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STUDIES ON BETA LACTAMASE PRODUCING MULTIDRUG RESISTANT *KLEBSIELLA* SPECIES ISOLATED AT DR JAGDALE MAMA HOSPITAL AND RESEARCH CENTER BARSHI DISTRICT, SOLAPUR (MAHARASHTRA)

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ABSTRACT

Introduction: Increased use of antibiotics in hospitals led to multidrug resistant organisms (*Klebsiella* species). The purpose of this study was to know prevalence of extended spectrum beta - LACTAMASE (ESBL) producing multidrug resistant *Klebsiella* species from clinical isolate from patients admitted to Dr. Jagdale Mama Hospital and Research centre, Barshi. Dist Solapur (MS) **Methodology:** A total 310 specimens including urine, pus blood was isolated and identified by standard methodology. An antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. For detection of extended spectrum beta -lactamases producing isolates combination disk method was done **Result:** A total of 310 samples (200 urine, 60 pus, and 50 bloods) were included in study. Out of total samples 120(38.8%) samples precede showed growth in e Gram- negative bacteria. Amongst these significant growths 70(58.4%) *Klebsiella* species, 50(71.4%) *Klebsiellapneumoniae* and 20(28.5%) *Klebsiellaoxytoca* were isolated. Amongst these total isolated *Kpneumoniae*, 35(70%) multidrug resistant *Klebsiellapneumoniae* and 13(65%) multidrug resistant *Klebsiellaoxytoca* were isolated. A total of 23(65.7%) *Kpneumoniae* and 6(46.1%) *Koxytoca* isolates were found to be extended spectrum beta-lactamase producers.

KEYWORDS

MDT, ESBL and Antibiotics susceptibility testing.

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INTRODUCTON

Klebsiellapueumoniae producing ESBL rarely reported as a cause of septicemia out break among pediatric patients in medical literature. Organisms are a potential cause of severe infection in intensive care units and among pediatric patients (Podschun R. *et al*, 1998)[1]. An increased use of extended spectrum cephalosporins leads to development of

resistance strains. Outbreaks due to resistance strain have been associated with higher morbidity and mortaility (Rice L. B. *et al*, 2009)[2]. Spreading of extended spectrum beta lactamase (ESBL) producing *Klebsiellapueumoniae* in hospital may be a complex event. ESBL producing *Klebsiellapueumoniae* spread by several modes such as dissemination of several unrelated strains or the propagation of a single clone from patients to patients (Bingen E. H. *et al*, 1994)[3]. *Klebsiellapueumoniae* is a clinically most important member of *Klebsiella genus of enterobacteriaceae family. Klebsiellapueumoniae* have been identified as an important common pathogen for nosocomial *pneumonia* (7 to 14% of all cases) septicaemia (4% to 15% of all cases), neonatal septicemia (3 to 30 % of all cases) (Podschun R, *et al*, 1998)[1]. It also causes bacteremia and heptatic infections. It is isolated from number of unusal infections including endocarditis, primary gas containing mediastinal abscess, cholecyctitisdiarrchoea, peritonitis, crepitantmyonecrosis, pyomyositis, necrotizing fasciitis, osteomyelitis, meningitis. They are also important opportunistic pathogens (Purva M *et al*, 2002[4], Rabindranath R. N. *et al*, 2012). *Klebsiellapueumoniae* isolated from clinical samples generally resistant to wide range of antibiotics and always naturally resistant to amphicilin and amoxicillin. Beta lactum antimicrobial agents are most common treatment option for such infections. The constant exposure of bacterial strain to a multitude of beta lactum induces dynamic continuous production and mutation of beta lactamases in these bacteria. Such bacteria show their activity even against newly developed beta lactum antibiotics and called extended spectrum beta lactamases (ESBL). *Klebsiellapueumoniae* and *E. coli* are major ESBL producing organisms isolated worldwide which are recommended to be routinely tested for and reported by CLSI (Clinical Laboratory Standards Institute) guidelines (Jadhav Savita *et al*, 2011[5], Avasthi T. S *et al*, 2011[6]).

