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RESEARCH ARTICLE
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IDENTIFICATION AND CHARACTERIZATION OF KLEBSIELLA PNEUMONIAE IN DIFFERENT CLINICAL SAMPLES AT PIMS HOSPITAL ISLAMABAD PAKISTAN.

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ABSTRACT:
The main objective of the present study is the identification and characterization of klebsiella pneumoniae in different clinical samples in PIMS hospital Islamabad, Pakistan. There are 90 samples including 12 sputum samples, 21 tracheal swabs, 30 pus and 27 urine samples. Sample size: 90 subjects Duration: 6 months i.e., 01-07-2022 to 31-12-2022. The current study was conducted at PIMS hospital Islamabad. Gram staining, microscopy, sputum, tracheal swab, pus, and urine cultures, biochemical tests, and antibiotic susceptibility testing were performed for all samples. Statistical analysis was performed by using SPSS Software. Result of the current study show that among the 90 clinical samples, 50 (55.6%) were positive for K. pneumonia and 10 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: Klebsiella pneumoniae, pneumonia, urinary tract infections, sensitivity patterns

INTRODUCTION

In 1882, Carl Friedländer made a groundbreaking discovery in the realm of pneumonia. By extracting bacteria from the lungs of deceased pneumonia patients, he identified a previously

unknown encapsulated bacillus. This marked a significant step forward in understanding the microbial world and its role in respiratory infections. Friedländer's work laid the foundation for further exploration and research into K. pneumonia. (1). K. pneumonia, a gram-negative, non-motile, encapsulated bacterium, has been associated with pneumonia, particularly in individuals with alcoholism or diabetes. It tends to colonize human mucosal surfaces in the oropharynx and gastrointestinal tract. The unique characteristics of this bacterium make it a notable player in respiratory infections, especially in susceptible populations. (3). Polymorphonuclear granulocytes play a crucial role as the backbone of the host's defense against bacterial invasion. These cells, armed with complement proteins, engage in the phagocytosis of bacteria, contributing significantly to the body's immune response. Their ability to engulf and neutralize invading bacteria underscores their importance in the host's defense mechanism.(4). The alternative complement activation cascade demonstrates heightened activity in the defense against K. pneumoniae infection. Neutrophils, equipped with lipopolysaccharide-binding protein and myeloperoxidase, actively contribute to the host's defense mechanism against this bacterial invasion (5). The pathogenicity of microorganisms hinges on the presence of a polysaccharide capsule, comprising intricate acidic polysaccharides. This structural component plays a pivotal role in defining the virulence of these microorganisms. (6). In the medical realm, β-lactams, and various antibiotics with potency against Enterobacteriaceae are frequently employed to treat infections in hospitalized or immunocompromised individuals. This standard practice reflects the effectiveness of these medications in addressing such infections within this specific patient population. (7). Hypervirulent strains of Klebsiella pneumoniae and those resistant to antibiotics have independently emerged in various regions across the globe. The evolution of these distinct strains reflects the dynamic nature of microbial adaptation and poses unique challenges in addressing infections caused by Klebsiella pneumoniae.(8). Recent advancements in molecular techniques have unveiled a noteworthy revelation: some clinical isolates, previously classified as Klebsiella pneumoniae, belong to different Klebsiella species (9). Known for causing a range of infections, Klebsiella pneumoniae is a prevalent hospital-acquired bacterium responsible for urinary tract infections, nosocomial pneumonia, gastrointestinal infections, surgical wound infections, and blood diseases. This recognition underscores the importance of molecular insights in refining our understanding of bacterial classifications and their implications in clinical settings (10). Additionally, Klebsiella pneumoniae poses a viral infection risk within the community. Statistics suggest that Klebsiella spp is implicated in approximately 8% of hospital-acquired infections and contributes to 3% of epidemic diseases. These figures highlight the potential gravity of untreated infections caused by Klebsiella species and emphasize the importance of prompt and effective medical intervention (11).

