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ORIGINAL ARTICLE

Antibiogram and Extended Spectrum Beta-lactamase (ESBL) production among *Klebsiella pneumoniae* isolated from sputum

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Abstract:

Background & Aims: *Klebsiella pneumoniae is one of the major causes of respiratory tract infection. It has been found that this organism is being increasingly resistant to broad spectrum antibiotics especially â- lactam antibiotics mediated by extended Spectrum â-lactamase (ESBL) enzymes. The present study was undertaken to determine the antibiogram and the incidence of ESBL production among Klebsiella pneumoniae strains isolated from sputum.*

Materials & Methods: *sputum specimens from patients were subjected to culture as per Clinical Laboratory Standard Institute (CLSI) guidelines. All specimens were inoculated on to Blood agar, Chocolate agar and MacConkey agar plates. Klebsiella pneumoniae were identified by the standard biochemical procedures. Detection of ESBL production by isolated Klebsiella pneumoniae strains was done by Double Disc Synergy Test which is a phenotypic confirmatory test for ESBL production. Antibiotic susceptibility testing of isolates was also done and described as per CLSI guidelines. The study was conducted from 1st August, 2014 to 31st July, 2015.*

Result: *A total of 124 Klebsiella pneumoniae strains were isolated of which 40 (32.25%) isolates were ESBL producers. Susceptibility of isolates to Cephalosporins tested except Ceftriaxone , was not satisfactory. All isolates were sensitive to Imipenem. More than 80% sensitivity was found only to Gentamicin (83.89%), Tazobactam- Piperacilin combination (82.25%) and Doxycyclin (81.45%). Moderate number of isolates were sensitive to Colistin and Ciprofloxacin.*

Conclusion: *A high prevalence of respiratory tract infections by Klebsiella pneumoniae was observed in our hospital setting and the rate of ESBL production was moderately high. Bacteria is gaining resistance to many commonly used antibiotics.*

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Introduction:

The discovery and development of antibiotics was one of the greatest advances of modern medicine. But antibiotic resistant bacteria has emerged as a threat to this advancement. Antibiotics are among the most commonly used and misused of all drugs. The inevitable consequence of this has been the emergence of antibiotic resistant pathogens[1].

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Klebsiella pneumoniae is gram negative, non motile, encapsulated, lactose fermenting bacteria belonging to Enterobacteriaceae family. They are ubiquitously present and reported worldwide[2]. In recent years *Klebsiella pneumoniae* has become an important pathogen for both nosocomial and community acquired infections. In addition to causing various extra-pulmonary infections, it is one of the predominant organism of primary pneumonia[1,2]. Extensive use of broad-spectrum antibiotics especially Penicillin and Cephalosporin in hospitalized patients has led to both increased carriage of *klebsiella pneumoniae* and the development of multidrug resistant (MDR) strains[3]. Evidence from researches have proved that multidrug resistant *Klebsiella pneumoniae* are emerging world-wide causing serious infections which are difficult to eradiate using available antibiotics. This situation is alarming in both developing as well as developed countries which has led clinicians in the state of paucity of antibiotics to treat the organism[1, 4, 5 6]. Pharmacological industries are trying to combat this by producing large number of newer antibiotics. Microorganisms develop resistance to these newer antibiotics even. In Gram negative bacteria like *Klebsiella pneumoniae*, production of Extended spectrum â-lactamase (ESBL) is perhaps one of the most important causes of such resistance pattern. ESBLs are plasmid mediated enzymes that cause hydrolysis of â- lactam antibiotics, like Penicillin, Cephalosporins, Monobactams conferring resistance to these drugs. ESBL enzymes have been identified in large number in various *Klebsiella pneumoniae* strains[3]. On the other hand plasmids carrying genes of ESBL enzymes also carry resistance genes to other antibiotics including Aminoglycosides, Sulphonamides, Trimethoprim, Tetracyclin, Ciprofloxacin[7]. As plasmids are mobile genetic material so they can carry resistance genes against a number of antibiotics between bacteria. As such ESBL producing strains are associated with resistance to other non â-lactam drugs also. Infection with such multidrug resistant bacterial strains leads to increased patient mortality and morbidity when antibiotics inactive against the strain are used[8]. Also ESBL producing organisms are threat to infection control and there is a potential for transfer of such organism to other patients[8]. The microbiology laboratory plays a central role in the decision to select a particular antibiotic by appropriate identification of causative organism of infection and by rational selection of class of antibiotic likely to work on the patient[1]. The present study was therefore conducted with a view to see the antimicrobial susceptibility profile and the prevalence of ESBL production among *Klebsiella pneumoniae* isolated from sputum.

