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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF KLEBSIELLA SPECIES FROM VARIOUS CLINICAL SAMPLES IN TERTIARY CARE HOSPITAL, JAIPUR, RAJASTHAN

Microbiology

Dr. Chirag P. Kukadia* Assistant Professor, Department of Microbiology Jaipur National University Institute Of Medical Sciences And Research Centre, Jagatpura, Jaipur *Corresponding Author

Dr. Preeti Srivastava Professor & Head, Department of Microbiology Jaipur National University Institute Of Medical Sciences And Research Centre, Jagatpura, Jaipur

ABSTRACT

Introduction:- Klebsiella is one of the most important bacteria causing infections. This bacterium threatens public health and leads to increased hospital costs and mortality rate. The purpose of this study was to determine the pattern of antibiotic resistance in Klebsiella. Klebsiella shows resistance to antimicrobial agents like cephalosporins, quinolones and aminoglycoside. Both morbidity and mortality is increased when infection is caused by these drug resistant organisms. Antibiotic sensitivity pattern may change from time to time and place to place. Therefore updated knowledge of the drug resistance pattern in a particular region is useful in clinical practice. This work gives an account of isolation of Klebsiella from various clinical samples like pus, sputum, urine and other various samples, and their antibiogram and presence of resistant strains in various samples.

Methods:- This study was performed on 159 samples of Klebsiella isolates from 3496 samples in all units of JNU hospital in 7 months. The antibiotic resistance of Klebsiella to 19 antibiotics was performed by Kirby Bauer disk diffusion method.

Results:- The frequency of Klebsiella among 3496 samples was determined 159 isolates (4.55%). The highest number of Klebsiella was isolated from the ICUs 42 (27.67%). As per samples wise highest number of Klebsiella isolated from urine (32.70%) and than from sputum 42 (26.42%), pus 34 (21.38%), tracheal aspirate 12 (7.55%), BAL 7 (4.40%), blood 6 (3.77%), ET secreation 3 (1.89%) and throat swab 3 (1.89%), respectively. Klebsiella resistance to antibiotics included: cefazoline (82.46%), cefuroxime (81.13%), ceftazidime (78.62%), ceftriaxone (77.99%), norfloxacin (U) (75.00%), cefepime (71.70%), ciprofloxacin (66.04%), levofloxacin (64.78%), amoxyclav (59.75%), pipracillin tazobactum (58.49%), nitrofurantoin (U) (57.69%), gentamicin (56.60%), cotrimoxazole (54.09%), amikacin (52.83%), meropenem (51.57%), doxycyclin (46.54%), imipenem (35.22%), fosfomycin (U) (17.31%) and colistin (0%).

Conclusion:- Doxycyclin, imipenem and fosfomycin (for UTI patients) shows more than 50% of sensitivity. Colistin is only one drug that shows 100% of sensitivity. Other antibiotics shows less than 50% sensitivity and has the lowest effect on the treatment of Klebsiella infections.

KEYWORDS

Klebsiella, AST, resistance

INTRODUCTION :-

In 1883 Friedlander isolated a capsulated bacillus from the lungs of patients who had died of pneumonia [1]. This was named after him as Friedlander's bacillus. Later on this organism was given the generic name of Klebsiella, which is ubiquitously present and reported worldwide.

Klebsiella is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family [2]. It is the second most popular member of the aerobic bacterial flora of the human intestine. It is the most common causative agent of nosocomial and community acquired infections.

It has even replaced Escherichia coli in some centres as a nosocomial pathogen. It causes pneumonia, urinary tract infections, other pyogenic infections, septicaemia and rarely diarrhoea [3].

Biochemically typical strains of Klebsiella are resistant to a wider range of antibiotics than are most Escherichia coli strains. They are nearly always naturally resistant to ampicillin [1]. Resistance of Klebsiella to previously sensitive antibiotics is also increasing in the recent years due to overuse and misuse of antimicrobial agents and or natural causes.

Of particular concern is the Extended Spectrum Beta Lactamase (ESBL) producing Klebsiella that have been steadily increasing over the past years and rapidly spreading worldwide that pose a serious threat for healthcare associated infections. Increasingly the ESBL Klebsiella are also showing co-resistance to other antimicrobial agents like quinolones and aminoglycoside antibiotics. Both morbidity and mortality is increased when infection is caused by these drug resistant organisms. Antibiotic sensitivity pattern may change from time to time and place to place.

