxacin in the ESBL-producing positive isolates.

Osagie et al., (2017), isolated 167 strains of K. pneumoniae from inpatients at Soeradji Tirtonegoro Hospital Klaten for analysis of colony shape, microscopic examination, antibiotic susceptibility, and biofilm-producing capacity (June 2016 to May 2017). Antibiotic resistance was widespread among Klebsiella pneumoniae isolates. Meropenem, amikacin, and piperacillin- tazobactam have 1.20%, 4.79%, and 10.53% antibiotic resistance, respectively. 148 (85.63%) of the 167 isolates produced biofilms.

Gootz et al., (2010) proposed that nosocomial infections caused by resistant gram-negative organisms, notably K. pneumoniae strains, have become a major challenge at a time when potential novel antimicrobial medicines are few, Carbapenem use rose in response to the frequency of extended-spectrum-lactamases, and Carbapenem resistance quickly followed. In North Carolina, researchers discovered a novel -lactamase known as "K. pneumoniae carbapenemase (KPC-1). KPC-producing Klebsiella species were soon discovered in New York

City and then upstate New York. All antibiotic drugs examined, including Carbapenem, polymyxin B, and tigecycline, were resistant to the isolates. To our knowledge, no cases of pan resistant K. pneumoniae infection had previously been documented in the United States.

Chen et al., (2004), isolated 193 species of K. pneumoniae bacteraemia from diabetic patients in northern Taiwan in between January 2001 and December 2003. Community-acquired pneumonia was defined as bacteraemia. In 193 instances, 147 (76.2%) had infections obtained in the community, while 46 (23.8%) had illnesses acquired in the hospital. The nosocomial group was older and had greater rates of malignancy, leukopenia infection with the extended-spectrum- lactamase-producing strain (ESBL infection), and mortality than the community-acquired group.

Yu et al. (2006) obtained 120 K. pneumoniae strains from patients with pneumonia at ten Japanese medical facilities in between April 2004 and April 2006. Ten serotype k2 strains were obtained and included in the study 30 years ago. Multilocus sequence typing (MLST) was used to characterise these isolates, and characteristics of their virulence factors such RmpA, aerobactin synthesis, and hypermucoviscosity phenotype were compared between patients with and without bacteraemia. MLST analysis was done on 120 isolates from pneumonia patients, and genetic lineages were identified for several sequence type groups.

Chen et al. (2012) suggested that Carbapenemase enzymes, which were capable of inactivating Carbapenem, were produced most commonly by K. pneumoniae. Due to significant infections in hospitalised patients and high mortality in the United States, Greece, Italy, and Israel, carbapenemase-producing K. pneumoniae has generated a public health catastrophe of global proportions. KPC-producing clones of K. pneumoniae have been found, although they were difficult to detect in a clinical microbiology lab.

Khadri et al. (2011) studied 45 patients with K. pneumoniae liver abscess from a tertiary teaching hospital in China were retrospectively assessed for clinical and microbiological characteristics in June 2008 and June 2010. Antimicrobial agents were effective against the vast majority of strains. These strains belong to the serotypes ki and k2. Only one of the 45 strains tested positive for ESBL. All isolates were resistant to ampicillin and sensitive to amikacin, amoxicillin-clavulanate, aztreonam, ceftazidime, cefoperazone-sulbactam, imipenem, and meropenem, as determined by antimicrobial susceptibility.

Garza-Ramos et al. 2018 Studied between 2012-2015 K. pneumoniae isolates were screened from patients admitted in American university of Beirut medical centre. The inclusion criterion was non-susceptibility, defined as resistant or intermediate phenotypes to at least one of the three clinically tested carbapenems, namely imipenem, ertapenem, or meropenem following the Clinical and Laboratory Standards Institute (CL.SI) guidelines 13. All of the isolates were non- susceptible to ertapenem, 85.3% (29/34) to imipenem, and 70.6% (24/34) to Meropenem. Resistance to meropenem was observed only when the isolate was simultaneously resistant to the remaining two carbapenems. In all cases where an isolate was resistant to only two carbapenems, these were ertapenem and imipenem.

Brisse et al. (2014) proposed in 2014, 51 samples of Klebsiella isolates from 500 patients in three units of Aleshtar hospital in 9 months for checking antibiotic resistance of K pneumoniae 18 antibiotics was performed by Kirby Bauer disk diffusion method. The largest number of K pneumoniae was isolated from the infectious unit (49.02%). The frequency of K. pneumoniae based on the source of infection for urine was 22 cases (43.14%), sputum 17 (33.33%), stool 6 (11.77%), wound 4 (7.84%), blood 2 (3.92%), and cerebrospinal fluid 0 (0%), respectively.

Klebsiella pneumoniae resistance to antibiotics included: ceftriaxone (94,12%), ciprofloxacin (90.20%), ofloxacin (86.27%), cefotaxime (78.43%), nalidixic acid (58.82%), nitrofurantoin (56.86%), aztreonam (54.90%), ampicillin (50.98%).

