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Research Article

EXTENDED SPECTRUM β-LACTAMASE PRODUCING MULTIDRUG-RESISTANT *KLEBSIELLA* SPECIES ISOLATED AT NATIONAL MEDICAL COLLEGE AND TEACHING HOSPITAL, NEPAL

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ABSTRACT

Introduction: Extended spectrum β- lactamases (ESBL) producing multidrug resistant (MDR) *Klebsiella* species resulting in limitation of therapeutic option. The present study has been undertaken to detect the presence of extended spectrum β- lactamases producing multidrug resistant *Klebsiella* species in various clinical specimens.

Methodology**:** A total of 300 specimens including Urine, Pus and Blood were processed according to the standard methodology. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method following Clinical and Laboratory standard institute (CLSI) guidelines. Combination disk method was done for the detection of extended spectrum β- lactamases producing isolates.

Result**:** A total of 300 samples (200 urine, 45 bloods and 55 pus) were included in the study. Out of total samples proceed 110(36.7%) samples showed significant growth of Gram-negative bacteria. Amongst these significant growth of 65(59.1%) *Klebsiella* species, 45(69.2%) *Klebsiella pneumonia* and 20(30.8%) *Klebsiella oxytoca* were isolated. Amongst these total isolated *Klebsiella* species 30(66.7%) multidrug resistant *Klebsiella pneumonia* and 9(45.0%) multidrug resistant *Klebsiella oxytoca* were isolated. A total of 21(70.0%) *Klebsiella pneumonia* isolates and 4(44.4%) *Klebsiella oxytoca* isolates were found to be extended spectrum β- lactamases producers.

Conclusion: This study shows that *Klebsiella pneumonia* and *Klebsiella oxytoca* recovered from clinical specimens in this region produces extended spectrum β- lactamases in much higher number. Such isolates are also resistant to Floroquinolones, Aminoglycosides, Tetracycline and Cotrimoxazole. Further studies to investigate the factors which determine the emergence and persistence of multidrug resistant extended spectrum β- lactamases producing Klebsiella species in this region and their impact on clinical and economic outcomes at such institutions would be useful.

Keywords: Antibiotic susceptibility testing, MDR, ESBL, Combined disk assay,

INTRODUCTION

Among the human pathogenic bacteria Klebsiella species are very notable. They are gram negative, non motile, encapsulated lactose fermenting, facultative anaerobic and rod shaped bacterium found in the normal flora of the mouth, skin and intestine1. Klebsiella are ubiquitously present and reported worldwide. In recent years Klebsiella have become important pathogens in noscomial infections. Epidemic and endemic noscomial infections caused by Klebsiella species are leading causes of morbidity and mortality2. In addition to being the primary cause of respiratory tract infections like pneumonia, rhinoscleroma, ozaena, sinusitis and otitis it also causes infections of the elimentary tract like enteritis, appendicitis and cholycystitis. It is also commonly involved in acute pyelonephritis in pregnant women with urinary tract abnormalities such as urolithiases, hydronephrosis or congenital deformities. They are opportunistic pathogen and under certain conditions may cause serious infections3. Antibiotics are often used against diseases caused by Klebsiella species. But these pathogens are becoming increasingly antibiotic-resistant so that many are now labeled as Multi-drug resistant (MDR) Klebsiella species4-5. An increasing prevalence of multidrug resistant strains of K. pneumonia which possess extended spectrum beta-lactamases (ESBL) enzymes, encoded by plasmid-borne genes which confer resistance to broad spectrum Cephalosporins and other antibiotics used to treat serious infection has been widely reported6. Epidemic strains of Cephalosporin resistant K. pneumonia have been associated with increased morbidity and mortality in hospitalized patients7. Since 1983 noscomial outbreaks of ESBLs producing K. pneumonia infections in Europe, 8 the United States and South America have been described9-10. Between 1990 and 1992, 5% of K. pneumoniae clinical isolates produced ESBLs11. Multidrug-resistance contributes to unfavorable clinical-outcomes, impacts the utilization of hospital resources, and increases the burden of effective infection control practice and the overall health economic cost12, 6. Thus it is an important task for the researcher to find out alternative medicine.

We have started our studies to identify ESBLs producing multidrug resistance Klebsiella species isolated from patients of National Medical College & Teaching Hospital, Nepal

MATERIALS & METHODS

Isolation and identification of Klebsiella species from different clinical laboratory samples.

Collection of clinical samples: Clinical samples which include mid stream urine, blood, and pus were collected from prescribed patients. The samples were collected and labeled in medical laboratory unit of the hospital. These samples were analyzed within 30 minutes to 1 hour of collection.