Klebsiella organisms can lead to a wide range of diseases, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhoea, and soft tissue infections (Podschun and Ullmann; 1998)[1]. The majority of human *Klebsiella* infections are caused by *K pneumoniae*, followed by *K oxytoca*. Infections are common the very young, very old, and those with other underlying diseases, such as cancer (Bagley S; 1985)[7]. The emergence and global expansion of hypervirulent and multidrug resistant (MDR) clones of *Klebsiella* species have been increasing reported I community-acquired and nosocomial infections. Despite this, the population genomics and epidemiology of MDR *Klebsiella* eat the national level are still poorly understood. The constant exposure of bacterial strain to a multitude of beta-LACTAMASE lactam induces dynamic continuous production and mutation of lactamases in these bacteria. Such bacteria shows their activity even against newly developed beta - lactam antibiotics and called extended spectrum beta-lactamases (ESBL). lt has been observed that there are many organisms that are able to produce ESBLs, with *Klebsiella* species being the most common to produce ESBL. Bacteria that are able to produce ESBLs are not susceptible to treatment with third generation cephalosporin s, which poses a problem in effectively treating patients who are infected an ESBL producing organism. This it is an important task for the researcher to find out alternative medicine. We have started our studies to identify ESBLs producing multidrug resistant *Klebsiella* species isolated from patients admitted to Dr Jagdale Mama Hospital and Research centre, Barshi. Dist Solapur, Maharashtra.

MATERIAL AND METHODS

All the samples included in this study were collected from Dr Jagdale Mama Hospital and Research Center, Barshi, Dist Solapur, Maharashtra, India. The isolates were collected during January 2018 to December 2018. Clinical samples include Midstream urine, Blood and pus. Samples were collected from prescribed patients and analysed within 30 minutes to 1 hour of collection.

Isolation of *Klebsiella* species

The isolates were cultured on Mac monkey's agar and blood agar plates and incubated at 37°C for 24 hours. The Samples where significant pure growth

obtained were included in this study. Isolates were subcultured and preserved in glycerol agar for further analysis.

Identification of *Klebsiella* species: A series of morphological and biochemical tests were performed to identify the suspected *Klebsiella* species of isolates. The tests include Gram-staining, motility, catalase production, oxidase activity, ox-ferm test, different biochemical studies like sugar fermentation, IMViC test and urease production test. All tests were conducted according to the Bergey's Manual of Determinative Bacteriology. (13, Bergey DH, John GH).

Bacterial isolates: A total of 310 clinical isolates from various specimen including urine (200), Blood (50) and pus (60) were collected from January 2018 to December 2018 and enrolled in this study. Out of these total samples proceeds only 120(38.8%) samples showed significant growth. Amongst these significant growths 70(58.4%) *Klebsiella* species (50 *Klebsiellapneumoniae* and 20 *Klebsiellaoxytoca*) were isolated.

Antibiotics susceptibility testing

Antimicrobial susceptibility testing was performed by following Kirby Bauer's disc diffusion method, (Baur A W, *et al*, 1966)[8] as recommended by clinical and laboratory standards institute (CLSI) (Clinical and Laboratory Standards Institute 2005). The zones of growth inhibition were measured and interpreted using the standard chart and organism reported as susceptible, intermediate or resistant accordingly. The antibiotics tested were cefepime, ceftazidime, ceftaxime, cefprodoxime, Ciprofloxacin, levofloxacin chloramphenicol, Tetracycline, Gentamicin, Amikacin, cotrimoxazole, Nitrofurantoin Imipenem and meropenem. Criterion for Multidrug R Resistance: In present study the defining criterion for an isolate to be Multidrug Resistance (MDR) was set as resistance to two oi more drugs of different structural classes.

ESBLS Detection Methi: Each *Klebsiellapneumoniae* and *Klebsiellaoxytoca* isolate should be considered a potential ESBL producer if the test results as follows.

Disc diffusion

Cefotaxime-<\=28.
Cefpodoxime-<\=23.
Cefotaxime-<\=22.
Ceftriaxone<\=26.
Aztreonam-<\=27.

The screen is then followed by a phenotype confirmatory test.

Double disc diffusion synergy test

Muller Hinton agar plates are inoculated with standardized inoculum to form lawn, 16 Augmentin disk (Amoxicillin-clavulanate) is placed in the Center of the plate and disc containing one of the oxyimino-beta lactam antibiotic is placed 30mm from center to center from the Augmentin disk. The test organism is considered to produce ESBLs if the zone z size around the test antibiotic disc increases towards the Augumentin disc (Abigail S *et al*, 1995)[9].

RESULTS AND DISCUSSION

Out of total 310 specimens processed urine 200(200/310, 64.5%) followed by pus 60(60/320, 19.3%), blood 50(50/310, 16.1%). As shown in Table No.1 prevalence of *Klebsiella* infection was high in old age group followed by middle age group and adults.