Fang et al., (2015) From January to July 2015, collected 128 sputum samples for testing antibiotic sensitivity in the Department of Microbiology at Akash Institute of Medical Sciences and Research Centre, Devanahalli, Bangalore rural. K. pneumoniae was found in 100% of the 128 sputum samples, and it was sensitive to Amikacin (66%), Ciprofloxacin (68%), Gentamicin (62%), Cefepime (60%), Imipenem (56.66%), and Aztreonam (56.66%). (52.63%). Ticarcillin clavulanic acid (81%) and Tobramycin (58%) resistance was found in isolates, as well as Co-trimoxazole
resistance (50%).

Hamdan et al. (2015) carried out a biochemical and genotypic approach for the phenotypic identification of 250 isolates of K. pneumoniae in April 2015 at four hospitals of Sudan's Khartoum state. Culture, colony morphology, and Gram stain results are used to identify K. pneumoniae from urine, swab, and wound samples. Only 139 isolates (55.6%) were genotypically verified as K pneumoniae, whereas 44.4% were classified as non-K. pneumoniae.

Yadav et al., (2015) identified K. pneumoniae from a patient with severe anaemia at Iraq's biology department using a swab from the oral cavity and tongue. Antibiotic resistance was determined using the VITEC device, which revealed that it was resistant to a wide range of medicines and was the source of hospital-acquired pneumoniae.

In 2017 Nepal et al., (2017) isolated K. pneumoniae from different clinical samples (pus. blood, body fluids, urine, sputum) for testing antimicrobial susceptibility by using Kirby-Bauer dise diffusion method at Om Hospital and Research Centre, Kathmandu, Nepal. Total isolates 61 were ESBL producers and 7 isolates were found to be MBL. producers. The lowest rates of resistance were seen toward imipenem followed by piperacillin/tazobactam, amikacin and cefoperazone.

Pragasam et al. (2017) described that increasing incidences of colistin resistance among nosocomial K. pneumoniae isolates have been reported from Europe, Asia, North America, and South America. Many isolated case reports and outbreaks of MDR K. pneumoniae infections were
reported from different parts of India. High mortality rate (approximately 69%) in bloodstream infections due to carbapenem- and colistin resistant K. pneumoniae was also noted among Indian patients.

III. MATERIALS AND METHODS

Study Area
Samples were collected from different laboratories of District Peshawar, KPK Pakistan.

Samples Collection
Total 30 clinical samples of urine, swab and puss were randomly collected from different labs of Peshawar and transported to the Central laboratory of Women University Mardan for further studies. This study was conducted between Oct 2021 to May 2021.

Chemicals Used in Research Work
Different chemicals which were used in research work included Crystal violet, gram iodine solution, safranin, decolourizer, ethanol, H_2O2, oxidase reagent, wood oil, distil water, nutrient agar, nutrient broth, MacConkey agar, Muller Hinton agar, phenol red solution, sucrose, glucose.

Apparatus Used in Research Work
Laminar flow hood. Digital balance, autoclave, incubator, refrigerator, graduated cylinder. Durham's tubes, wire loop, Petri plates, funnels, aluminium foil, burner, test tubes, slides, conical flasks and beakers.

Isolation of Bacterial Strains

For the isolation of bacterial strains, the samples were inoculated on Nutrient Broth (NB) and incubated at 37°C for 24hrs. After overnight incubation, Streaking was done on prepared nutrient agar plates and again kept for incubation in an incubator for 24hrs at 37°C in order to obtain pure cultures as shown in (figure 3.1).

Figure 3.1 isolation of pure culture

Sub Culturing

The isolated bacterial colonies were further sub-cultured for pure colonies on MacConkey agar plates through streaking methods as shown in (figure 3.2).

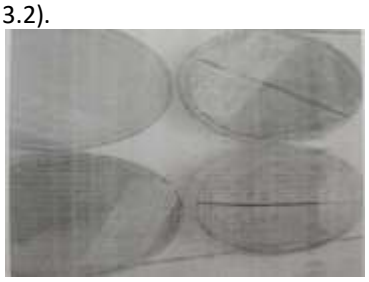

Figure 3.2 Sub Culturing plates

Isolated Bacterial Strains

The isolated colonies were labelled as Rkpl, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6 respectively. The appearance of bacterial colonies was pink in sub-culturing as shown in (figure 3.3).

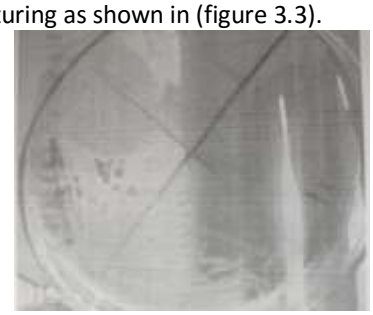

Figure 3.3 Morphology of isolated bacterial strains

Biochemical Identification and Characterization of Bacterial Isolates

For morphological characterization and biochemical identification, different biochemical tests were performed in the lab such as Gram Staining, Microscopy, Catalase Test, Oxidase Test, Indole Test, Sugar Fermentation Test.

Gram Staining

Gram staining involves the ability of bacterial cell wall to retain the primary stain during solvent treatment.

