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ORIGINAL ARTICLE

Identification and Characterization of Klebsiella Pneumoniae in Different Clinical Samples at Tertiary Care Hospital, Lahore

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ABSTRACT

Aim: To identify and characterization of klebsiella pneumoniae in different clinical samples in tertiary care hospital, Lahore
Methodology: These 90 samples include 12 sputum samples, 21 tracheal swab, 30 pus and 27 urine samples from LGH, Lahore.
Sample size: 90 subjects
Duration: 6 months i.e. 01-07-2022 to 31-12-2022
Study place: LGH, Lahore
Gram staining, microscopy, sputum, tracheal swab, pus, and urine cultures, biochemical tests, and antibiotic susceptibility testing was performed for all samples. Statistical analysis was performed by using SPSS Software.
Results: Among the 90 clinical samples, 50 (55.6%) were positive for K. pneumoniaand10 cases (11.1%) with other bacteria. 30 (33.3%) cases showed no growth. Colistin, meropenem, amikacin, and imipenem (8.3%) were sensitive to sputum samples. Colistin 38.1%, ceftazidime 4.7%, meropenem 4.7%, amikacin 9.5%, sulfamethoxazole 4.7%, gentamycin 9.5%, and imipenem 28.5% were sensitive in tracheal swab samples.
Conclusion: The highest sensitivity was observed incefoperazone, cefotaxime, and ceftriaxone (53.3%) isolates in pus samples.
Keywords: Klebsiellapneumoniae, pneumonia, urinary tract infections, sensitivity patterns.

INTRODUCTION

K. pneumonia was first recognized by "Carl Friedlander" in 1882. After extracting the bacteria from the lungs of patients who had deceased of pneumonia, he classified it as an encapsulated bacillus[1.]The bacteria were first known as "Friedlander's bacillus" and weren't given the name Klebsiella until 1886[2]. It is a gram-negative, non-motile, and encapsulated bacterium that has been linked to pneumonia in patients with alcoholism or diabetes. Human mucosal surfaces of the oropharynx and GI tract are commonly colonized by bacteria[3].

Polymorphonuclear granulocytes, which are complement proteins and phagocytose bacteria are the backbone of the host's defens against bacterial invasion. The alternative complement activation cascade is more active. Lipopolysaccharide-binding protein and myeloperoxidase in neutrophils aid in the defense against K. pneumoniae infection[4]. The pathogenicity of microorganisms is determined by a polysaccharide capsule composed of complicated acidic polysaccharides.

β-lactams and other antibiotics (potent for Enterobacteriaceae) are commonly used to treat these infections in hospitalized or immunocompromised individuals[5]. Hypervirulent Klebsiella pneumoniae and antibiotic-resistant strains, on the other hand, have evolved in different parts of the world. In addition, recent developments in molecular techniques have revealed that certain clinical isolates formerly recognized as Klebsiella pneumoniae are different Klebsiella species[6,7].

Klebsiella pneumoniae is a common hospital-acquired bacteria, which causes urinary tract infections, nosocomial pneumonia, gastrointestinal infections, surgical wound infections, and blood diseases. All of these diseases can spread to other forms of stroke and death if left untreated in an aggressive manner. Klebsiella pneumoniae is also a viral infection in the community. It is estimated that Klebsiella spp causes 8% of hospital-acquired infections and 3% of epidemic diseases[8].

METHODS

A total of 90 cases were included in this study. Out of 90 samples, 11 were sputum, 30 were pus, 21 were tracheal swab and 27 were urine samples. Before collecting samples and IRB permission all the study subjects were briefed about the sample collection methods. Out of 90, 45 were male and 31 were female participants.
Study design: Cross sectional study
Inclusion criteria: Both sexes were included with age between 15-- 60 years
Exclusion criteria: Subjects with ages below 15 and above 60 years were excluded

All the clinical samples which include (pus, urine, sputum, tracheal swab) had inoculated on the respective media and incubated over night at 37°C. After incubation, observed the colony appearance.Out of 90 samples, 11 were sputum, 30 were pus, 21 were tracheal swab and 27 were urine samples. The participants of hospital- acquired UTI and pneumonia collected from both male and female were included, with prior consent from their participants and attendants.The samples were preserved by using swabs, syringes, containers and blood bottles according to transport and preservative medium.

