Frequency and Susceptibility Pattern of K. Pneumoniae Recovered from Human Clinical Specimens in Lahore Population

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Abstract:

Klebsiella pneumoniae is gram negative, capsulated and non-motile organism, associated with different infections causing disease in both immunocompetent and immunocompromised hosts with a vast range including urinary, respiratory and gastro intestinal tracts.

Objective:

To investigate the frequency and prevalence of Klebsiella pneumoniae recovered from different human clinical specimens and their antibiotic susceptibility pattern in population of Lahore.

Methods:

A total of 150 clinical samples were studied for the isolation of K. pneumoniae. Out of 150, 30 samples from urine, 30 from pus, 30 from sputum, 30 from bronchial wash and 30 from pleural aspirates. The identification of K. pneumoniae was done by different biochemical tests i.e urease, indole, citrate etc. Extended spectrum beta lactamases (ESBL) screening was done by double disc diffusion method by placing cephalosporin/clavulanate combination discs.

Results:

Out of 150 samples 29 were isolated as K. pneumoniae. K. pneumoniae isolates were mostly carbapenem sensitive i.e imipenam (86.20%) and meropenam (86.20%). The most resistant drugs were ampicillin and cefuroxime 100% followed by cefotxime93.10%. A high prevalence of ESBL producing bacteria 37.9% exists among in-patients in Lahore.

Conclusions:

Emerging antimicrobial resistance is currently the main concern of the medical community, because such resistant bacteria are becoming more difficult to treat.

Keywords:

Bacteria, Klebsiella pneumoniae, Extended spectrum beta lactamases (ESBL), Resistant, Sensitive, Antibiotics.

Introduction:

Klebsiella species are capsulated, gram negative, rod shaped and non-motile. These opportunistic human commensals have placed in the family of Enterobacteriaceae where Klebsiella pneumoniae and Klebsiella oxytoca, are two species that took part in the majority of infections [1, 2]. K. pneumoniae are associated with different infections causing disease in both immunocompetent and immunocompromised hosts with a vast range including urinary, respiratory and gastro intestinal tracts. Furthermore, they cause septicaemia, bacteremia, and different soft tissue and organ diseases. Klebsiella further divided into a number of species such as K. pneumoniae, K. oxytoca, K. granulomatosis, K. mobilis, K. planticola, K. ornithinolytica, K. terrigena, K. trevisanii and K. singaporensis [3,4].

Edwin Kleb first described Klebsiella pneumoniae in 1875, and in 1882 Carl and Friedlander Formally described the species[5]. Gram-negative pathogens that produce extended spectrum β-lactamase (ESBL) enzymes that cause the major type of antibiotic resistant [6,7]. Increasing antibiotic resistance is one of the large-scale hazard to global health[7]. K. pneumoniae have many virulence factors, for example, capsular polysaccharides, lipopolysaccharide (LPS). Major risk factors with ESBL producing organisms are serious illness, continuous intensive care unit stay, long-standing antibiotic exposure, nursing home residency, and living in an institution with large

amount of third generation cephalosporin and ceftazidime usage[8]. Drug resistance has been enhanced in recent years due to extended spectrum beta lactamases (ESBLs) production by distinct organisms. These organisms show resistivity to various drugs such as extended spectrum penicillin, monobactams and including third generation cephalosporin. Fluoroquinilones, aminoglycosides and other agent's susceptibility is variable. Only the active class of antibiotics against these strains are Carbapenems[9]. Escherichia coli and Klebsiella both are predominantly produce ESBL, while it can also view in other Enterobacteriaceae[10]. The early most ESBL isolates were detected in Germany in the mid-1980s and afterwards in the United States of America in the late 1980s soon after the introduction of ceftazidime and cefotaxime into clinical proceeding[11].

It is necessary to find out the antimicrobial susceptibility pattern of K. pneumoniae so that suitable steps can be taken to limit the fast spreading of different drugs resistance. (The present study will be helpful to know about the antimicrobial susceptibility of K. pneumoniae and also the prevalence ESBLs producing K. pneumoniae.

Methods:

Samples were collected from patient of different wards in Gulab Devi hospital within Lahore city from Jan 2018 to Jun 2018. A total 150 different samples were used for the isolation of K. Pneumoniae were collected and processed for the isolation and evaluation of the susceptibility pattern of isolated pathogens. For example urine, pus, sputum, bronchial wash and pleural aspirates.

Following are the media used for the isolation as well as for sensitivity pattern of K. pneumoniae.