Out of 310 samples processed 120(120/300, 38.7) samples showed significant growth amongst which 70(70/120, 58.4%) Klebsiella species (50 *Klebsiellapneumoniae* and 20 *Klebsiellaoxytoca*) were isolated of which urine specimens 81(81/200, 40.5%) showed significant growth among which 39(81/300, 48.1%) were *Klebsiellapneumoniae* and 15(81/15, 18.5%) were *Klebsiellaoxytoca*. Similarly, 22(60/22, 30%), 17(50/17, 34%) specimens of blood and pus respectively showed growth, among which 5/22, 22.7%) were *Klebsiellapneumoniae* and 3(22/3, 13.6%) were *Klebsiellaoxytoca* isolates from blood and 6(17/6, 35.2%) *Klebsiellapneumoniae* and 2(17/2, 11.7%) *Klebsiellaoxytoca* isolates from pus (Table No.2 and Table No.3) Most of the isolates showed resistant to many commercial antibiotics and refereed as "Multidrug Resistant Organisms (Madrid). Out of the total isolates

Klebsiellapneumoniae 35(50/35, 70%) isolates were multidrug resistant similarly, 13(20/13, 65%) isolates were of *Klebsiellaoxytoca* were multidrug resistant. The majority of ESBLs producing *Klebsiellapneumoniae* urine 19(29/19, 65.5%), pus 3(4/3, 75%) and blood 1(2/1, 50%). Majority of the ESBLs producing *Klebsiellaoxytoca* isolates were from urine 5(10/5, 50%) pus 0(0/1, 0%) and blood 1(2/1, 50%).

As mentioned in Table No.4 the *Klebsiella* species isolates showed variable results in antibiotic susceptibility patterns against different antibiotic disc tested. According to the susceptibility pattern Imipenem is most effective antibiotics against isolated *Klebsiella* species in this region. While *Klebsiella* species are highly resistant to cepepime (74%).

Discussion

In the present research study, total 310 clinical samples of different types were collected and then processed for the isolation of the pathogenic bacteria. The different types of clinical samples included blood, urine, pus, sputum, stool, catheter tips, etc. Das Pradip Kumar and Jayanta Debnath (2015)[10] studied various clinical samples Viz. urine, sputum, blood and pus sent to the Microbiology laboratory were further processed for the isolation of *Klebsiellapneumoniae* followed by their antibiogram.

In the present study out of 90 burns swabs there were 42 isolates were identified *as K. pneumoniae*. This result is in agreement with Amna *et al*, (2014)[11] and Majid *et al*, (2014)[12] who reported that *Klebsiellapneumoniae* and *Pseudomonas* were the most gram negative bacteria that causing urinary tract infection, wounds, burns, blood and respiratory tract infection with the percentage range 12% , 10% and 15%, respectively.

In the current study *Klebsiellapneumoniae* was found to be highly resistance to the antibiotics especially to beta lactam and 3rd generation of cephalosporins. These results are in accordance with the many studies such of Coyle M. B. (2005)[13] and Iroha *et al*, (2011)[14] who reported that *Klebsiellapneumoniae* was resistance to many antibiotics like: Gentamicin (74%), Ceftazidime (98%), Amoxiclav with percentage 96%, Cefotaxime (96.7%) and Tobramycin (50%). But in the study reported by Mariya and Sunil (2015)[15], *Klebsiellapneumoniae* was resistance to nitrfurantoin with percentage (52.78%), amikacin (31.95%), cefotaxime (61.1%), ceftriaxone (54.17%) and ceftazidime (62.5%). *Klebsiellapneumoniae* strains encompass a high degree of resistance to the third-generation cephalosporins (92%). The cephalosporins are used as a first-line therapy for septicemia and burns infections (Khosravi *et al*, 2013[16], Ariadnna *et al*, 2014). *Klebsiellapneumoniae* is well known for the high resistance to different antibiotics. This bacterium has a series of antibiotic resistance genes which can be transferred horizontally to the other gram negative bacteria (Piddock LJV, 2006)[17] and coupled with a series of nosocomial infections in the hospitals (Lewis *et al*, 2007[18], Chikere *et al*, 2008)[19].

