Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 912-923

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 912-923**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.507.103

Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-Lactamse Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated at a Tertiary Care Institute in North West Region of Rajasthan, India

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Keywords

ESBL, beta-lactamases, resistance, *Escherichia coli*, *Klebsiella pneumoniae*

Article Info

Accepted:
25 June 2016
Available Online:
10 July 2016

A B S T R A C T

The incidence of extended spectrum beta lactamase producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options available.8,16Therefore we conducted this study to determine the prevalence and antibiotic susceptibility pattern of ESBL producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates in the north west region of the state, as the prevalence and antimicrobial susceptibility of ESBL-producing pathogens varies from one region to another. This study was carried out in the Department of Microbiology, Sardar Patel Medical college, Bikaner from April 2009 to May 2010 on a total of 700 clinical isolates including 520 *Escherichia coli* and 180 *Klebsiella pneumoniae*. In the present study ESBL production was noticed in 65.57% isolates with maximal incidence in *Escherichia coli* (66.92%) followed by *Klebsiella pneumoniae* (61.67%) isolates. The antimicrobial resistance was significantely higher in ESBL producer than non-ESBL producer. Among ESBL producer high rate of resistance to various antibiotics was seen (cefotaxime, ceftazidime, ceftriaxone, norfloxacin, cotrimoxazole). These high rates of resistance to various antibiotics may be due to various factors like-plasmid mediated transmission of ESBLs, poorly directed therapy and over-the-counter sales of antibiotics.

Introduction

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in the community and hospital settings. β-lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by β-lactamases, but latter on *Klebsiella* spp. was found to be resistant to cephalosporin. The mechanism of this resistance was production of extended spectrum β-lactamases (ESBLs) (Chaudhary *et al*., 2004).

Over the last 20 years, many new β-lactam antibiotics have been developed that were

specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to that class of the drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β-lactamase. One of these new classes was the oxyimino-cephalosporins, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s. Not surprisingly, resistance to these expanded-spectrum β-lactam antibiotics due to β-lactamases emerged quickly (Bradford, 2003).

Extended spectrum beta-lactamases (ESBLs) represent a major group of of β-lactamases currently being identified world wide in large numbers, most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli* but also occur in other gram negative bacteria (Agrawal *et al*., 2008; Kumar *et al*., 2006; Rodrigues *et al*., 2004).

ESBLs are derivative of common beta-lactamases that have undergone one or more amino acid substitution near the active site of enzyme, thereby increasing their affinity and hydrolytic activity against third generation cephalosporins and monobactams. However these plasmid mediated enzyme have no detectable activity against carbapenems (Kader *et al*., 2005; Shobha *et al*., 2009; Bishara *et al*., 2005; Briggs *et al*., 2005).

Being plasmid mediated they are easily transmitted among members of Enterobacteriace thus facilitating the dissemination of resistance not only to β-lactam but to other commonly used antibiotics such as quinolones and aminoglycosides.

ESBLs are specifically inhibited by β-lactamase inhibitors like clavulanic acid, and this property is commonly utilized for the detection and confirmation of ESBLs in the laboratory (Agrawal *et al*., 2008; Tsering *et al*., 2009).

As no enough study has been undertaken on prevalence and antibiotic susceptibility pattern of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* in the state of Rajasthan, with the prevalence and antimicrobial susceptibility of ESBL-producing pathogens varying from one region to another, therefore we conducted this study to determine the prevalence and antibiotic susceptibility pattern of ESBL producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates in the north west region of the state.

Materials and Methods

This study was carried out in the Department of Microbiology, Sardar Patel Medical college, Bikaner from April 2009 to May 2010 to detect Extended spectrum beta-lactamase producing strains of *Escherichia coli* and *Klebsiella pneumoniae* in a total of 700 clinical isolates. 520 *Escherichia coli* and 180 *Klebsiella pneumoniae* were isolated from various clinical specimens such as urine, blood, pus, wound, sputum and other respiratory tract specimen, body fluids, high vaginal swab, stool, semen, prostatic secretion and CSF etc. received from patients attending various outpatient departments, admitted in wards at P.B.M. hospital and associated group of hospitals.