Requirements

Gram staining kit, sterilised slide, distilled water, pipette, wire loop.

Procedure

Sterile slide was taken and added 1µL of distilled water to it through a pipette and took a single colony of bacteria through a sterile wire loop and mixed it properly after mixing and left the slide until drying. A drop of Crystal violet was then applied to that prepared smear slide for 50 sec to Imin, washed with distilled water and then dried the slide through air dry or Bunsen burner. Gram iodine was dropped on the slide for 1.5minute, washed with acetone decolourizer, left for 15 secs, then washed with distilled water and air dried the slide. After drying, covered the smear on safranin which was used as a secondary dye for 30 secs then washed with distilled water

and dried the slide as shown in (figure 3.4). The same procedure was followed for each of the bacterial isolates (Rkp2, Rkp3, Rkp4, Rkp5, Rkp6).

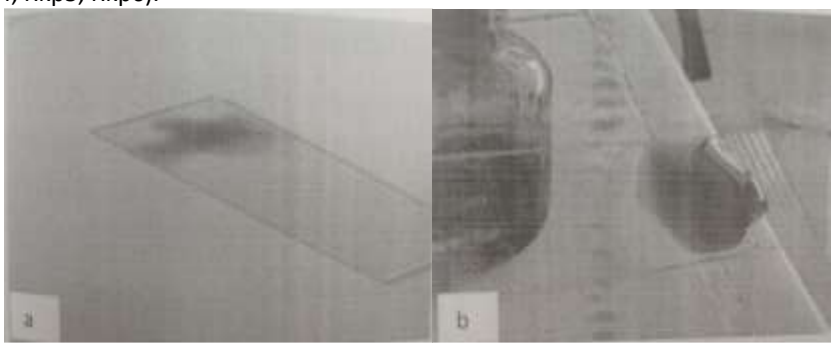

Figure 3.4 Procedure for Gram Staining(a)Crystal violet applied (b) Safranin.

Microscopy

The slide was then observed under a compound microscope. To get a clear image, the slide was observed under 100X oil immersion lens as shown in (figure 3.5). Under microscope the colour of gram-positive colonies appeared purple or bluish. However, the colour of colonies which appeared pink or reddish were termed as gram negative.

Figure 3.5 Microscopy

Catalase Test

A Catalase test was done to check that either bacterial isolates converted the hydrogen peroxide into water and oxygen or not.

Requirement

Ethanol, sterilised glass slide, H2O2, wire loop, spirit lamp.

Procedure

For catalase test, 20µl of 3% H2O2 was added on the clean glass slide. Fresh bacterial colonies were taken through a sterilised inoculating loop and mixed in the drop of H2O2 as shown in the figure 3.6

Figure 3.6 adding H202 on slides

Oxidase Test

Oxidase test was done to check that either bacterial isolates could produce cytochrome c oxidase enzymes or not.

Requirement

Petri plate, filter paper, oxidase reagent, micro pipette.

Procedure

For oxidase test sterile filter papers were taken and placed on the petri plates. Drop of oxidase reagent was added on the filter paper as shown in figure 3.8 (a) and then the fresh culture of the bacterial isolates was mixed with a drop of oxidase reagent as shown in figure 3.7

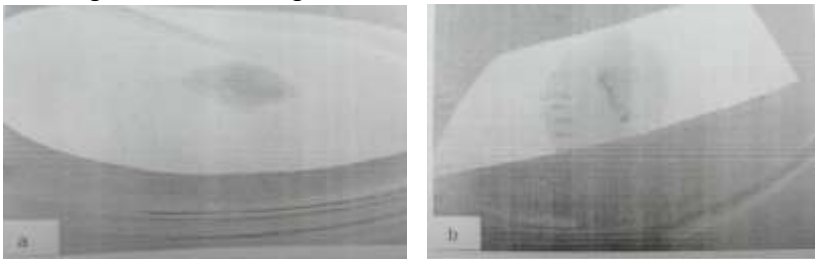

Figure 3.7 (a) oxidase test reagent added (b) Bacterial isolates was mixed

Indole Test

Indole test was done to check the ability of an organism regarding to tryptophan utilisation and indole ring formation

Requirements

Tryptone broth, sterilised tubes, kovacs reagent.

Procedure

4 ml of prepared tryptophan broth was taken in sterilised tubes and inoculated bacterial isolates in to the tube and incubated in an incubator for 24hrs at 37°C. After incubation Kovac reagent was added in order to check either red ring was produced or not, as an indicator for positive or negative
result for indole test as shown in figure 3.8

Figure 3.8 Tryptophan broth taken for indole test

Sugar Fermentation Test

Sagar fermentation test was done in order to check the type of sugar fermented by bacterial isolates.

Requirement

Nutrient broth, sugar, phenol red, Durham's tubes, sterilised tubes, phenol red.

Procedure

Sugar fermentation test was performed by inoculating a loop full of nutrient broth culture of the organism into the tubes containing different sugar media like sucrose and glucose. Then added phenol red as a PH indicator in the test tube and incubated for 24hrs at 37°C as shown in (figure 3.9).