Isolation of Klebsiella species

Samples were processed according to the sample nature type. All the samples were cultured in MacConkey Agar, Blood agar plates and incubated at 37°C for 24 hours. The sample where significant pure growth of pathogen obtained was included in this study. Isolates were subcultured and colonies were screened for gram negative isolates.

MacConkey Agar media: This media is selective media for the growth of the lactose fermenting organisms in some times contaminating organisms also grows so, further identification is essential.

Identification of Klebsiella species: A series of morphological and biochemical tests were performed to identify the suspected Klebsiella species isolates. The test included Gram staining, motility, oxidase activity, catalase production, oxidation-fermentation 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

Bacterial isolates: A total of 300 non duplicate clinical isolates from various clinical specimen including urine (n=200), blood (n=45), Pus (n=55) were collected from August 2011 to March 2012 and enrolled in this study. Out of these total samples proceeds only 110 (36.67%) samples showed significant growth. Amongst these significant growth 65 (59.09%) Klebsiella species (45 Klebsiella pneumonia and 20 Klebsiella oxytoca) were isolated.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed by following Kirby Bauer's disc diffusion technique,14 as recommended by clinical and laboratory standard institute (CLSI)15. The zone of inhibition was measured and interpreted using the standard chart and organism reported as susceptible, intermediate or resistant accordingly. Different antibiotic disc used in this study were Chloramphenicol, Tetracycline, Gentamycin, Amikacin, Cefpodoxime, Cefotaxime, Ceftazidime, Ciprofloxacin, Ofloxacin. Co-trimoxazole, Nitrofurantoin,

Criterion for Multidrug Resistance: In the present study the defining criterion for an isolate to be Multidrug Resistant (MDR) was set as resistance to two or more drugs of different structural classes.

ESBL Detection methods: Each Klebsiella pneumonia and Klebsiella oxytoca isolate should be considered a potential ESBL producer if the test results are as follows.

Disk diffusion

Cefpodoxime: - ≤ 22mm

Ceftazidime: - ≤ 22mm

Cefotaxime: - ≤ 27mm

Ceftriaxone: - ≤ 25mm

Aztreonam: - ≤ 27mm

The screen is then followed by a phenotypic confirmatory test.

Double disk diffusion synergy test: Muller Hinton agar plates are prepared and inoculated with standardized inoculums to form a lawn culture,16 Augmentin disk (Amoxicillin-Clavulanate) is placed in the centre of the plate and disk containing one of the oxyimino-β lactam antibiotic is placed 30 mm from center to center from the Augumentin disk. The test organism is considered to produce ESBLs, if the zone size around the test antibiotic disc increases towards the Augumentin disc17.

RESULT

Out of total 300 specimens processed urine 200 (200/300, 66.7%) consisted the most followed by pus 55 (55/300, 18.3%), blood 45 (45/300, 15.0%). As shown in table 1 prevalence of Klebsiella infection was high in old age group followed by middle age group and adults.

Table1: Age wise distribution of ESBLs producers

Age group	Total no. of isolates	ESBLs producing *Klebsiella pneumonia* (%)	ESBLs producing *Klebsiella oxytoca* (%)
Pediatrics(1-10)	02	00 (0.0)	00(0.0)
Teenage(11-20)	04	01 (25)	00(0.0)
Adults(21-30)	15	04(26.7)	01(6.7)
Middle age(31-40)	16	05(31.2)	01(6.3)
Late middle age(41-50)	08	02(25.0)	00(0.0)
Old age(50 onwards)	20	05(25.0)	02(10.0)
Total	65(21.6)	17(26.1)	04(6.15)

Of the 300 samples processed 110(110/300, 36.7) samples showed significant growth amongst which 65(65/300, 21.7) *Klebsiella* species (45 *Klebsiella pneumonia* and 20 *Klebsiella oxytoca*) were isolated. Of which urine specimens, 69(69/200, 34.5%) showed significant growth, among which, 30(69/30, 43.4%) were *Klebsiella pneumoniae* and 14(69/14, 20.2) were *Klebsiella oxytoca*. Similarly, 18(18/45, 40.0%), and 13(13/55, 23.6%) specimens of blood and pus respectively showed growth, among which 10(10/18, 55.6%) were *Klebsiella pneumonia* and 3(3/18, 16.7%) were *Klebsiella oxytoca* isolated from blood and, 5(5/13, 38.5%) *Klebsiella pneumonia* and 3(3/13, 23.1%) *Klebsiella oxytoca* were isolated from pus (Table 2 & 3). Most of the isolates exhibited resistant to multiple commercial antibiotics and refereed as "Multidrug-resistant organisms" (MDROs). Out of the total isolated *Klebsiella pneumonia* 30(30/45, 66.7) isolates were multi drug resistant similarly, 9(9/20, 45.0) isolates of *Klebsiella oxytoca* were multidrug resistant. The majority of the ESBLs producing *K. pneumoniae* isolates were from urine specimens (17/22, 77.3%), pus (3/4, 75.0%) and blood (1/4, 25.0%) similarly the majority of the ESBLs producing *K. oxytoca* isolates were from urine specimens (3/7, 42.9%), pus (1/1, 100.0%) and (0/1, 0.0%) none of the isolates were ESBLs producers isolated from blood.