Cystein Lactose Electrolyte Deficient Agar (CLED) (Oxoid), Blood Agar (Oxoid), Chocolate Agar (Oxoid), MacConkey Agar (Oxoid), Meullar Hinton agar (Oxoid) The antibiotics used for antibiogram determination of the collected strains along with their Classes are shown in Table 1.

Class	Antibiotics
Carbapenems	Meropenem (MEM), Imepenem(IPM)
Cephalosporins	Cefotxime (CTX) , Ceftazidime (CAZ), Cefipime (CPE), Cefpodoxime(CEP)
Flouroquinolones	Ciprofloxacin (CIP)
Aminoglycosides	Gentamycin (CN), Amikacin (AK),
Penicillin	Amoxicillin+Clavulanic Acid (AMC), Piperacillin-tazobactam (TZP), Cefuroxime (CXM), Ampicillin (AM)
Fosphonic Acid Derivatives	Fosfomycin (FF)
Monobactam	Aztreonam (ATM)

Table 1: Classes of antibiotics

Identification of bacteria was done by various methods:

First of all, the morphology of bacterial colonies were analyzed which includes size, color, shape, translucency, surface elevation and margins.

Then their cultural characteristics were analyzed in which fermentation of lactose, their odor and the pattern of growths were also included. For the terminal identification, Citrate Test, Catalase Test and Gram Staining will be used.

On blood and chocolate agar pathogens were produced large grey color colonies. On CLED agar pathogens were produced large yellow mucoid colonies. Whereas follow on MacConkey agar pathogens are produced large pink mucoid lactose fermenter colonies when incubated aerobically at 37°C for 24 hours (Cheesbrough 2000).

Results:

A total 150 specimens that were used for the isolation of K. pneumoniae were as follow

30 urine, 30 pus, 30 sputum, 30 bronchial wash, 30 pleural aspirates. 0ut of 150 sample, 72 samples were from male and 78 samples were from female.

Out of 150 clinical specimens 29 (19%) isolates were recovered as K. pneumoniae, 33 (22%) isolates were identified as No pathological Growth (NPG), 83 (56%) specimen showed No growth (NG) and 5 (3%) isolates were recovered as Coliform as shown in Figure 1.

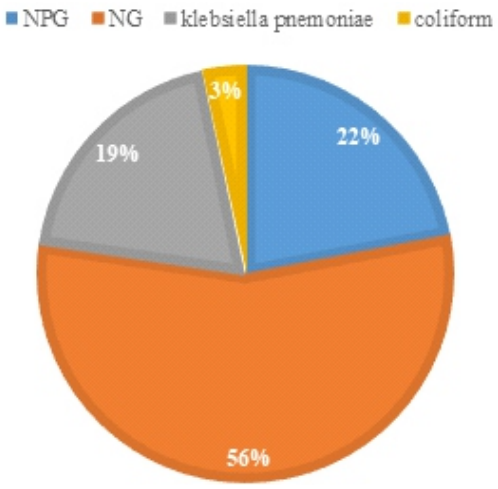

Figure 1: Percentage of clinical isolates from total sample

Antimicrobial susceptibility of K. pneumonia against important drugs like Ampicillin (AM) shows 0(0%) sensitivity and 29(100%) resistant , IPM shows 25(86.2%) sensitivity and 4(13.8%) resistant, AMC 4(13.8%) sensitivity and 25(86.2%) resistant, CN 14(48.3%) and 15(51.7%) resistant and CIP shows 11(37.9%) sensitivity and 18(62.1%) resistant.

Antibiotics	Sensitive %	Resistant %	Total
Ampicillin(AM)	0 (0%)	29 (100%)	29
Imepenem(IPM)	25 (86.2%)	04 (13.8%)	29
Meropenem(MEM)	25 (86.2%)	04 (13.8%)	29
Aztreonam(ATM)	06 (20.7%)	23 (79.3%)	29
Amoxacillin+Clavulanic acid(AMC)	04 (13.8%)	25 (86.2%)	29
Cefpodoxime (CEP)	07 (24.1%)	22 (75.9%)	29
Cefuroxime (CXM)	0 (0%)	29 (100%)	29
Fosamycin (FF)	17 (60.7%)	11 (39.3%)	28
Amikacin(AK)	24 (82.8%)	05 (17.2%)	29
Gentamycin(CN)	14 (48.3%)	15 (51.7%)	29
Piperacillin-tazobactam (TZP)	16 (55.2%)	13 (44.8%)	29
Ciprofloxacin (CIP)	11 (37.9%)	18 (62.1%)	29
Cefotxime (CTX)	02 (6.9%)	27 (93.1%)	29

Table 2: Antimicrobial susceptibility of K. pneumonia against different drugs

Out of total 150 samples from different human clinical specimens, K. pneumoniae was recovered from 29 specimens. Out of which 11 are positive for ESBL production, Figure 2.

