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Antimicrobial Resistance Pattern and Extended Spectrum Beta Lactamase Production in Klebsiella Isolates from Various Clinical Samples

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Abstract

Background: Klebsiella spp. is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family. They are ubiquitously present, reported worldwide and popular member of aerobic bacterial flora of human intestine. They are common causative agents of variety of nosocomial and community acquired infections. These bacteria have become important nosocomial pathogens and have replaced Escherichia coli in many hospitals. Epidemic and endemic nosocomial infections caused by Klebsiella spp. are leading causes of morbidity and mortality. **Subjects and Methods:** This is a Prospective study was conducted in the Department of Microbiology, Guntur Medical College, Guntur over a period of 1 year among 100 isolates of Klebsiella from various clinical samples. Antibiotic sensitivity tests were done by Kirby-Bauer disk diffusion method. The test organism is subcultured into peptone water and incubated for 4-6 hours at 370c. The turbidity is standardized with 0.5 McFarland, and is swabbed over 90mm Muller Hinton agar plate. Antibiotic disks were placed 15mm from the edge of the plate and disks are evenly placed and incubated at 370c for 18-24 hours. Zone of inhibition were measured with a ruler and interpreted as per NCCLs guidelines. The commercially available antibiotic disks supplied by High media (Mumbai) were used. **Results:** The present study reveals prevalence of Klebsiella isolates as 40% from pus samples. Present study reveals prevalence of Klebsiella from sputum as 10%. The present study reveals prevalence of Klebsiella isolates from blood samples as 10%. Present study reveals that all Klebsiella isolates found to be 100% sensitive to Imepenem in all samples, followed by amikacin and ciprofloxacin. Highest resistance pattern was observed among ampicillin, amoxyclav, norfloxacin, aztreonam and third generation cephalosporins. Present study shows isolates from pus samples are 32% resistant to Amikacin, 28% resistant to Ciprofloxacin, 100% resistant to amoxycillin, aztreonam and 93% third generation cephalosporins. In Present study urinary isolates shows highest sensitivity to imipenem followed by amikacin and norfloxacin. **Conclusion:** The present study was undertaken to know the prevalence of Klebsiella pneumonia antimicrobial resistance pattern and ESBL production among those isolates from the all clinical isolates at Hospital. Screening and confirmation of ESBL producers in clinical microbiology laboratories should include efficient and inexpensive methods that cover the different needs and complexities of the local and regional epidemiology.

Keywords: Antimicrobial Resistance, Extended Spectrum Beta Lactamase, Klebsiella Isolates.

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Introduction

Klebsiella spp. is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family.[1] They are ubiquitously present, reported worldwide and popular member of aerobic bacterial flora of human intestine. They are common causative agents of variety of nosocomial and community acquired infections. These bacteria have become important nosocomial pathogens and have replaced Escherichia coli in many hospitals. Epidemic and endemic nosocomial infections caused by Klebsiella spp. are leading causes of morbidity and mortality.[1,2] In addition to being the primary cause of respiratory tract infection like Pneumonia, Rhinoscleroma, Ozaena, Sinusitis and Otitis, they also cause Urinary tract infection, Septicemia, Pyogenic infections and even infection of the alimentary tract.[3]

Recently, WHO warned the community that multi-drug resistant bacteria are emerging worldwide, which is a big challenge to healthcare system.[4] In India, the reasons for development of antimicrobial resistance could be due to higher prevalence of infection, irrational use of antibiotics, over the counter availability of higher or broader antimicrobial agents, and poor monitoring of Antibiotic susceptibility in hospitals.[5]

Extensive use of broad spectrum antibiotics in hospitalized patients has led to both increased carriage of Klebsiella pneumoniae and development of multi-drug resistant (MDR) strains that produce Extended Spectrum Beta Lactamase (ESBL). Epidemic strains of Cephalosporin resistant Klebsiella pneumoniae have been associated with

increased morbidity and mortality in hospitalized patients.[6,7]