Table2: Specimens wise distributions of multidrug resistant ESBLs producing *Klebsiella pneumoniae*

Specimens (No.)	Significant growth (%)	*K .pneumoniae* (%)	MDRs (%)	ESBLs producers (%)
Urine(200)	69(34.5)	30(43.4)	22(73.3)	17(77.3)
Pus(55)	13(23.6)	05(38.5)	04(80.0)	03(75.0)
Blood(45)	18(40.0)	10(55.6)	04(40.0)	01(25.0)
Total(300)	110(36.7)	45(40.9)	30(66.7)	21(70.0)

Table3: Specimens wise distributions of multidrug resistant ESBLs producing *Klebsiella oxytoca*

Specimens (No.)	Significant growth (%)	*K .oxytoca* (%)	MDRs (%)	ESBLs producers (%)
Urine(200)	69(34.5)	14(20.2)	07(50.0)	03(42.9)
Pus(55)	13(23.6)	03(23.1)	01(33.3)	01(100.0)
Blood(45)	18(40.0)	03(16.7)	01(33.3)	00(0.0)
Total(300)	110(36.7)	20(18.2)	09(45.0)	04(44.4)

As mentioned in table 4 the *Klebsiella* species isolates showed variable result in their antibiotic sensitivity pattern against commercial antibiotic discs tested. According to the susceptibility pattern ofloxacin is the most effective antibiotics against isolated *Klebsiella* species in this region.

Table 4: Resistance rate of *Klebsiella pneumonia* and *Klebsiella oxytoca* to different antibiotics

Antibiotics class	Antibiotics (Symbol)	*K. pneumonia* (%)	*K.oxytoca* (%)
Amphenicol	Chloramphenicol (C)	28(62.3)	8(40.0)
	Tetracycline (TET)	20(44.4)	6(30.0)
Aminoglycosides	Gentamycin (G)	20(44.4)	7(35.0)
	Amikacin (AK)	19(42.2)	5(25.0)
β-lactamase (Cephalosporins)	Cefpodoxime (CPD)	24(53.3)	8(40.0)
	Cefotaxime (CTX)	26(57.8)	7(35.0)
	Ceftazidime (CTZ)	28(62.2)	7(35.0)
Quinolones	Ciprofloxacin (CIP)	28(62.2)	9(45.0)
	Ofloxacin (OF)	05(11.1)	2(10.0)
Sulphonamides & Trimethoprim	Cotrimoxazole (CO)	20(48.9)	6(30.0)
Others	Nitrofurantoin (NF)	14(31.1)	4(20.0)

DISCUSSION

The emergence and rapid spread of multidrug resistant isolates causing nosocomial infections are of great concern worldwide; among them, extended spectrum β-lactamase-producing

Enterobacteriaceae has been the subject of concern. The proportion of isolates of *K. pneumoniae* exhibiting the ESBLs phenotype has increased progressively. In this report, we describe the distribution of *Klebsiellae* speciess and their antimicrobial susceptibilities during a 7 month period. The data generally reflect the seriousness of the antimicrobial resistance among bacterial pathogens in Nepal. Globally increasing resistance trends to multiple antibiotics in *K. pneumoniae* have complicated the management of these infections. We also noted an increased resistance to the commonly used antibiotics against *K. pneumoniae* infections. More concerning was the emergence and increase in the isolation rates of ESBLs producing *K.pneumoniae* over the years. Studies from both developed and developing countries have reported an increasing trend in the isolation of ESBLs positive *K. pneumoniae* [18].