Materials and Methods:

This prospective observational study was carried out in the Department of Microbiology, DNMCH, Dhaka for a period of one year from 1st August, 2014 to 31st July, 2015. Total 896 sputum specimens from 896 patients with suspected respiratory tract infection were collected which included both indoor and outdoor patients. For sputum collection, patients were asked to take a deep breath & then expel the expectorate directly into a sterile leak-proof plastic container. The specimens were inoculated on to Blood agar, Chocolate agar and MacConkey agar plates and incubated overnight at 37°c. *Klebsiella pneumoniae* isolates were identified by their morphology and biochemical characteristics like Gram staining, negative indole test, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose[9]. Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolates was carried out using various antimicrobial disks (shown in table I) by Kirby-Bauer disk diffusion method[10]. Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth from isolated colony of *Klebsiella pneumoniae* selected from 18-24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of Mueller – Hinton agar plate by streaking the swab over it. For even distribution of the inoculum, the swab was streaked two more times at 60° angle over the surface. After 3-5 minutes antibiotic disks were applied and pressed down to ensure complete contact with agar surface. The disks were distributed evenly to ensure a minimum distance of 24 mm from centre to centre. Within 15 minutes the plates were inverted and kept in incubator for

aerobic incubation at 37°c. The diameter of zone of inhibition for individual antimicrobial agent was measured in millimeter with the help of a ruler and described as sensitive, intermediate & resistant according to CLSI 2012 guideline[11]. Detection of ESBL: Isolated *Klebsiella pneumoniae* were tested for ESBL production by Double Disk Synergy test (DDST), which is a phenotypic confirmatory test of ESBL production. For this, a lawn culture of isolated bacteria was made on Mueller Hinton Agar and disks containing 30ìg Ceftriaxone and 30ìg Ceftazidime were placed with a disk of Amoxicillin-Clavulanic acid (20 ìg /10 ìg) in between. The distance between the disks was 30mm centre-to-centre. The plate was incubated overnight. A clear extension of the edge of any Cephalosporin inhibition zone toward the disk containing Clavulanic acid was interpreted as synergy, indicating the presence of ESBL[8].

Result:

During the study period a total of 896 sputum specimens were processed. Sputum samples from patients of all age group of both sexes were processed. 247 sputum specimens were couture positive. (Table II). Table III shows different organisms isolated from sputum specimens. *Klebsiella pneumoniae* is the most frequent isolate (46.10%), which is followed by *Pseudomonas spp.* (20.44%), *Staphylococcus aureus* (11.15%), *Moraxella catarrhalis* (9.29%) and other organisms like *E. coli, Acinetobacter spp.*, *S. pneumoniae*, *H. influenzae*, *Candida spp.* Isolation rate of *Klebsiella pneumoniae* is highest (34.69%) among 46-60 years age group patients and least (2.41%) among patients <16 years of age. 32% of *Klebsiella pneumoniae* isolates were ESBL producer. ESBL production is highest in isolates obtained from sputum specimen of patients of more than 60 years of age. From Table V we observe that *Klebsiella pneumoniae* are most sensitive to Imipenem (100% sensitivity) followed by Gentamicin (83.87%), Tazobactam+Piperacilin combination (82.25%), Doxycyclin (81.45%) and Amikacin (72.58%). It is seen that the organism is moderately sensitive to Ceftriaxone, whereas sensitivity to other Cephalosporins is not at all satisfactory. *Klebsiella pneumoniae* is moderately sensitive to Colistin and Ciprofloxacin.