ESBLs are typically plasmid mediated, Clavulanate susceptible enzymes that hydrolyze Pencillins, extended spectrum cephalosporins (Cefotaxime, Ceftrioxone, Ceftazidime and others) and aztreonam.[8] Several risk factors are responsible for emergence of extended spectrum beta lactamases like indiscriminate use of cephalosporins, long duration of hospital stay, severity of the underlying illness, instrumentation etc. ESBL producing organisms can cause a wide spectrum of diseases like urinary tract infection, sepsis, pneumonia, peritonitis, meningitis etc.[9,10]

Various diagnostic methods are now available for the detection of ESBLs like double disk diffusion method, Phenotypic confirmatory disk diffusion test and various commercial tests like E-test, Microscan panels and Vitek system.[11]

ESBLs are more commonly found in Klebsiella spp. This may probably be due to the adaptation of the Klebsiella which can survive longer on hands and environmental surfaces facilitating cross-infection with in hospital. The present study gives an account of isolation of Klebsiella pneumoniae from various clinical specimen, their antibiogram and detection of extended spectrum beta lactamase production among the isolated Klebsiella spp.[12]

Subjects and Methods

This is a Prospective study was conducted in the Department of Microbiology, Tertiary Care Teaching Hospital over a period of 1 year among 100 isolates of Klebsiella from various clinical samples.

Inclusion Criteria

- Various clinical samples of Hospitalized patients from different wards of OGH.
- Isolates of Klebsiella from various clinical (urine, pus, sputum, blood) samples.

Exclusion Criteria

- Specimens from Health care personnel including doctor, nursing staff.
- Specimens from out patients.
- Isolates of Gram positive bacteria.
- Isolates of non-fermenting bacteria and other Enterobacteriaceae

Methods

1. Standard Bacteriological culture methods to identify Klebsiella.
2. Antimicrobial susceptibility test by Kirby Bauer Disc diffusion method.
3. To identify ESBL production following CLSI guidelines.

Antibiotic Sensitivity Tests

Antibiotic sensitivity tests were done by Kirby-Bauer disk diffusion method. The test organism is subcultured into peptone water and incubated for 4-6 hours at 370c. The turbidity is standardized with 0.5 McFarland, and is swabbed over 90mm Muller Hinton agar plate. Antibiotic disks were placed 15mm from the edge of the plate and disks are evenly placed and incubated at 370c for 18-24 hours. Zone of inhibition were measured with a ruler and interpreted as per NCCLs guidelines. The commercially available antibiotic disks supplied by High media (Mumbai) were used.

Two muller-Hinton agar plates were used, one for reporting routine and antibiotic susceptibility, the other for identifying ESBL producing Klebsiella using indicator antibiotic discs viz. ceftriaxone and ceftazidime.

Identification of EXTENDED SPECTRUM OF BETALACTAMASES (ESBL) in Klebsiella pneumoniae

- The accurate and timely detection of ESBL is vital in the treatment and in the control of their spread.
- Clinical and Laboratory Standards Institute 8 has developed screening tests for identifying the ESBL – producing Klebsiella species.
- Zone of inhibition of ≤22mm for ceftazidime, ≤27mm cefotaxime, and ≤25mm for ceftrioxone were selected as screening tests for ESBL.

ESBL Confirmatory Tests

- Double Disc Synergy Test(DDST)
- Phenotypic Confirmatory Disc Diffusion Test(PCDDT)

Double Disc Synergy Test (DDST) or (Double Disc Diffusion test or Double Disk approximation Method): 0.5 McFarland standardized inoculums was swabbed onto Muller-Hinton agar plate.