Figure 3.9Showing sugar fermentation test by adding Phenol Red

Antibiotic Susceptibility Testing

The Kirby Bauer disc diffusion method was used to determine antibacterial properties of Klebsiella pneumoniae strains using Muller Hinton agar. This method was performed according to CLSI guidelines (2020). The following antimicrobial dises were Vancomycin (VA). Amoxicillin+Clavulanate (AMC), Ampicillin (AMP), Amikacin (AK),

Cefepime (FEP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Cefotiam (CN), Chloramphenicol (C), Doxycycline (DO), Imipenem (IPM, Piperacillin (PRL), Ticarcillin (TIC), Polymyxin B (PB), Tazobactam Piperacillin (TZP).

Procedure

The isolated colonies of pure culture were spread on prepared Muller Hinton agar plates with the help of sterile cotton swabs. After 5mints, antibiotic discs were placed in order to check the antimicrobial susceptibility. Incubated the plates in the incubator for 24hrs at 37°C after incubation the zone of inhibition was measured as shown in (figure 3.10).

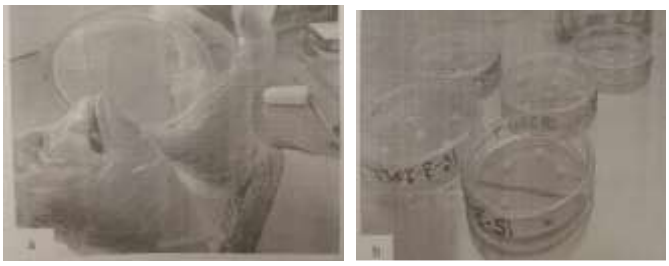

Figure 3.10 (a) Spreading Klebsiella strains(b) Antibiotic discs applied on plates

Table 3.1 Potency and Abbreviation of different antibiotics dose used to check Klebsiella pneumoniae antibiotic susceptibility.

S. No	Antibiotics	Abbreviation of antibiotics	Potency (ug)
1	Vancomycin	VA	30
2	Amoxicillin+Clavulanate	AMC	30
3	Ceftriaxone	CRO	10
4	Cefotiam	CN	10
5	Imipenem	IPM	30
6	Chloramphenicol	C	30
7	Doxycycline	DO	30
8	Amikacin	AK	30
9	Ticarcillin	TIC	75
10	Polymyxin B	PB	300
11	Cefepime	FEB	30
12	Tazobactam Piperacillin	TZP	110
13	Ciprofloxacin	CIP	5
14	Ampicillin	AMP	10

IV. RESULTS

Selection of Klebsiella pneumoniae strains

Among 30 bacterial isolates obtained from urine, puss, swab samples, only 6 isolates were further studied and labelled according to their sequencing number i-e Rkpl, Rkp2, Rkp3, Rkp4, Rkps and Rkp6 respectively. The selected isolates showed their morphological and biochemical characterization.

Morphological Characterization

Morphology of bacterial colonies was observed, the colonies were off-white or pink in colour. lactose fermenting mucoid colonies on MacConkey agar.

Gram Staining

Gram staining showed the result of Rkpl, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6. All were gram negative (pink colour) and rod shaped as shown in the table 4.1

Table 4.1 Gram staining of isolated bacterial strains

S. No	Bacterial isolates	Shapes of isolates	Colour of isolates	Gram staining
1	Rkp1	Rod shape	Pink	Negative
2	Rkp2	Rod shape	Pink	Negative
3	Rkp3	Rod shape	Pink	Negative
4	Rkp4	Rod shape	Pink	Negative
5	Rkp5	Rod shape	Pink	Negative
6	Rkp6	Rod shape	Pink	Negative

Catalase Test

The Catalase test showed the results of Rkpl, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6. All bacterial isolates formed bubbles and formation of these bubbles indicated that catalase activity is positive.

Oxidase Test

Oxidase test showed the result of Rkp1, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6. All bacterial isolates were oxidase negative because no change in colour was observed on the filter paper.

Indole Test

All bacterial isolates Rkp1, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6 were found as indole negative because of no ring formation.

Sugar Fermentation Test

Rkpl, Rkp2, Rkp3, Rkp4, Rkp5, and Rkp6 strains produced yellow colour in the medium that showed positive sugar fermentation result.

Antibiotic Susceptibility Test

A total of 6 positive isolates were screened for antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and interpreted as per CLSI guidelines (2020). According to CLSI guideline an organism was classified as susceptible if the diameter of the zone inhibition was greater than 19mm, intermediate if diameter was 15-18mm, and resistant if diameter was less than 13mm. The antibiotic disc which was choose for the study were; Vancomycin (VA), Amoxicillin+Clavulanate (AMC), Ceftriaxone (CRO), Imipenem (IPM), Chloramphenicol (C), Doxycycline (DO), Amikacin (AK), Piperacillin (PRL), Ticarcillin (TIC), Polymyxin B (PB), Cefepime (FEP), Cefotiam (CN), Tazobactam Piperacillin (TZP) and Ampicillin (AMP).