In our study, twenty one (70.0%) ESBLs producing *Klebsiellae pneumoniae* and 4(44.4%) *K. oxytoca* isolates were detected. This is very high as compared to developed countries such as 0 to 25% in USA[19] and 51% in China.[20] However, results are significantly better compared to India (86.6%)[16]. A study done at Kasturba Medical College Mangalore, [21] in their studies sixteen ESBLs producing *Klebsiella* isolates (26.66%) were detected. Overall ESBLs prevalence Elsewhere in Asia, the percentage of ESBL production in *E.coli* and *K. pneumoniae* varies like 8.5% in Taiwan [22,4] and 12% in Hong Kong[22]. Similarly, at the University Hospital of the West Indies, a tertiary care hospital in Jamaica, 18.2% of the total *K. pneumoniae* was found to be ESBLs producers while there was no ESBL producing *E. coli* [23]. A study done in a general hospital in Saudi Arabia showed that 6% of all isolates were MDR and 4.8% were positive for ESBLs[24]. Whereby antibiotic overuse, prescription of drugs with proper sensitivity test and over dosing may have created this problem in developing nations. As emphasized by various authors, prevalence of ESBLs positive strains in a particular region or even hospital is variable and is associated with frequency of treatment of bacterial infections with β-lactam antibiotics as well as with colonization of patients hospitalized for over 10 days by ESBLs positive strains.[25] In summary, high antibiotic resistance and ESBLs rates among *K. pneumoniae* towards commonly used antibiotics are the major reasons for prolonged infections, increased hospitalization, increased cost of therapy and enhanced morbidity mortality rates. Special care must be taken regarding treatment of infections especially in taken regarding treatment of infections especially in developing countries like Nepal. Moreover physician must change their prescription priorities towards alternative treatments in management of enteric infections specially *Klebsiella* spp.

Antibiotic resistance is also related to age groups because in very young age or old age groups when a patient becomes immune-compromised there are greater chances that they suffer from infections (Table 1). Such infections are of many types but in patients with low immunity it is difficult to treat such infections hence, the risk in becoming more serious or non-treatable[26]. This is the leading cause of ESBLs infection in old age as suggested by data. A study done in Pakistan also revealed that multidrug resistance was more common in the age group of 50+ in both males and females[27].

Resistance to Ciprofloxacin is high in *Klebsiella* (Table 4). We recorded 62.2% *K. pneumoniae and* 45% *K. oxytoca* isolates were resistant to Ciprofloxacin and 44.4% *K. pneumoniae and 30% K. oxytoca* isolates resistant to Tetracycline. This is in agreement with other studies[28].

Cephalosporins, particularly second and third generation cephalosporins have been used for *Klebsiella* infections[29]. In our study, 62.2%, 57.8% and 53.3% *K.pneumoniae* isolates and 40%, 35% and 35% *K.oxytoca* isolates were resistant to the third generation cephalosporin (Ceftazidime, Cefotaxime and Cefpodoxime respectively). In the study conducted in Pakistan have recorded a total of 54.3% isolates were resistant to the third generation cephalosporins (ceftazidime and cefotaxime)[30]. Study from India have recorded 84% resistance to cefotaxime[31].

In the study conducted in US 95% of the *K. pneumoniae* isolates showed resistance to at least one of the three third generation cephalosporin [3GC (ceftazidime, cefotaxime, ceftriaxone)] used for the study. 87% of the *K. pneumoniae* isolates showed resistance to all the three third generation cephalosporin antibiotics and this resistance to all the three 3GC was found to coexist with resistance to other antibiotics[32].

Aminoglycosides have good activity against clinically important gram negative bacilli[33]. Amikacin showed good activity with 47.8% *K. pneumoniae* isolates and 65% *K.oxytoca* isolates being susceptible and 45.6% *K. pneumoniae* isolates and 75% *K.oxytoca* were susceptible to gentamicin. This is lower than the study conducted in Doha Qatar. In the study done in Doha Qatar showed that all gram negative bacilli were sensitive to amikacin and resistance of GNB to gentamicin was 20%[34]. Similarly the study from India has reported 39.10% activity of amikacin and 16.7% activity of gentamicin in *Klebsiella* [35]. This may be due to increased use of amikacin and gentamicin in India and as compared to Nepal. Pattern of resistance to aminoglycosides is affected by selective pressure in different regions[36]. The observed resistance in *Klebsiella pneumoniae* to ciprofloxacin and Ofloxacin was 62.2% and 11.1% and in *K.oxytoca* the resistance is 45% and 10%. This is lower than studies conducted in India, [33, 31] and higher than those of USA[36]. We recorded 48.9% *Klebsiella pneumonia* and 30% *K. oxytoca were* resistance to co-trimoxazole. This is in contrast to data from USA, where a much lower resistance was recorded in *Klebsiella* that is 1.8% only[36]. Doxycycline and co-trimoxazole have been used for infections caused by *Klebsiella* [37-38].

CONCLUSION

Increasing prevalence of ESBL producing isolates of *K.pneumoniae*, *K.oxytoca* and emergence of extensively resistant isolates with resistance to second line antibiotics is a call for concern and mandates strict adherence to antibiotic selection and restriction policies in community and hospitals in Nepal. Prudent and potent antibiotic use will help prevent emergence and spread of antibiotic resistant strains, which in turn will benefit the patient and Nation as a whole.

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