An amoxicillin with clavulinic acid disc is placed in the center of the plate and one ceftrioxone disc and ceftazidime disc were placed at a distance of 20mm (Center to Center) from the amoxicillin with clavulinic acid disk. The plates were incubated overnight at 370c and results were read. Enhancement of zone of inhibition of the cephalosporin disc towards clavulinic acid containing disc was inferred as synergy and the strain considered as ESBL producer.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT): 0.5 McFarland standardized inoculums was swabbed onto Muller Hinton agar plate. Ceftazidime disc containing 30µg and ceftaziime with clavulinic acid disc 20+10 µg were placed at a distance of 30mm from each other. Plates were incubated overnight at 370c and results were read. Increase in zone diameter ≥5mm with ceftazidime with clavulinic acid disc was inferred as positive test and the organism considered ESBL producer.

Results

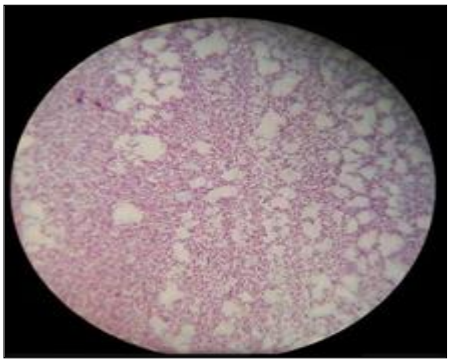

Figure 1: Gram stain showing GNB

Figure 2: Klebsiella species on BA and MA plates

Figure 3: Biochemical reaction showing Klebsiella species

Table 1: Distribution of males and female among various clinical samples

Sex	Male	Female
Pus	38	12
Urine	11	19
Sputum	6	4
Blood	6	4
Total	61	39

Table 2: Age incidence among klebsiella isolates from various samples

	pus	urine	sputum	blood	total	Percentage
<20yrs	8	0	1	3	12	12%
21-40yrs	20	7	1	2	30	30%
41-60yrs	16	15	6	3	40	40%
61-80yrs	6	7	2	2	17	17%
>81yrs	0	1	0	0	01	1%

Highest among 41-60 yr age group.

Table 3: Distribution of various organisms from culture positive

	CASES
Klebsiella	100
Escherichia coli	54
Staphylococcus	43
Cons	20
Pseudomonas	14
Proteus	6
Enterococci	7
Streptococcus	4
Citrobacter	5
Candida	3

Table 4: Antibiotic resistance pattern among pus isolates

Antibiotic	Resistance
Ampicillin	100%

Amoxyclav	52%
Ceftazidime	98%
Aztreonam	98%
Ciprofloxacin	28%
Imipenem	0
Amikacin	32%

Table 5: antibiotic resistance pattern among urinary isolates

Antibiotics	Resistance
Ampicillin	100%
Norfloxacillin	46%
Ceftazidime	93.3%
Amikacin	30%
Imipenem	0
Aztreonam	93.3%

Table 6: Antibiotic resistance pattern among sputum samples

Antibiotic	Resistance
Ampicillin	100%
Aztreonam	100%
Ceftazidime	100%
Azithromycin	80%
Cotrimoxazole	50%
Ofloxacillin	30%
IMIPENEM	0

Table 7: Antibiotic resisatance pattern among blood samples

Antibiotic	Resistance
Ampicillin	100%
Aztreonam	90%
Ceftazidime	80%
Amikacin	40%
Ciprofloxacillin	20%
Imipenem	0%

Table 8: Antimicrobial Susceptibility Pattern of Klebsiella Isolates

Antibiotics	Sensitive(%)	Resistance(%)
Imipenem	100	0
Amikacin	60	40
Ciprofloxacin	52	48
Cotrimaxazole	50	50
Cephalosporines	7	93
Azithromycin	20	80
Ampicillin	0	100
Aztronam	0	100
Gentamycin	23	72
Norfloxacin	54	46
Ofloxacin	70	30

Table 9: Prevalence of ESBL producers from variuos samples by different methods

	Total no..isolates	DDT	PCDDT	Percentage
PUS	50	24	24	48%
URINE	28	18	18	64.2%
SPUTUM	10	4	4	40%
BLOOD	8	4	6	75%
TOTAL	96	50(52.1%)	52(54.1%)	54.1%

Discussion

A study was done to show the prevalence and antibiogram of ESBL producers. They use the combination disk method and double disk approximation method to detect the ESBL producers.[13] Likewise in our study, we also detect the ESBL producers by double disk approximation test for screening the potential ESBL producers and phenotypic confirmatory test with combination disc method was used to confirm the ESBL producers.