Material and Methods:

A total of 120 cases were included in this study. Out of 120 samples, 26 were sputum, 33 were pus, 25 were tracheal swab and 32 were urine samples. Before collecting samples and IRB permission all the study subjects were briefed about the sample collection methods. Out of 120, 82 were male and 38 were female participants. 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 120 samples, 21 were sputum, 35 were pus, 26 were tracheal swab and 32 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 isolates were 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 Klebsiella pneumoniae in different samples.

Culture Appearance	**N=**	**%age**
Sputum		
Mucoid Colonies	6	17.7%
White Colonies	5	18.7%
No Growth	11	69.7%
Pus		
Mucoid Colonies	19	55.3%
Other Colonies	4	3.6%
No Growth	16	46.3%
Tracheal Swab		
Mucoid Colonies	25	93.5%
Other Colonies	3	1.5%
No Growth	6	11.5%
Urine		
Mucoid Colonies	16	49.1%
White Colonies	3	4.7%
Golden Colonies	4	8.4%
Greenish Colonies	3	3.7%
No Growth	8	27.9%
Mixed Growth	6	13.1%

Fig 1: Mucoid Colonies of Klebsiella pneumonia on Blood agar

Table 2: Mucoid colonies in different samples

Samples	n=	Growth present
Pus	34	19
Sputum	26	06
Tracheal Swab	28	24
Urine	32	17
Total	120	50

Table 3: Morphological Characterization

Bacteria	n	Morphology			
		Colony Shape	Color	Margins	Consistency
Klebsiella pneumonia	56	Large circular	Pinkish red	Entire	Mucoid
Pseudomonas aeruginosa	04	Circular	Colorless	Irregular	Mucoid
E coli	05	Circular	Pink	Entire	Smooth

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

Table 8: Sensitivity for urine samples

Name of Drug	Sensitive	Resistant
Cefepime	13	7
Amoxicillin	6	16
Tazobactam	5	16
Cefuroxime	4	15
Tetracycline	6	17
Fosfomycin	17	18
Ceftriaxone	8	11
Ciprofloxacin	6	13
Levofloxacin	5	13
Ceftazidime	6	11
Meropenem	11	8
Amikacin	13	5
Sulfamethoxazole	4	13
Gentamycin	9	7
Imipenem	11	7
Cefotaxime	7	10

DISCUSSION

This study shows infection rate in 55(58.6%) cases out of 120 participants. In this study, all samples (sputum, tracheal swab, pus, and urine) are taken from hospitalized infection patients at PIMS hospital Islamabad. In the present study, cefepime28.5%, amoxicillin 9.5%, tazobactam 11.5%, cefuroxime13%, Fosfomycin 29.5%, ceftriaxone 16%, ciprofloxacin 9.5%, levofloxacin 8.5%, ceftazidime 14.5%, meropenem 21%, amikacin 29.5%, 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 14/29 isolates (28.5%). The antibiotic resistance was in descending order fosfomycin (39.5%) > amoxicillin >sulfamethoxazole> ciprofloxacin > levofloxacin and tetracycline (33.5%) >tazobactam and cefuroxime (33%) >ceftazidime (29.5%) > ceftriaxone and cefotaxime (28%)>meropenem (24%) >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 (13). In the patients of wound infections caused by Klebsiella pneumonia colistin 16.7%, amoxicillin 3.3%, cefoperazone, cefotaxime, and ceftriaxone 53.3%, ciprofloxacin 3.3%, levofloxacin and ceftazidime 7.67%, meropenem 28.7%, amikacin 43.3%, sulfamethoxazole 8.67%, 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 86.5%, ceftriaxone 85%, cefeclor 89.5%, cephradine (100%), lincomycin (100%), followed by quinolones (gatifloxacin 15%, moxifloxacin 27%, norfloxacin 36%, nalidixic acid 43.5%, ofloxacin 49.5%, ciprofloxacin (58%), clavulanic acid/amoxicillin 13.5%, and carbapenams (meropenem, imipenem) with the minimum resistance at 11.5% (14).

CONCLUSION

It was concluded that 65 positive cases out of 120 subjects in Public Health Care Facility were identified for the presence of hospital- acquired infections caused by Klebsiella pneumaniae.

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