Inclusion Criteria

All consecutive, non-duplicate isolates of *Escherichia coli* and *Klebsiella pneumoniae* were collected from various clinical specimens.

Medical and demographic data of the patients were collected using a questionnaire. Data recorded were: demographic characteristics (age, gender); underlying lung diseases, acute suppurative otitis media, diabetes mellitus, chronic renal failure, nephrotic syndrome, connective tissue disease, malignancy, immuno-compromisation, septicemia, burn, eclampsia, puerperal sepsis. pyometra, peritonitis, fractures etc. Presence of intravascular or urinary catheters; prolong hospitalization (> one week); history of intensive care unit (ICU) stay; nursing home residency; being on mechanical ventilation; prior antibiotic use ; and recent surgery (within one month); poor nutritional status; haemodialysis etc. were also noted.

Exclusion Criteria

Isolation of three organism types with no predominating organism and repeated isolate from same patient were excluded from this study.

The samples were processed for the identification of organisms on the basis of conventional microbiological procedures and were screened for ESBLs. All isolates were cultured on Mac Conkey Agar and Blood Agar and urinary isolates on Hichrome UTI media (obtained from Hi-Media, Mumbai, India) also and incubated at 37°C for 24 hrs. They were identified to species level by their characteristic appearances on the media, Gram's stain, Oxidase test, Motility and the pattern of the biochemical reactions. Flow chart was used for preliminary identification of organisms as shown in chart 1

Antimicrobial susceptibility of the various isolates was performed as per the Clinical and Laboratory Standards Institute (CLSI) guidelines by the Kirby Bauer disk diffusion method on Mueller Hinton agar with a 0.5 McFarland's turbid inoculum as per CLSI recommendation (CLSI).

The zone of inhibition was measured and reported as Susceptible, Intermediate or Resistant according to standard zone size. For statistical purposes, data were categorized as susceptible and non-susceptible (including intermediate and resistant groups).

Control: *Escherichia coli* ATCC 25922 & *Staphylococcus aureus* ATCC 25923

Following antibiotic discs (obtained from Hi-Media, Mumbai, India) were used for antimicrobial sensitivity testing: Amikacin (30 µg), Amoxicillin+Clavulanic acid (20/10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cotrimoxazole (1.25/23.75 µg), Doxycycline hydrchloride (30 µg), Gatifloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg)

The inhibition zone diameter was measured in mm with the help of a special measuring scale and results recorded for each isolate separately as Sensitive, resistant, intermediate (S,R,I) according to the given standard zone size below for enterobacteriaceae.

Screening and confirmation of ESBL production by the phenotypic confirmatory (combination disc method) test was done as per the guidelines recommended by CLSI. Control strains, *Escherichia coli* ATCC 25922 (Beta-Lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used for quality control.

Initial Screening Tests (Agrawal *et al*., 2008; Winn *et al*., 2006): Inoculum was prepared by suspending few colonies of test

strain in 0.9 % sterile saline and turbidity was adjusted to 0.5 Mc Farland turbidity tube. A lawn culture was made from the inoculum using a sterile cotton swab on the surface of Mueller-Hinton agar medium and Ceftazidime (30µg) and Cefotaxime (30µg) discs were applied with all sterile precautions. The plates were incubated for 18-24 hours at 37°C.

According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg) and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

Phenotypic confirmatory test with combination disc: In this test, a third-generation cephalosporin, Ceftazidime (30µg) alone and in combination with clavulanic acid (10 µg) was used. Both the discs were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured.

When there was an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disc alone, was considered as confirmed ESBL producer.