Sample # 01

First sample of Klebsiella pneumoniae strain was labelled as Rkpl, formation of zone inhibition through disk diffusion method showed susceptibility to Imipenem (10µg) with zone of 26mm and were resistance to Ticarcillin (75µg) and Cefoperazone (5µg) having zone of inhibition 0mm and 7mm respectively as shown in figure 4.1 and table 4.2.

Figure 4.1 RKP1 Antibiotic zone of inhibition against Klebsiella pneumoniae

Table 4.2 Measurement of zone inhibition of RKP1

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Imipenem	IPM	6mm	26mm	≤ 23	20-22	≤19
2	Cefepime	FEP	6mm	15mm	≤18	15-17	≤14r
3	Polymyxin B	PB	6mm	12mm	≤12	9-11	≤8i
4	Cefoperazone	CP	6mm	7mm	≤21	16-20	≤15
5	Ticarcillin	TC	6mm	0mm	≤20	15-19	≤14

Sample # 02

Second sample of Klebsiella pneumoniae strain was labelled as Rkp2, formation of zone inhibition through disk diffusion method showed susceptibility to Chloramphenicol (30µg) with zone of 26mm and were resistant to Vancomycin (30µg). Amoxicillin+Clavulanate (30µg), and Ticarcillin (75µg) having 0mm 0mm and 12mm zone of inhibition as shown in figure 4.2 table 4.3.

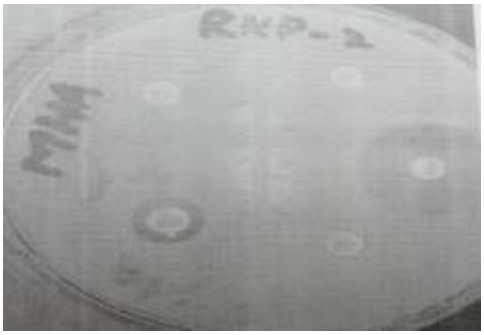

Figure 4.2RKP2 Antibiotic zone inhibition against Klebsiella pneumoniae

Table 4.3 Measurement of zone inhibition of RKP218

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Chloramphenicol	C	6mm	26mm	≤18	13-17	≤12
2	Vancomycin	VA	6mm	0mm	≤17	15-16	≤14
3	Amoxicillin	AMC	6mm	12mm	≤18	14-17	≤13
4	Piperacillin	PRL	6mm	9mm	≤21	18-20	≤17
5	Ticarcillin	TC	6mm	0mm	≤19	15-18	≤14

Sample #03

Third sample of Klebsiella pneumoniae strain was labelled as Rkp3, formation of zone inhibition through disk diffusion method showed susceptibility to Amikacin (30µg), Tazobactam (110µg) having zone of inhibition with 21mm and 23mm and were resistance to Doxycycline (30µg). Ceftriaxone (30µg), and Cefotiam (10µg) having 8mm, 11mm and 10mm zone of inhibition as shown in figure 4.3 and table 4.4.

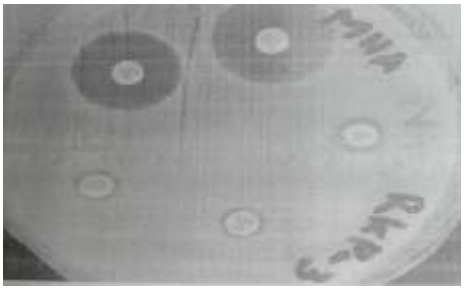

Figure 4.3 RKP3 Antibiotic zone inhibition against Klebsiella pneumoniae

Table 4.4 Measurement of zone inhibition of RKP3

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Amikacin	AK	6mm	21mm	≤17	15-16	≤14
2	Tazobactam	TZP	6mm	23mm	≤21	18-20	≤17
3	Ceftriaxone	CRO	6mm	10mm	≤25	16-24	≤15
4	Doxycycline	DO	6mm	8mm	≤14	11-13	≤10
5	Cefotiam	CN	6mm	11mm	≤16	12-14	≤11

Sample #04

Fourth sample of Klebsiella pneumoniae strain was labelled as Rkp4, formation of zone inhibition through disk diffusion method showed susceptibility to Tazobactam (110µg) with 23mm zone of inhibition and were resistance to Ceftriaxone (30µg). Cefotiam (10µg) and Amoxicillin (30µg) having 12mm zone of inhibition respectively as shown in figure 4.4 and table 4.5.