Plasmids resistance is the significant source of extended spectrum beta lactamase transmission. The transferable elements bestowing resistance to the antibiotics other than beta-lactams pass through on or alongside the extended spectrum beta lactamase having plasmids, leading to the multidrug resistance in bacteria. It is also that mechanism other than, additionally, plasmid mediated transfer of many types of resistance factors account for the phenomenon of co-resistance observed (Varsha K.V, 2011)[20].

In our study, we have found that out of forty two clinical isolates, all clinical isolates were found positive for *Klebsiellapneumoniae* at biochemical characterizations. When these clinical isolates of *Klebsiellapneumoniae* were further tested for the antimicrobial sensitivity then most of them were found to be multidrug resistant. The isolates were greatly susceptible quinolones and the aminoglycosides.

Table No.1: Age wise distribution of ESBLS producers

S.No	Age Group	Total No. of F isolates	ESBL's producing *Klebsiella Pneumonia* (%)	ESBL's producing *Klebsiella Oxytoca* (%)
1	Pediatrics (1-10)	02	00 (00.00)	00 (00.00)
2	Teenage (11-20)	05	2 (40.00)	00 (00.00)
3	Adults (21-30)	16	6 (37.40)	02 (12.5)
4	Middle Age (31-40)	17	7 (41.4)	01 (5.8)
5	Late Middle Age (41-50)	09	3 (33.3)	00 (00.00)
6	Old Age (50 onwards)	32	5 (23.8)	03 (9.5)

Table No.2: Specimens wise distribution of multidrug resistant ESBLs producing *K. Pneumoniae*

S.No	Specimens (No)	Significant Growth (%)	*K. Pneumoniae* (%)	MDR's (%)	ESBLs Producers (%)
1	Urine (200)	81 (41.50)	39 (48.10)	29 (74.30)	19 (65.50)
2	Pus (55)	17 (34.00)	06 (35.20)	04 (66.66)	03 (75.00)
3	Blood (45)	22 (36.00)	05 (22.70)	02 (40.00)	01 (50.00)
4	Total (300)	120 (38.80)	50 (71.40)	35 (70.00)	23 (65.70)

Table No.3: Specimens wise distribution pf multidrug resistant ESBLs producing *K. Oxytoca*

S.No	Specimens (No)	Significant Growth (%)	*K. Oxytoca* (%)	MDR's (%)	ESBLs Producers (%)
1	Urine (200)	81 (40.00)	15 (18.50)	10 (66.66)	05 (50.00)
2	Pus (55)	17 (34.00)	02 (11.17)	01 (50.00)	00 (00.00)
3	Blood (45)	22 (36.00)	03 (13.60)	02 (66.66)	01 (50.00)
4	Total (300)	120 (38.80)	20 (28.50)	13 (65.00)	06 (46.10)

Table No.4: Resistance rate of *Klebsiella Pneumoniae* and *Klebsilella Oxytoca*

S.No	Antibiotics Class	Antibiotics (Symbol)	*K. Pneumoniae* (%)	*K. Pneumoniae* (%)
1	Cephalosporins	Cefepime (CPM)	37 (74)	13 (65)
2	β -lactamase	Ceftazidime (CTZ)	31 (62)	07 (35)
		Cefotaxim (CTX)	28 (56)	09 (45)
		Cefprodoxime (CPD)	26 (52)	06 (30)
3	Quinolones	Ciprofloxacin (CIP)	34 (68)	08 (40)
		Levofloxacin (LVX)	15 (30)	07 (35)
4	Amphenicol	Chloramphenicol (C)	33 (66)	09 (45)
		Tetracycline (TET)	21 (42)	07 (35)
5	Aminoglycoside	Gentamycin (G)	23 (46)	08 (40)
		Amikacin (AK)	21 (42)	04 (20)
6	Sulphonamides	Cotrimoxazole ()	24 (48)	05 (25)
7	Synthetic Drugs	Nitrofurantoin ()	16 (32)	03 (15)
8	Carbapenems	Imipenem ()	10 (20)	05 (25)
		Meropenen ()	17 (34)	07 (35)

CONCLUSION

Extended spectrum beta-lactamase enzyme is responsible y multidrug resistance in *Kpneumoniae* and *K oxytoca* isolates from I samples Isolates are alt resistant to tetracycline cotrimoxazole Amino glycosides and Floroquinolones. Further studies on emergence and persistence of multidrug resistant extended spectrum beta-lactamases producing *Klebsiella* species and their impact on social clinical and economic out coms such institutions would be useful.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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