Results and Discussion

Out of 700 isolates of *Escherichia coli* and *Klebsiella pneumoniae*, 459 (65.57%) were found ESBLs producers. Out of 520 *Escherichia coli* isolates 348 (66.92%) were found ESBLs producers and of the 180 *Klebsiella pneumoniae* isolates 111 (61.67%) were found ESBLs producers.

ESBL producing *E. coli* & *K. pneumoniae* strains were most frequently recovered from urine 62.36% (217/348) , 32.43% (36/111) followed by sputum & respiratory tract specimens 15.23% (53/348), 27.03% (30/111) respectively.

During past 60 years, bacteria have demonstrated a remarkable ability to resist almost every antibiotic that has been developed.(11,12,13) Extended spectrum beta-lactamases (ESBLs) represent a major group of beta-lactamases, currently being identified worldwide in large numbers, most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli*.

The present study was conducted on 700 clinical isolates (520 *Escherichia coli* and 180 *Klebsiella pneumoniae*) recovered from various clinical specimens, from all ages and both sexes attending various outpatient departments and admitted in wards at P.B.M. hospital and associated group of hospitals.

ESBL production was noticed in 65.57% isolates with maximal incidence in *Escherichia coli* (66.92%) followed by *Klebsiella pneumoniae* (61.67%). This high incidence of ESBL production agrees with Mohanty S *et al.,* (2003)(18) where ESBL strains were observed in 71.5% isolates (60.7% *Escherichia coli* and 78.7% *Klebsiella pneumoniae*), Rajini E *et al*., (2008)(Rajini *et al*., 2008) where ESBL production was noticed in 57% isolates (*E. coli* 72% followed by *Klebsiella pneumoniae* 38.4%), Sasirekha B *et al*., (2010)(Sasirekha *et al*., 2010)where 53.9% isolates were found to be ESBL producer (61.1% *E.coli* followed by *K. pneumoniae* 40.6%).

ESBL-producing *E. coli* strains were recovered most frequently from urine

(62.36%) followed by sputum & Respiratory tract specimens (15.23%) and pus & other wound discharges (13.79%)(Table -2). Similar observations were made by Sasirekha *et al*., (2010) where ESBL producing *E. coli* were most frequently obtained from urine (76%) followed by sputum (18.7%) and pus (5.2%), Wani K A *et al*., (2009) ESBL producing *E. coli* isolates were most commonly recovered from urine (72.9%) followed by pus 9.3% and blood 7.6% , Agarwal P *et al*., (2008) with ESBL-positive isolates highest among urinary isolates (70%) followed by pus (22.5%) and blood (5%).

ESBL-producing *K. pneumoniae* strains were recovered most frequently from urine (32.43%) followed by sputum & Respiratory tract specimens (27.03%), pus & other wound discharges (26.13%) and blood (6.31%)(table.3). Similar observations were made by Sasirekha *et al*., (2010) who reported maximum incidence in urine (60%) followed by sputum (28.5%) and pus (11.4%), Kusum *et al.*, (2004) reported urine (44%) followed by sputum (42%) and blood (14%). In other studies, Waiwarawooth *et al*., (2006) reported higher incidence of ESBL production in sputum (44.69%), followed by urine (21.60%), pus (18.24%) and blood (10.28%) and El Astal *et al*., (2008) higher incidence in pus (48.4%) followed by urine (25.8%), sputum (17.2%) and blood (5.7%).