Different Clinical Samples were aseptically inoculated to Blood agar, MacConkey agar and CLED agar incubated overnight at 37°C. The isolateswere identified by their morphology and biochemical characteristics. Morphology of Klebsiella identified would have large dome shaped colonies on Blood and lactose fermenting mucoid colonies on MacConkey agar.

After the growth of samples on Different agar morphological character of the colonies were observed by direct examination of colonies such as forms, elevations,margins,appearance texture, optical property, pigmentation of colonies. All the single colonies were selected and marked with respective colony numbers that were isolated from each sample.

Gram staining was performed for all isolated colonies. Firstly, the smear was prepared by mixing the colony with water in circular motion, then heat fix the slide by passing it over a spirit lamp flame. Primary stain crystal violet was rinsed to the slide for 60 second. Slide was rinsed with running water for 5 seconds and gram iodine was added to on slide for 60 seconds then again slide was rinsed gently with water. Decolorizer was added on slide for 10 seconds to remove the gram negative bacteria. At the end counter stain Safranin was added on the slide to stain gram negative bacteria. The slide was then again washed with water and then slides were dried and observed at 100X with oil immersion lens.

RESULTS

The detail of results is given in tables

Table1: Growth of Klebsiellapneumoniae in different samples.

Culture Appearance	N=	%age
Sputum		
Mucoid Colonies	2	16.7%
White Colonies	2	16.7%
No Growth	8	66.7%
Pus		
Mucoid Colonies	16	53.3%
Other Colonies	1	3.3%
No Growth	13	43.3%
Tracheal Swab		
Mucoid Colonies	19	90.5%
Other Colonies	0	0.0%
No Growth	2	9.5%
Urine		
Mucoid Colonies	13	48.1%
White Colonies	1	3.7%
Golden Colonies	2	7.4%
Greenish Colonies	1	3.7%
No Growth	7	25.9%
Mixed Growth	3	11.1%

Fig 1: Mucoid Colonies of Klebsiella pneumonia on Blood agar

Table 2: Mucoid colonies in different samples

Samples	n=	Growth present
Pus	30	16
Sputum	12	02
Tracheal Swab	21	19
Urine	27	13
Total	90	50

Table 3: Morphological Characterization

Bacteria	n	Morphology			
		Colony Shape	Color	Margins	Consistency
Klebsiella pneumonia	50	Large circular	Pinkish red	Entire	Mucoid
Pseudomonas aeruginosa	01	Circular	Colorless	Irregular	Mucoid
E coli	02	Circular	Pink	Entire	Smooth

Table 4: Biochemical tests

Bacteria	n	Catalase Test	Oxidase Test	Citrate Test	Motility Test	Urease Test
Klebsiella pneumonia	50	Positive	Negative	Positive	Negative	Positive
Pseudomonas aeruginosa	01	Positive	Positive	Positive	Positive	Negative
E coli	02	Positive	Negative	Negative	Positive	Negative

Table 5: Sensitivity for Sputum Samples

Antibiotics	Sensitive	Resistant
Colistin	1	1
Amoxicillin	0	2
Cefoperazone	0	2
Ceftriaxone	0	2
Ciprofloxacin	0	2
Levofloxacin	0	2
Ceftazidime	0	2
Meropenem	1	1
Amikacin	1	1
Sulfamethoxazole	0	2
Gentamycin	0	2
Imipenem	1	1

Table 6: Sensitivity for Tracheal Swab Samples

Drugs	Sensitive	Resistant
Colistin	8	8
Amoxicillin	0	19
Cefoperazone	0	19
Ceftriaxone	0	19
Ciprofloxacin	0	19
Levofloxacin	0	19
Ceftazidime	1	18
Meropenem	1	18
Amikacin	2	17
Sulfamethoxazole	1	18
Gentamycin	2	17
Imipenem	6	13

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Table 7: Sensitivity for Pus Samples