Therefore updated knowledge of the drug resistance pattern in a particular region is useful in clinical practice. This work gives an account of isolation of Klebsiella from various clinical samples like pus, sputum, urine and other various samples, and their antibiogram and presence of resistant strains in various samples.

MATERIALS AND METHODS :-

Place of study was JNU Hospital, Microbiology Department, Jaipur, Rajasthan. Various clinical samples such as blood, urine, sputum, Endo-tracheal secretion, tracheal aspirates, pus, body fluids including ascitic fluid, CSF, pleural fluid, gastric aspirate, synovial fluid, peritoneal fluid etc obtained from patients of any age and either sex from in-patient and out-patient wards from various clinical specialties of JNU hospital, Jaipur, Rajasthan. This study includes two species of Klebsiella – Klebsiella pneumoniae and Klebsiella oxytoca.

Bacteriological Procedures:-

1. Macroscopic and Microscopic Examination:

Colour and turbidity was observed in all clinical samples macroscopically.

Gram stain was observed microscopically in all clinical samples except urine samples. Klebsiella is short, plump, straight capsulated gram negative rods[4].

2. Bacterial isolation and identification procedures:

I.Isolation: Isolation of uropathogens was performed by a surface streak culture on high chrom UTI agar media by using calibrated loop (delivering 0.001ml of urine) for semi quantitative method and incubate aerobically at 37 ^{0}C for 24 hrs. For isolation of pathogens from CSF samples culture was done on chocolate agar, blood agar and on Mac conkey agar. For isolation of pathogens from other remaining samples culture was done on blood agar and on Mac conkey agar.

II.Identification: Based on the colony morphology on agar plates as on High chrom UTI agar media Klebsiella gives blue to purple, mucoid colonies; on Mac Conkey agar Klebsiella produces large dome shaped mucoid, sticky, pink colour, lactose fermenting colonies[4] ; on blood agar Klebsiella produces non-haemolytic, grey white, mucoid colonies[2] . Then further gram's staining and biochemical tests namely catalase test, oxidase test, triple sugar iron test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, urease production test, sugar fermentation tests were performed. K. oxytoca is biochemically similar to K. pneumonia, but differs from latter by being

indole positive[4]. Klebsiella was isolated under sterile conditions with controls being satisfactory.

III.Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility of all identified Klebsiella was done by the Kirby-baur disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guide lines.

In this method discs imbued with antibiotics were laid over the carpet culture of test organisms, antibiotics diffused radially from the disc into the medium. After overnight incubation, the culture was examined for areas of no growth around the disc. Bacterial strains sensitive to the antimicrobial were inhibited in a circle around the discs whereas resistant strain grew up to the edge of the disc. Interpretation of the results was done using the zone of inhibition sizes provided the antibiotic disc manufacturer (Hi-Media). Results were interpreted as sensitive (S), intermediate resistant (I) or resistant (R) based on CLSI guidelines. Quality control strain of Escherichia coli – ATCC – 25922 is used to validate the results of the antimicrobial discs[5].

Klebsiella isolates were selected to determine their susceptibility patterns against the first line antimicrobial agents, by the disc diffusion method of Kirby- Bauer as described by Clinical Laboratory Standards Institute (CLSI). The following antibiotic discs were used for testing bearing the concentrations: Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Cefepime (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Amoxyclav (10 µg), Pipracillin + Tazobactum (100 µg/10 µg), Meropenem (10 µg), Imipenem (10 µg), Doxycycline (30 µg), Co-trimoxazole (1.25/23.75 µg), Colistin (10 µg) [5].

For Urine samples following antibiotic discs were used; Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Norfloxacin (10 µg), Cefepime (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Amoxyclav (10 µg), Pipracillin + Tazobactum (100 µg/10 µg), Meropenem (10 µg), Imipenem (10 µg), Doxycycline (30 µg), Co-trimoxazole (1.25/23.75 µg), Colistin (10 µg) Nitrofurantoin (300 µg), Fosfomycin (200 µg) [5].