The present study reveals prevalence of Klebsiella isolates as 40% from pus samples. A study conducted by Namratha et al,[14] in 2015 also showed 40% of Klebsiella isolates

from pus samples. The present study correlates with the study of Namratha et al.[18] Another study conducted by Chakrabourthy et al,[15] in 2016 and Orhue et al in 2015 reveals 10% and 1% respectively.

Another study conducted by Thosar et al,[16] in 2014 isolates 60% Klebsiella isolates from 130 pus samples. Various studies shows different rates of prevalence of Klebsiella isolates from pus samples. All these studies reveals hospital environment, Klebsiella may be more responsible for wound infections(pus) and respiratory infections, unless otherwise there is direct hematogenous spread.

Present study reveals 30% Klebsiella isolates from urinary tract infections. A study conducted by Gayathri et al,[17] (2016) shows similar prevalence rate as 30% little higher than the Namratha et al,[18] (26%) study. Another study conducted by Thosar et al. shows 42.86% prevalence from 165 cases.

Present study reveals prevalence of Klebsiella from sputum as 10% which correlates with the study of Chakrabourthy et al,[19] (7%) and Orhue et.al20(8%.). Our study far away from the studies of the Olowe et al,[21] (29%), Thosar et al,[22] (45%) and Namratha et al,[23] (26%) studies.

The present study reveals prevalence of Klebsiella isolates from blood samples as 10%. Another study conducted by R M Praveen et al. in 2011 reveals prevalence of Klebsiella from blood samples as 16% and pamba et al showed it as 15.5%. Our study co-insides with these studies.

Present study reveals Klesiella isolates predominantly isolated from male population this was co-insides with the studies conducted by Chakrobourthy et al. in 2016 and R.K.Shah et al in 2010. These studies also shows male predominance as 58% and 62% respectively.

Our study correlates with the study of Chakraborthy et.al (2016),[24] in their study klebsiella isolates are more predominant in >40 yrs age group. Present study shows Klebsiella pneumonia and Klebsiella oxytoca are the two common isolates obtained from various samples. From pus samples 3(3%) and from urinary isolates 3(3%) of Klebsiella oxytoca are obtained.

Present study reveals that all Klebsiella isolates found to be 100% sensitive to Imepenem in all samples, followed by amikacin and ciprofloxacin. Highest resistance pattern was observed among ampicillin, amoxyclav, norfloxacin, aztreonam and third generation cephalosporins. Another study conducted by susethra,[25] et al. (2016), Mohmad Akram,[26] et al (2007) reported similar findings.

Present study shows isolates from pus samples are 32% resistant to Amikacin, 28% resistant to Ciprofloxacin, 100% resistant to amoxycillin, aztreonam and 93% third generation cephalosporins. Our study correlates with the study of Sharathbabu,[27] et al conducted in (2012). In their study they show amikacin resistance as 33.3% from pus samples.

In Present study urinary isolates shows highest sensitivity to imipenem followed by amikacin and norfloxacin. Amikacin shows resistance 30% which correlates with the study of Sharathbabu et al.[28] (2010) (28.8%), norfloxacin shows resistance 46.6% correlates with Sharathbabu et al (2008) (44.57%). among 30 isolates two isolates shows (100%) sensitivity to all antibiotics including cefotaxime, ceftrioxone, ceftadizime and aztreonam.

Conclusion

The present study was undertaken to know the prevalence of Klebsiella pneumonia antimicrobial resistance pattern and ESBL production among those isolates from the all clinical isolates at Hospital. Screening and confirmation of ESBL producers in clinical microbiology laboratories should include efficient and inexpensive methods that cover the different needs and complexities of the local and regional epidemiology.

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