In the present study ESBL producing *E. coli* isolates were found to be 100% susceptibile to imipenem & meropenem similar to observations made by Agarwal *et al*., (2008), El Astal *et al*., (2008), Fazlay Bazzaz *et al*., (2009), Wani *et al*., (2009) and Sasirekha *et al*., (2010). 60.35% ESBL producing *E. coli* isolates were found to be susceptible to amikacin, with variable susceptibility observed in other studies as Bishara *et al*., (2005) 75%, El Astal *et al*., (2008) 77.8% and Wani *et al*., (2009) 78.2%. ESBL producing *E. coli* isolates from urine showed 69.12% susceptibility to nitrofurantoin slightly on lower side as compared to other studies Puberza *et al*., (2007) 92.3% and Wani *et al*., (2009) 91.5% . Susceptibility to gatifloxacin was seen in 51.44 % of ESBL producing *E. coli* isolates while Wani *et al*., (2009) observed 64.1% susceptibility. 20.11% susceptibility to doxycycline hydrochloride was reported which agrees with El Astal *et al*., (2008) reporting 22.2% susceptibility. Cotrimoxazole susceptibility was seen in 11.21% of ESBL producing *E. coli* isolates with variation in other studies like Tsering DC *et al*., (2009) 21.52%, El Astal *et al*., (2008) 22.2% and Shobha *et al*., (2007) 42%. 32.5% ESBL producing *E. coli* isolates were found to be susceptible to amoxicillin+clavulanic acid similar to the studies by Bishara *et al*., (2005) 33%, Gupta *et al*., (2007) 31%, El Astal *et al*., (2008) 22.2%. High resistance to norfloxacin of 0.92% was seen in ESBL producing *E. coli* isolates from urine with other studies showing higher susceptibility. High resistance was also shown for IIIrd generation cephalosporin with only 0.86% and 0.29% ESBL producing *E. coli* being susceptibile to ceftriaxone and cefotaxime, respectively while all strains being resistant to ceftazidime. This corresponds to study of El Astal *et al*., (2008) where none of the tested isolates were susceptible to cephalosporins and Wani *et al*., (2009) who reported 0.8%, 0.8%, 2.5% susceptibility of cefotaxime, ceftazidime and ceftriaxone respectively.

ESBL producing *K. pneumoniae* isolates were found to be 100% susceptible to imipenem & meropenem similar to observations made by Gupta *et al*., (2007), El Astal *et al*., (2008), Fazlay Bazzaz *et al*., (2009), Mehrgan *et al*., (2010). 55.86%

ESBL producing *K. pneumoniae* isolates were found to be susceptibile to amikacin which agrees with observations made by Agarwal *et al*., (2008) 44%, and El Astal *et al*., (2008) 67.5%. ESBL producing *K. pneumoniae* isolates from urine showed 66.67% susceptibility to nitrofurantoin which was also observed by Mehrgan *et al*., (2010) 60% and Puberza *et al*., (2007) 72.2% respectively. 63.03% susceptibility to gatifloxacin was seen in ESBL producing *K. pneumoniae* isolates. 27.03% of ESBL producing *K. pneumoniae* isolates were susceptibile to doxycycline hydrochloride similar to El Astal *et al*., (2008) who reported 20% susceptibility. 5.41% susceptibility to cotrimoxazole was observed in ESBL producing *K. pneumoniae* isolates with marked variation in other studies like Brigg *et al*., (2005) 5%, Tsering *et al*., (2009) 21.52% and Mehrgan *et al*., (2010) 47.1%. High resistance to amoxicillin+clavulanic acid was seen with only 2.7% of ESBL producing *K. pneumoniae* isolates being susceptible . Similar observations were made by Mehrgan *et al*., (2010) 4.5% and Bishara *et al*., (2005) 5% respectively. ESBL producing *K. pneumoniae* isolates were found to be 8.33% susceptibile to norfloxacin similar to Shobha *et al*., (2007) who reported 6% susceptibility. High resistance to IIIrd generation cephalosporin, with only 0.9%, 1.8% and 4.5% ESBL producing *K. pneumoniae* isolates being susceptibile to cefotaxime, ceftazidime and ceftriaxone, respectively. Similar observations were made by El Astal *et al*.,(2008) where none of the tested isolates were susceptible to cephalosporins and Mehrgan *et al*., (2010) who reported 0.6%, 1.9%, 0.6% susceptibility of cefotaxime, ceftazidime and ceftriaxone respectively while other studies showed variable higher susceptibility.