Figure 4.4 RKP4 Antibiotic zone inhibition against Klebsiella pneumoniae

Table 4.5 Measurement of zone inhibition of RKP4

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Amoxicillin	AMC	6mm	12mm	≤17	15-16	≤14
2	Tazobactam	TZP	6mm	23mm	≤21	18-20	≤17
3	Ceftriaxone	CRO	6mm	9mm	≤25	16-24	≤15
4	Doxycycline	DO	6mm	8mm	≤14	11-13	≤10
5	Cefotiam	CN	6mm	11mm	≤16	12-14	≤11

Sample #05

Fifth sample of Klebsiella pneumoniae strain was labelled as Rkp5, formation of zone inhibition through disk diffusion method showed susceptibility to Amikacin (30 µg), Tazobactam (110µg) with zone of 28mm and were resistance to Doxycycline (30µg) and Ceftriaxone (30µg) having zone of inhibition with 8mm and 12mm as shown in figure 4.5 and table 4.6

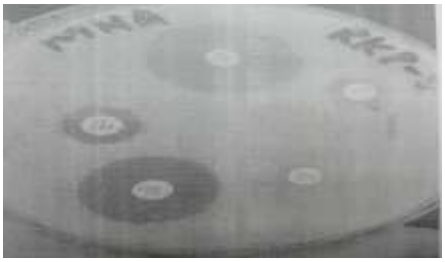

Figure 4.5 RKP5 Antibiotic zone inhibition against Klebsiella pneumoniae

Table 4.6 Measurement of zone inhibition of RKPS

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Amikacin	AK	6mm	22mm	≤17	15-16	≤14
2	Tazobactam	TZP	6mm	28mm	≤21	18-20	≤17
3	Ceftriaxone	CRO	6mm	12mm	≤25	16-24	≤15
4	Doxycycline	DO	6mm	8mm	≤14	11-13	≤10
5	Amoxicillin	AMC	6mm	12mm	≤17	15-16	≤14

Sample # 06

Sixth sample of Klebsiella pneumoniae strain was labelled as Rkp6, formation of zone inhibition through disk diffusion method showed susceptibility to Chloramphenicol (30µg) having 21mm zone of inhibition and showed resistance to Amikacin (30µg). Polymyxin B (300µg), and Doxycycline (30µg) with 7mm, 9mm and 12mm respectively as shown in figure 4.6 and table 4.7.

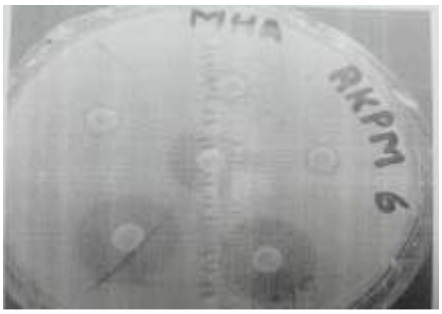

Figure 4.6 RKP6 Antibiotic zone inhibition against Klebsiella pneumoniae

Table 4.6 Measurement of zone inhibition of RKP6

S. No	Antibiotics	Ab	Diameter of disc	Zone of inhibition	Susceptibility	Intermediate	Resistance
1	Amikacin	AK	6mm	20mm	≤17	15-16	≤14
2	Tazobactam	TZP	6mm	18mm	≤21	18-20	≤17
3	Polymyxin	PB	6mm	7mm	≤12	9-11	≤8

	B						
4	Doxycycline	DO	6mm	9mm	≤14	11-13	≤10
5	Amoxicillin	AMC	6mm	12mm	≤17	15-16	≤14
6	Chloramphenicol	C	6mm	21mm	≤18	12-17	12≤

V. DISCUSSION

Community acquired infection and hospital acquired infections are mainly caused by contaminated areas and by transmission of person-to-person contact. K. pneumoniae is one of the most common bacteria that cause infections in patients. In the current study 30 different samples of urine, swab, puss was taken and screened for specific bacteria and their antibiotic susceptibility. Total of 6 bacterial isolates were purified and antibiotic susceptibility was tested. Biochemical tests were performed for identification of bacterial isolates and the result revealed that all of 6 bacterial isolates were K pneumoniae. Total of 6 bacterial isolates were gram negative rods, non-motile, indole negative and catalase positive. Podschun et al. (2013) proposed the same study. 39 isolates were collected from patients with community-acquired infections in the hospitals of Guerrero, Mexico. The samples were obtained from all outpatients that were considered as an infection. A molecular test was performed to all isolates that were non motile, oxidase negative, rod-shaped gram negative and to be considered as K. pneumoniae spp.

All of these K. pneumoniae isolates were taken from clinical sites. For checking bacterial resistance and susceptibility antibiotic tests were performed through disc diffusion method. According to recent studies, K. pneumoniae resistance has spread to almost all antibiotics. Rate of antibiotic resistance is high according to antibiotic susceptibility tests. In present study, Antibiotics that were susceptible according to antibiotic susceptibility test through CLSI guideline were imipenem (10µg), chloramphenicol (30µg), amikacin (30µg) and tazobactam (110µg). Nirwati et al. (2019) conducted the same study for identification and antibiotic susceptibility tests of K. pneumoniae strains. For Identification of K. pneumoniae isolate, Gram staining and biochemical testing were performed. Kirby Bauer disc diffusion method was used to perform antibiotic susceptibility tests. The Clinical and Laboratory Standards Institute 2015 was used to classify as sensitive, intermediate or resistant bacteria. Klebsiella pneumoniae had only a good sensitivity to Meropenem, levofloxacin, amikacin, piperacillin-tazobactam and ciprofloxacin.