Table-I

Antimicrobial disc used & their zone diameter interpretative for Klebsiella pneumoniae

Antimicrobial disc	Disc potency	S	I	R
Imipenem	10µg	≥23	20-22	≤19
Amikacin	30µg	≥17	15-16	≤14
Gentamicin	10µg	≥15	13-14	≤12
Ciprofloxacin	5µg	≥21	16-20	≤15
Ceftriaxone	30µg	≥23	20-22	≤19
Ceftazidime	30µg	≥21	18-20	≤17
Cephradine	30µg	-		- -
Cefixime	5µg	≥19	16-18	≤15
Cefuroxime	30µg	≥18	15-17	≤14
Trimethoprim+ Sulphamethoxazole				
25µg	≥16	11-15	≤10	
Doxycycline	30µg	≥14	11-13	≤10
Tazobactam+piperacilin				
10/100µg	≥21	18-20	≤17	
Colistin	10µg	≥11	-	≤10

Note: S=Sensitive, I= Intermediate, R= Resistant

Table-II

Number of culture positive specimen among different age groups

Age in years	No. of specimens	No. of culture positive specimen(%)
<16	58	12 (20.69)
16-30	266	41 (15.41)
31-45	218	68 (31.20)
46-60	234	79 (33.76)
>60	120	47 (39.17)
Total	896	247 (27.57)

Table-III

Organisms isolated from sputum specimen

Organism	Number of organism isolated
Klebsiella pneumoniae	124 (46.10)
Pseudomonas spp.	55 (20.44)
Staphylococcus aureus	30 (11.15)
Moraxella catarrhalis	25 (9.29)
Others	35 (13.02)
Total	269 (100)

Table-IV

Frequency of Klebsiella pneumoniae isolation & ESBL production in different age groups

Age in years	No. of *Klebsiella pneumoniae* isolated (%)	No. of ESBL producer (%)
<16	3 (2.41)	00 (00)
16-30	22 (17.74)	04 (18.18)
31-45	31 (25)	07 (22.58)
46-60	43 (34.69)	13 (30.23)
< 60	25 (20.16)	16 (64)
Total	124 (100)	40 (32.25)

Table-V

Antimicrobial susceptibility of isolated Klebsiella pneumoniae

Antimicrobial agents	Sensitive (%)	Resistant (%)
Imipenem	124(100)	00 (00)
Amikacin	90(72.58)	34(27.42)
Gentamicin	104(83.87)	20(16.13)
Ceftriaxone	81(65.32)	43(34.68)
Ciprofloxacin	75(60.48)	49(39.52)
Cephradine	26(20.96)	98(79.04)
Ceftazidime	45(36.29)	79(63.71)
Cefixime	71(57.25)	53(42.75)
Cefuroxime	44(35.48)	80(64.52)
Trimethoprim+ Sulphamethoxazole	24(19.35)	100(80.65)
Doxycyclin	101(81.45)	23(18.55)
Tazobactam+ Piperacilin	102(82.25)	22(17.75)
Colistin	84(67.74)	40(32.26)

Discussion

Antibiotic resistance is an important issue affecting public health drastically[12]. In case of Gram negative bacteria one important cause of antibiotic resistance is production of ESBL enzymes. During the past decade, ESBL producing *Klebsiella pneumoniae* have emerged worldwide as causative pathogen for serious infections in both hospital and community settings[1]. So, rapid detection of antimicrobial resistant organisms, especially those producing ESBL, in clinical laboratories is essential[12]. In this study an attempt was made to understand the antimicrobial sensitivity pattern and epidemiology of ESBL production of *Klebsiella pneumoniae* isolates in sputum. This study results revealed that *Klebsiella pneumoniae* was the predominant isolate in sputum constituting more than 46% of all isolated organism, followed by *Pseudomonas aeruginosa*. A previous study in Bangladesh showed predominance of *Klebsiella pneumoniae* isolation from sputum in diabetic patients, with isolation rate of 19.1%[13]. In Indian studies rate of *Klebsiella pneumoniae* isolation was in range of 10-39%[1,14,15,16], though some of these studies[15,16] revealed *S. pneumoniae* as the predominant isolate. Some patient factors like old age, smoking, concomitant illness such as COPD and Diabetes impair pulmonary defense and predispose to infection by Gram negative bacilli like *Klebsiella pneumoniae*[16]. This may be the reason of our finding. Infact, we found *Klebsiella pneumoniae* infection was most predominant among 46-60 years age group patients. The occurrence of ESBL production among clinical isolates of *Klebsiella pneumoniae* vary greatly world wide and geographically[12]. We found 32% of *Klebsiella pneumoniae* isolates were ESBL producer. A retrospective study in Saudi Arabia (year 2004-2005) reveals this rate as 13.7% and 3.1% among indoor & outdoor specimens respectively[4]. On the year 2013 in Europe, the rate of ESBL positive *Klebsiella pneumoniae* was 18.4%[17]. On contrary much higher rate of ESBL positive *Klebsiella pneumoniae* isolates were found in a tertiary care hospital in tehran, Iran (77%)[18]. Our finding of most frequent ESBL production in patients at the higher extreme of age is also in contrast to that study where high prevalence of ESBL was found in patients at the lower extreme of age. Prolonged hospital stay, poor nutritional status, previous use of broad spectrum antibiotics may be the risk factors for the high prevalence of ESBL among this age group patients as has been reviewed by Paterson & Bonomo[8]. Based on our in vitro findings, Imipenem was the most effective antibiotic against *Klebsiella pneumoniae* (100% sensitivity). 100% sensitivity to Imipenem was found in a previous study in Bangladesh also[19] and even ESBL producing strains were reported to be 100% sensitive to Imipenem by different research groups like Jones et al[20]. Many investigators prefer Imipenem and Meropenem as drugs of choice for life- threatening infections due to ESBL producing