Among ESBL producer high rate of resistance to various antibiotics was seen (cefotaxime, ceftazidime, ceftriaxone, norfloxacin, cotrimoxazole). These high rates of resistance to various antibiotics may be due to various factors like-plasmid mediated transmission of ESBLs, poorly directed therapy and over-the-counter sales of antibiotics.

Association of Risk Factors with Esbl Production

In the present study total in 459 ESBL producing isolates, 144 (31.37%) were isolated from patients with one or other type of serious illnesses followed by 112 (24.4%) in old age patients, 93 (20.26%) with prior exposure to antibiotics, 74 (16.12%) with history of recent surgery, 68 (14.81%) with invasive medical devices (urinary/arterial catheterization, central venous lines), 31 (6.75%) with poorly nourished patient (anaemia, malnutrition), 21 (4.58%) were associated with patient admitted in ICU, 09 (1.96%) with intubated and mechanically ventilated patients and 08 (1.74%) with urinary tract disease. All isolates recovered from ICU patients (21) and from intubated and mechanically ventilated (09) patients showed ESBL production.

Foley catheter, intravenous catheter, central venous catheter, intubation, surgery and mechanical ventilation were found as the risk factors for the acquisition of *E. coli* and *K. pneumoniae* with ESBLs Ozqunes *et al*., (2006).

Chart.1 Flowchart for identification of Gram-negative lactose fermenting organisms