Name of Drug	Sensitive	Resistant
Colistin	5	11
Amoxicillin	1	15
Cefoperazone	16	14
Cefotaxime	16	14
Ceftriaxone	16	14
Ciprofloxacin	1	15
Levofloxacin	2	14
Ceftazidime	2	16
Meropenem	8	10
Amikacin	13	5
Sulfamethoxazole	2	16
Gentamycin	4	14

Table 8: Sensitivity for urine samples

Name of Drug	Sensitive	Resistant
Cefepime	11	5
Amoxicillin	3	13
Tazobactam	3	12
Cefuroxime	4	12
Tetracycline	3	13
Fosfomycin	11	15
Ceftriaxone	6	10
Ciprofloxacin	3	13
Levofloxacin	3	13
Ceftazidime	5	11
Meropenem	8	8
Amikacin	11	5
Sulfamethoxazole	3	13
Gentamycin	8	7
Imipenem	9	7
Cefotaxime	6	10

DISCUSSION

This study shows infection rate in 50(55.6%) cases out of 90 participants. In this study, all samples (sputum, tracheal swab, pus and urine) are taken from hospitalized infection patients in a public health care facility in Lahore.

In the present study, cefepime27.5%, amoxicillin 7.5%, tazobactam 7.5%, cefuroxime10%, fosfomycin 27.5%, ceftriaxone 15%, ciprofloxacin 7.5%, levofloxacin 7.5%, ceftazidime 12.5%, meropenem20%, amikacin 27.5%, sulfamethoxazole 7.5%, gentamycin 20%, imipenem 22.5%, and cefotaxime15%were sensitive for UTI associated with hospitalized patients.The highest sensitivity was to cefepime and amikacin 11/27 isolates (27.5%). The antibiotic resistance was in descending order fosfomycin (37.5%) > amoxicillin >sulfamethoxazole> ciprofloxacin > levofloxacin and tetracycline (32.5%) >tazobactam and cefuroxime (30%) >ceftazidime (27.5%) > ceftriaxone and cefotaxime (25%) >meropenem (20%) >imipenem and gentamycin (17.5%) >cefepime and amikacin (12.5%).

In the patients of pneumonia (sputum samples) caused by Klebsiella pnemuniaecolistin, meropenem, amikacin, and imipenem (8.3%) were sensitive In the patients of pneumonia (tracheal swab) caused by Klebsiella pnemuniaecolistin 38.1%, ceftazidime 4.7%, meropenem 4.7%, amikacin 9.5%, sulfamethoxazole 4.7%, gentamycin 9.5%, and imipenem 28.5% were sensitive. In a previous study, ESBL producers were found to be susceptible to imipenem 100%, nitrofurantoin 89%, and amikacin 86%. In ESBL producers, there was a high rate of related resistance to quinolones, co-trimoxazole, and gentamicin[9].

In the patients of wound infections caused by Klebsiella pnemuniae, colistin 16.7%, amoxicillin 3.3%, cefoperazone, cefotaxime, and ceftriaxone 53.3%, ciprofloxacin 3.3%, levofloxacin and ceftazidime 6.67%, meropenem26.7%, amikacin 43.3%, sulfamethoxazole 6.67%, and gentamycin 13.3% were sensitive. The highest sensitivity was to cefoperazone, cefotaxime, and ceftriaxone 53.3% isolates.

In another study, cephalosporins were found to be highly resistant (cephalexin 75%, cefotaxime 82.5%, ceftriaxone 85%, cefeclor 87.5%, cephradine 100%), lincomycin (100%), followed by quinolones (gatifloxacin 15%, moxifloxacin 25%, norfloxacin 35%, nalidixic acid 42.5%, ofloxacin 47.5%, ciprofloxacin 55%), clavulanic acid/amoxicillin 12.5%, and carbapenams (meropenem, imipenem) with the minimum resistance at 7.5%[10].

CONCLUSION

It was concluded that 50 positive cases out of 90 subjects in Public Health Care Facility were identified for the presence of hospital-acquired infections caused by Klebsiella pnemuniae.

Conflict of interest: Nothing to declare

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