In a recent study, Clinical K. pneumoniae strains, mostly community acquired pneumonia, were highly resistant to antibiotic vancomycin (30µg), amoxicillin (30µg), Ticarcillin (75µg). doxycycline (30µg), ceftriaxone (30µg), cefotiam (10µg), and polymyxin B(300µg). Babakhan er dal. (2015) studied 51 Klebsiella isolates from 500 patients in three Aleshtar hospital units over the course of nine months. The Kirby Bauer disc diffusion method was used to test K. pneumoniae antibiotic resistance to 18 antibiotics. In 500 samples, the frequency of K pneumoniae was determined in 51 instances (10.2%). The infectious unit had the highest number of K. pneumoniae isolates (49.02). Antibiotic susceptibility tests were performed that showed resistance to ampicillin and Ticarcillin, piperacillin, amoxicillin-clavulanic acid, ceftazidime, cefotaxime, cefoxitin. gentamicin, amikacin, tetracycline, and levofloxacin.

VI. CONCLUSION AND RECOMMENDATIONS

In a recent study, Clinical K. pneumoniae strains, mostly community acquired pneumonia, were highly resistant to antibiotic vancomycin (30µg), amoxicillin (30µg), Ticarcillin (75µg). doxycycline (30µg), ceftriaxone (30µg), cefotiam (10µg), and polymyxin B(300µg). Babakhan er dal. (2015) studied 51 Klebsiella isolates from 500 patients in three Aleshtar hospital units over the course of nine months. The Kirby Bauer disc diffusion method was used to test K. pneumoniae antibiotic resistance to 18 antibiotics. In 500 samples, the frequency of K pneumoniae was determined in 51 instances (10.2%). The infectious unit had the highest number of K. pneumoniae isolates (49.02). Antibiotic susceptibility tests were performed that showed resistance to ampicillin and Ticarcillin, piperacillin, amoxicillin-clavulanic acid, ceftazidime, cefotaxime, cefoxitin. gentamicin, amikacin, tetracycline, and levofloxacin.

VII. REFERENCES

1. Arivett. B. A, Ream, D.C., Fiester, S. E., Mende, K. Murray, C.K. Thompson, M.G....& Actis, L. A. (2015). Draft genome sequences of Klebsiella pneumoniae clinical type strain ATCC 13883 and three multidrug-resistant clinical isolates, Genome Announcements, 3(1), e01385- 14.
2. Babakhani, S., Shokri, S., & Baharvand, M. (2015). Antibiotic resistance pattern of Klebsiella pneumoniae isolated from nosocomial infections in Aleshtar hospital, Lorestan province. Report of Health Care, 1(2), 55-59.
3. Bina, M., Pournajaf, A., Mirkalantari, S., Talebi, M., & Irajian, G. (2015). Detection of the Klebsiella pneumoniae carbapenemase (KPC) in K. pneumoniae Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. Iranian journal of pathology. 10(3), 199.
4. Brisse S. Passet V, Haugaard AB et al. wzi gene sequencing, a rapid method for determination of capsular type for Klebsiella strains. J. Clin. Microbiol. 51(12), 4073-4078 (2014).
5. Chen, L. F., Anderson, D. J., & Paterson, D. L. (2012). Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC) resistance. Infection and drug resistance, 5. 133.
6. Chen, Y. J., Kuo, H. K., Wu, P. C., Kuo, M. L., Tsai, H. H., Liu, C. C., & Chen, C. H. (2004). A 10- year comparison of endogenous endophthalmitis outcomes: an east Asian experience with Klebsiella pneumoniae infection. Retina, 24(3), 383-390.
7. Chong, Y., Shimoda, S., & Shimono, N. (2018). Current epidemiology, genetic evolution and clinical impact of extended-spectrum B-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infection, Genetics and Evolution, 61, 185-188.
8. Comandatore, F., Sassera, D., Ambretti, S., Landini, M. P., Daffonchio, D., Marone, P..... & Gaibani, P. (2013). Draft genome sequences of two multidrug resistant Klebsiella pneumoniae ST258 isolates resistant to colistin. Genome Announcements, 1(1), e00113-12.
9. Fang, C. T., Chuang, Y. P., Shun, C. T., Chang, S. C., & Wang, J., T. (2004). A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. The Journal of experimental medicine, 199(5), 697-705.
10. Garza-Ramos, U., Barrios-Camacho, H.,
11. Moreno-Dominguez, S., Toribio-Jiménez, J., Jardón-Pineda, D.. Cuevas-Peña, J., ... & Roman-Roman, A. (2018). Phenotypic and molecular characterization of Klebsiella spp. isolates causing community-acquired infections. New microbes and new infections, 23, 17-27.
12. Gonzalez-Ferrer, S., Peñaloza, H. F., Budnick, J. A., Bain, W. G., Nordstrom, H. R., Lee, J. S., & Van Tyne, D. (2021). Finding order in the chaos: Outstanding questions in Klebsiella pneumoniae pathogenesis. Infection and Immunity, 89(4), e00693-20.
13. Gootz. T. D. (2010). The global problem of antibiotic resistance. Critical Reviews™ in Immunology, 30(1).
14. Hamdan, H. Z., Kubbara, E., Adam, A. M., Hassan, O, S., Suliman, S. O., & Adam, I. (2015). Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum. Sudan. Annals of clinical microbiology and antimicrobials, 14(1), 1-6.
15. Khadri, H. C., Surekha, S., Lakshmi, S., & Narasimha, G. (2007). Multi drug resistance and 1- lactamase production by Klebsiella pneumoniae African Journal of Biotechnology, 6(15).
16. Kislichkina, A. A., Lev, A. I., Komisarova, E. V., Fursova, N. K., Myakinina, V. P., Mukhina, T. N.. & Volozhantsev, N. V. (2017). Genome sequencing and comparative analysis of three hypermucoviscous Klebsiella pneumoniae strains isolated in Russia. Pathogens and Disease, 75(4), ftx024.
17. Ko, W. C., Paterson, D. L., Sagnimeni, A. J., Hansen, D. S., Von Gottberg, A., Mohapatra, S..... & Yu, V. L. (2002). Community-acquired Klebsiella pneumoniae bacteremia: global differences in clinical patterns. Emerging infectious diseases, 8(2), 160.
18. Lin. Y T.. Siu. L. K., Lin, J. C., Chen, T. L., Tseng, C. P. Yeh, K. M., & Fung, C. P. (2012). Seroepidemiology of Klebsiella pneumoniae colonising the intestinal tract of healthy Chinese and overseas Chinese adults in Asian countries. BMC microbiology. 12(1), 1-7.
19. Martin, R. M., & Bachman, M. A. (2018). Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Frontiers in cellular and infection microbiology, 8.4.
20. Nepal, K., Pant, N. D., Neupane, B., Belbase, A., Baidhya, R., Shrestha, R. K., & Jha, B. (2017). Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiella pneumoniae isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. Annals of clinical microbiology and antimicrobials, 16(1), 1-7.