RESULT :-

Total duration of this study was 7 months from 1st June 2019 to 31st December 2019. Total samples received in laboratory was 3496. Out of total 3496 samples 159 numbers of Klebsiella isolated and that accounts 4.55% from total samples. From total 159 isolates of Klebsiella, 58 (36.48%) were from females and 101 (63.52%) from males. The highest number of frequency for Klebseilla at the hospital units was related to ICUs, Surgery, Medicine, Respiratory medicine, Pediatrics and Obstetrics and gynecology (Table 1). According to the source of infection, most of Klebseilla was isolated from urine, sputum, pus, tracheal aspirate, BAL, blood samples, ET secreation and throat swab respectively (Table 2). Out of total 159 Klebsiella, 139 (87.42%) were Klebsiella Pneumoniae and 20 (12.58%) were Klebsiella Oxitoca (Table 3). The number of Klebseilla of resistant, susceptible are specified to 19 antibiotics tested in Table 4.

Table 1. Distribution of isolates of Klebsiella as per Medical units

Medical unit	Numbers of isolates	Percentage of isolates
ICUs	44	27.67%
Medicine	38	23.90%
Surgery	34	21.38%
Respiratory medicine	25	15.72%
Pediatrics	11	6.92%
Obstetrics and gynecology	7	4.40%

Table 2. Distribution of isolates of Klebsiella as per samples

Type of sample	Numbers of isolates	Percentage of isolates
Urine	52	32.70%
Sputum	42	26.42%
Pus	34	21.38%
Tracheal aspirate	12	7.55%
BAL	7	4.40%
Blood	6	3.77%
ET secreation	3	1.89%
Throat swab	3	1.89%

Table 3. Distribution of Klebsiella species

Species of Klebsiella	Numbers of isolates	Percentage of isolates
Klebsiella pneumoniae	139	87.42%
Klebsiella oxitoca	20	12.58%

Table 4. Antimicrobial susceptibility pattern of Klebsiella

Name of medicine (Code of medicine)	Sensitivity (%)	Resistance (%)
Ceftriaxone (CTR)	22.01 %	77.99 %
Ceftazidime (CAZ)	21.38 %	78.62 %
Cefuroxime (CXM)	18.87 %	81.13 %
Cefazoline (CZ)	17.54 %	82.46 %
Cefipime (CPM)	28.30 %	71.70 %
Amoxyclav (AMC)	40.25 %	59.75 %
Pipracillin Tazobactum (PIT)	41.51 %	58.49 %
Amikacin (AK)	47.17 %	52.83 %
Gentamicin (GEN)	43.40 %	56.60 %
Ciprofloxacin (CIP)	33.96 %	66.04 %
Levofloxacin (LE)	35.22 %	64.78 %
Meropenem (MRP)	48.43 %	51.57 %
Imipenem (IPM)	64.78 %	35.22 %
Doxycyclin (DO)	53.46 %	46.54 %
Cotrimoxazole (COT)	45.91 %	54.09 %
Colistin (CL)	100.00 %	00.00 %
Nitrofurantoin (For urine samples only) (NIT)	42.31 %	57.69 %
Fosfomycin (For urine samples only) (FO)	82.69 %	17.31 %
Norfloxacin (For urine samples only) (NX)	25.00 %	75.00 %

DISCUSSION :-

Klebsiella is one of the most common bacteria that causes infections in patients. Choosing the best antibiotic is very important for the treatment of infections caused by Klebsiella Based on the results obtained in this study, Klebsiella antibiotic resistance is high. This study revealed that colistin and imipenem are the most effective antibiotics for Klebsiella and for urinary tract infections Fosfomycin is effective other alternative. 15.72 % Klebsiella shows ESBL producers.

Babakhani S. et al[6] study in 2015 was performed on 51 samples of Klebsiella isolates from 500 patients in three units of Aleshtar hospital in 9 months. The antibiotic resistance to 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 highest number of K. pneumoniae isolated from urine samples (43.14%) and lowest number from blood samples (3.92%). K. pneumoniae shows highest resistance to ceftriaxone (94.12%), ciprofloxacin (90.20%) and lowest resistance to piperacillin/ tazobactam (19.61%), amikacin (15.69%), imipenem (5.88%), and meropenem (3.92%).