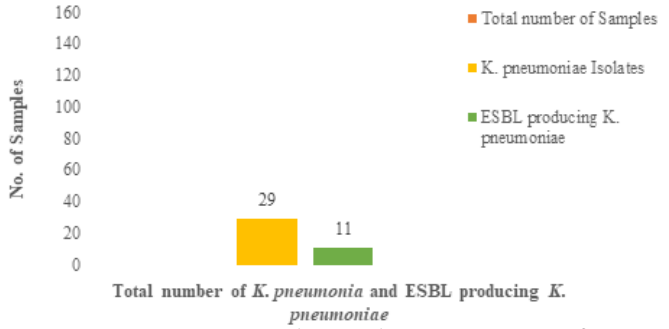

Figure 2: Human clinical specimens for K. pneumoniae

Figure 3 represents the number of K. pneumoniae and ESBL producing K. pneumoniae recovered from different human clinical specimens.

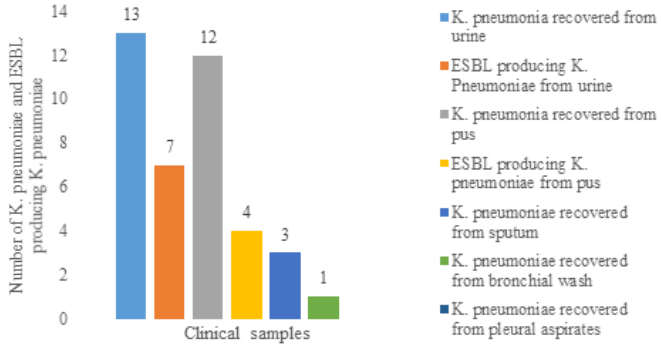

Figure 3: Clinical isolates

Discussion:

In present study, 150 samples from different human clinical specimens for example sputum, urine, pus, bronchial wash and pleural aspirate obtained. From which 29 are K. pneumoniae positive. Highest isolation of K. pneumoniae obtained 13(44.8%) from urine, 12(41.4%) from pus, 3(10.3%) from sputum and 1(3.5%) from bronchial wash that is different from another study which was conducted in popular diagnostic centre and medinova diagnostic centre of Sylhet branch, Bangladesh that showed the high prevalence of K. pneumoniae in case of urine sample (66%), in pus 10% cases and in sputum 7% cases.[12]

A study conducted in department of microbiology, RKDFMCH & RC, Bhopal, India for the K. pneumoniae isolates, the most effective antibiotics were imipenem (81.25%) followed by meropenem (78.75%)[13] but in this study

susceptibility pattern of meropenem and imipenem is 86.20% and 86.20% respectively.

A study in hospital of Madinah, Saudi arabia that shows Imipenem was the best antibiotic against K. pneumoniae with 99.5% affectability took after by CAZ with 98.6% affectability for K. pneumoniae [2] while according to this study the most effective drugs against K. pneumoniae are imepenem 86.20% and meropenem 86.20% followed by AK 82.8% and FF 60.7%. Another study conducted in Bandra west Mumbai, India, there results showed that K. pneumoniae were sensitive against amikacin 88%, gentamycin 79.6% and ciprofloxacin is 89.2%[14] while our results shows the quit similar results that K. pneumoniae is sensitive against amikacin 82.8% but against gentamycin and ciprofloxacin shows different results as 48.3% and 37.9% that shows high resistant.

A study in armed forces hospital soudi Arabia that showed the 100% sensitivity of imipenem against K. pneumoniae and showed 100% resistivity of ampicillin against K. pneumoniae[15]. while this study shows K. pneumoniae sensitivity rate against imipenam is 86.20% and resistivity rate against ampicillin is same 100%. For K. pneumoniae, ESBL-production rates in US are usually less than 5%[16]. ESBL-producing K. pneumoniae prevalence in Europe may be as high as 15% to 20%, or even higher in isolates from ICUs [17, 18] while our findings for ESBL producing K. pneumoniae prevalence is 37.9% in Lahore. A high ESBLs prevalence in Kuwait 31.7% and 41% in the United Arab Emirates have been noted [19,20].

Conclusions:

The antimicrobial resistance of K. pneumoniae to multiple numbers of antimicrobials drugs have been found to increase. To avoid the anomalies in future, antimicrobial drugs should be carefully prescribed only after confirmed pathological investigation and hence rational use of antimicrobials is to be ensured.

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