21. Nirwati, H., Sinanjung, K.. Fahrunissa, F., Wijaya, F., Napitupulu, S., Hati, V. P... & Nuryastuti, T (2019, December). Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. In BMC proceedings (Vol. 13, No. 11, pp. 1-8). BioMed Central.
22. Paterson, D. L., & Bonomo. R. A. (2005). Extended-spectrum ß-lactamases: a clinical update. Clinical microbiology reviews, 18(4), 657-686.
23. Peleg. A. Y., & Hooper, D. C. (2010). Hospital-acquired infections due to gram-negative bacteria. New England Journal of Medicine, 362(19), 1804-1813.
24. Podschun, R., & Ullmann, U. (2013). Klebsiella spp. as nosocomial pathogens: epidemiology. taxonomy, typing methods, and pathogenicity factors. Clinical microbiology renters, (4), 589-603.
25. Pragasam, A. K., Shankar, C., Veeraraghavan, B., Biswas, I., Nabarro, L. E., Inbanathan, F. Y., ... & Verghese, S. (2017). Molecular mechanisms of colistin resistance in Klebsiella pneumoniae causing bacteremia from India-a first report, Frontiers in microbiology. 7. 2135.
26. Rodriguez-Zulueta, P., Silva-Sánchez, J., Barrios, H., Reyes-Mar, J., Vélez-Pérez, F., Arroyo- Escalante, S., ... & Garza-Ramos, U. (2013). First outbreak of KPC-3-producing Klebsiella pneumoniae (ST258) clinical isolates in a Mexican Medical Center, Antimicrobial agents and chemotherapy, 57(8), 4086-4088.
27. Sably, H., Aucken, H., Benedi, V. J., Forestier, C., Fussing, V., Hansen, D. S., & Ullmann, U. (2004). Increased serum resistance in Klebsiella pneumoniae strains producing extended- spectrum B-lactamases. Antimicrobial agents and chemotherapy, 48(9), 3477-3482.
28. Sikarwar, A. S., & Batra, H. V. (2011). Prevalence of antimicrobial drug resistance of Klebsiella pneumoniae in India International Journal of Bioscience, Biochemistry and Bioinformatics, (3), 211.
29. Isay. R. W., Siu, L. K., Fung, C. P., & Chang, F. Y. (2002). Characteristics of bacteremia between community-acquired and nosocomial Klebsiella pneumoniae infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. Archives of internal medicine, 16219), 1021-1027.
30. Wyres, K. L., & Holt, K. E. (2018). Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Current opinion in microbiology, 45, 131-139.
31. Yadav, K. K., Adhikari, N., Khadka, R., Pant, A. D., & Shah, B. (2015) Multidrug resistant Enterobacteriaceae and extended spectrum [-lactamase producing Escherichia coli, a cross-sectional study in National Kidney Center, Nepal. Antimicrobial resistance and infection control, 4(1), 1-7.
32. Yang, F., Deng, B., Liao, W., Wang, P., Chen, P., & Wei, J. (2019). High rate of multiresistant Klebsiella pneumoniae from human and animal origin, Infection and Drug Resistance, 12. 2729.
33. Yu, W. L., Ko, W. C., Cheng, K. C., Lee, H. C., Ke, D. S., Lee, C. C., & Chuang, Y. C. (2006). Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clinical infectious diseases, 42(10), 1351-1358.