Ebadi AR. et al[7] study in 2017 shows that out of 325 samples tested, after conducting differential tests, 198 cases were positive regarding K. pneumoniae (60.92%), out of whom 119 were female (60.10%) and 79 cases were male (39.90%). Based on antibiotic susceptibility test, K. pneumoniae strains have the most resistance to gentamicin, tetracycline, chloramphenicol antibiotics, and the highest susceptibility to amikacin, cefotaxime, imipenem, ceftazidime, cefotaxime and ciprofloxacin. Ghanem S. et al[8] study in 2017 shows more resistance to amoxyclav, cotrimoxazole and more than 85 % sensitivity to amikacin, gentamycin, ceftazidime, ciprofloxacin and imipenem. The antibiotics colistin, nitrofurantoin and meropenem showed 100% sensitivity. Shilpa K et al[9] study in 2016 shows more resistance to cotrimoxazole and more than 50 % sensitivity to amikacin, gentamicin, ciprofloxacin and imipenem. Tarani Prasad P.[10] study in 2015 shows more resistance to ceftriaxone, ceftazidime, cefuroxime, cefazolin, amoxyclav, cotrimoxazole, colistin and more than 90 % sensitivity to cefipime, amikacin and ciprofloxacin. Table 5 shows the comparison of Klebsiella resistance of the present study with other studies.

Table 5. Comparison of antibiotic resistance of Klebsiella with other studies

Name of medicine (Code of medicine)	Present study	Babakhani S. et al[6] (2015)	Ebadi AR. et al[7] (2017)	Ghanem et al[8] (2017)	Shilpa K et al[9] (2016)	Tarani Prasad P.[10] (2015)
Ceftriaxone (CTR)	77.99 %	94.12 %				43.12 %

Ceftazidime (CAZ)	78.62 %		2.52 %	1.40 %		61.25 %
Cefuroxime (CXM)	81.13 %					42.50 %
Cefazolin (CZ)	82.46 %					53.12 %
Cefipime (CPM)	71.70 %	43.14 %			40.00 %	3.84 %
Amoxyclav (AMC)	59.75 %	50.98 %		33.90 %		58.12%
Pipracillin Tazobactum (PIT)	58.49 %	19.61 %				
Amikacin (AK)	52.83 %	15.69 %	0 %	13.40 %	33.33 %	3.75 %
Gentamicin (GEN)	56.60 %	41.18 %	32.83 %	12.40 %	37.50 %	
Ciprofloxacin (CIP)	66.04 %	90.20 %	18.18 %	3.90 %	31.03 %	7.50 %
Levofloxacin (LE)	64.78 %					
Meropenem (MRP)	51.57 %	3.92 %		0 %		
Imipenem (IPM)	35.22 %	5.88 %	1.52 %	0.50 %	43.33 %	
Doxycyclin (DO)	46.54 %					
Cotrimoxazole (COT)	54.09 %			37.30 %	50.00 %	54.37 %
Colistin (CL)	0 %	43.14 %		0 %		53.75 %
Nitrofurantoin (For urine samples only) (NIT)	57.69 %	56.86 %		0 %		
Fosfomycin (For urine samples only) (FO)	17.31 %					
Norfloxacin (For urine samples only) (NX)	75.00 %					

CONCLUSION :-

Antibiotic resistance is worrying in bacteria, and Klebseilla through the world has become a threat to public health. According to studies conducted in recent years, Klebseilla resistance is spread to most antibiotics. In these conditions, Klebsiella antibiotic resistance should be determined by tests. This attempt can help to treat Klebsiella infections and reduce mortality rate. Although, resistance to multiple antibiotics especially in cases of nosocomial organisms is not new, but it has increased in recent years. The frequency of resistance to other classes of antibiotics such as quinolones and aminoglycosides are also high. Therefore, in the treatment of patients with these multidrug resistance organisms other options including combination therapy or potentially toxic drugs such as colistin and fosfomycin (for UTIs patients) may be used. With increase resistance in Klebseilla pneumoniae, resistance among other species of Klebseilla mostly Klebseilla oxytoca also shows increasing trends of antimicrobial resistance. Cases of other species of Klebsiella mostly Klebseilla oxytoca are also increasing.

Ethical issues :-

Not applicable.

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