Enterobacteriaceae. However, to preserve the therapeutic value, the use of these drugs should be restricted[18]. Rather based on institutional pattern of susceptibility results, Tazobactam-Piperacilin combination, Fluoroquinolones or an Aminoglycoside would be preferable. In fact, with more than 80% sensitivity we found Gentamicin (83.89%), Tazobactam- Piperacilin combination (82.25%) and Doxycyclin (81.45%) to be moderately active against *Klebsiella pneumoniae* in our study. On the other hand we found only 60% isolates were sensitive to Ciprofloxacin. Such susceptibility pattern to Ciprofloxacin was comparable to previous studies[1,18,19]. Report has shown a close association between ESBL production and Ciprofloxacin resistance[3]. Though we found moderate activity of Gentamicin, some studies showed much less activity [1,2,18]. A previous study in Bangladesh also showed lesser sensitivity to Gentamicin[19]. In contrast to our finding of moderate sensitivity to Tazobactam- Piperacilin combination (82.25%), studies from Iran[18] and India[21] showed striking resistance to this drug. In fact, it is to be noted that therapeutic failure with this antibiotic combination have already been documented[3] and thus in vitro susceptibility may not necessarily predict in vivo efficacy. A moderately higher resistance to Amikacin (27.12%) and Colistin (32.20%) was observed in this study. There are reports covering high levels of resistance of *Klebsiella pneumoniae* to these antibiotics in other studies also[2]. But a study from India showed more than 92% sensitivity to Amikacin[1]. We found Ceftriaxone has better sensitivity (more than 65%) among Cephalosporins. Sensitivity to other Cephalosporins like Cephradin, Ceftazidime and Cefuroxime was disappointing. This finding is in accordance with previous studies [1,17]. This high resistance rate may be due to the production of â-lactamase enzymes which cause the hydrolysis of â- lactam rings resulting in inactivation of â-lactam drugs. In the present study with a sensitivity rate of as low as 19%, Trimethoprim+ Sulphamethoxazole combination has shown to be of no value in the treatment of infections by *Klebsiella pneumoniae*.

As the available treatment options are becoming limited, antibiotic control policies together with implementation of infection control measures remain of high importance. Due to the changing nature of ESBL enzymes, clinicians should be familiar with the clinical significance of these enzymes and clinical laboratories should adopt a technique most appropriate to them for their detection.

Conclusion:

High antibiotic resistance of *Klebsiella pneumoniae* towards commonly used antibiotics are the major reason for prolonged infections, increased hospitalization, increased cost of therapy and enhanced morbidity and mortality rates. Regular surveillance of antibiotic susceptibility pattern may help to overcome the indiscriminate use of antibiotics which is the major cause of emergence of drug resistance among pathogens and to develop antibiotic policies. The data of this study may be used to determine trends in antimicrobial susceptibility to formulate local antibiotic policy and thus may assists clinicians in the rational choice of antibiotic therapy.

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