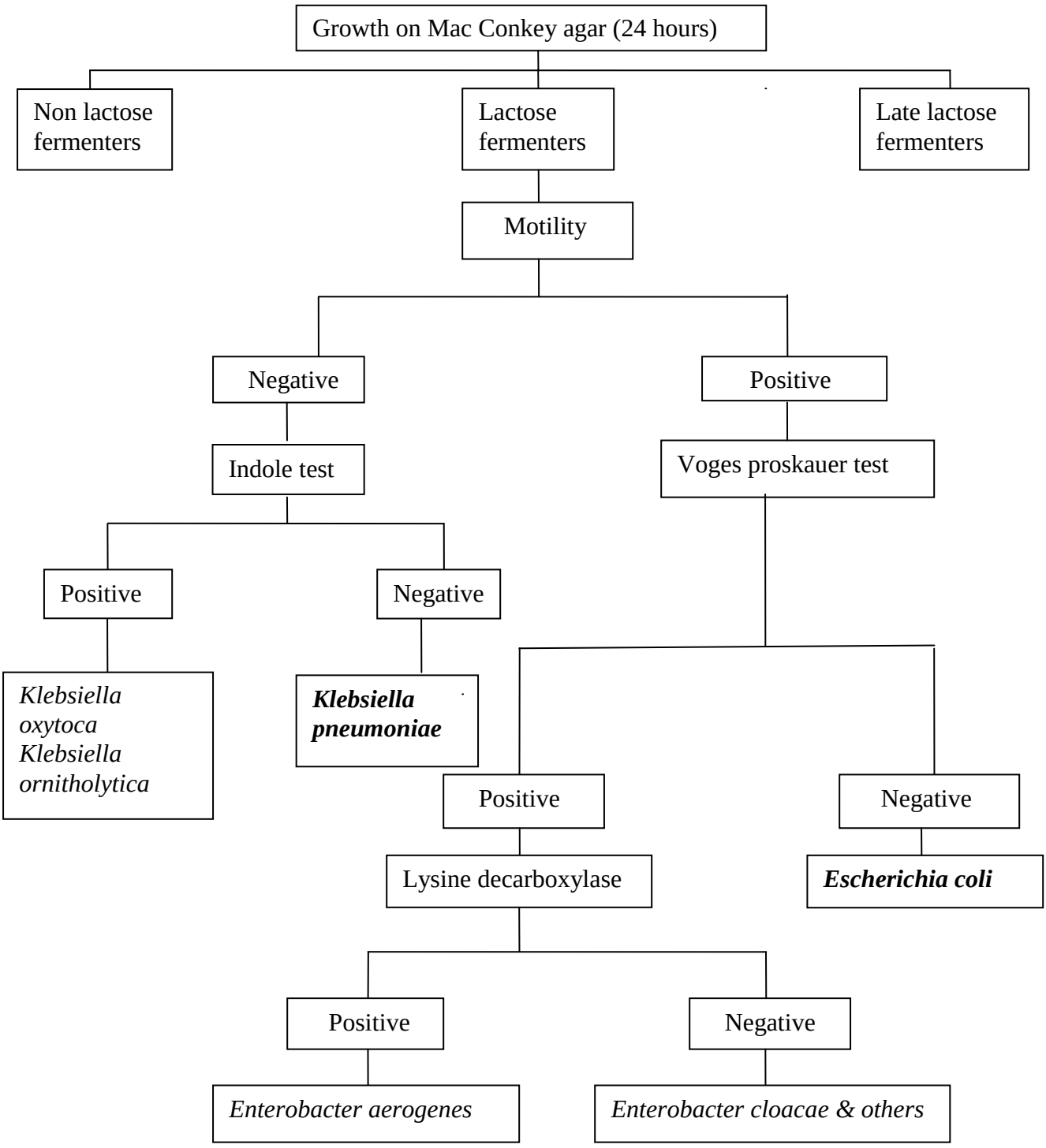

Table.1 Distribution of ESBL Producing Escherichia coli and Klebsiella pneumoniae.

S. No	Organism	Total numbers	ESBL producers	Percentage (%)
1	*Escherichia coli*	520	348	66.92 %
2	*Klebsiella pneumoniae*	180	111	61.67 %
TOTAL		700	459	65.57 %

Table.2 Distribution of ESBL producing and non ESBL producing Escherichia coli isolates from various clinical specimens

S.N.	Clinical sample	Total *E.coli* isolate (%)	*E.coli* ESBL positive isolate (%)	*E.coli* ESBL negative isolate (%)
1.	Urine	343 (65.96%)	217 (62.36%)	126 (73.26%)
2.	Sputum & Respiratory tract specimens	59 (11.35%)	53 (15.23%)	06 (3.49%)
3.	Pus & other wound discharges	65 (12.50%)	48 (13.79%)	17 (9.88%)
4.	High vaginal swab	21 (4.04%)	11 (3.16%)	10 (5.81%)
5.	Stool	20 (3.85%)	09 (2.59%)	11 (6.40%)
6.	Body fluids	07 (1.35%)	06 (1.72%)	01 (0.58%)
7.	Blood	02 (0.38%)	01 (0.29%)	01 (0.58%)
8.	Others	03 (0.58%)	03 (0.86%)	00(0.00%)
9.	TOTAL	520	348	172

Table.3 Distribution of ESBL producing and non ESBL producing Klebsiella pneumoniae isolates from various clinical Specimens

S.N.	Clinical sample	Total *K. pneumoniae* isolate (%)	*K. pneumoniae* ESBL positive isolate (%)	*K. pneumoniae* ESBL negative isolate (%)
1.	Urine	75 (41.67%)	36 (32.43%)	39 (56.52%)
2.	Sputum & Respiratory tract specimens	50 (27.78%)	30 (27.03%)	20 (28.99%)
3.	Pus & other wound discharges	35 (19.44%)	29 (26.13%)	06 (8.70%)
4.	Blood	08 (4.44%)	07 (6.31%)	01 (1.45%)
5.	Body fluids	05 (2.78%)	04 (3.60%)	01 (1.45%)
6.	High vaginal swab	04 (2.22%)	03 (2.70%)	01 (1.45%)
7.	Stool	01 (0.56%)	01 (0.90%)	00 (0.00%)
8.	Others	02 (1.11%)	01 (0.90%)	01 (1.45%)
9.	TOTAL	180	111	69

Table.4 Antimicrobial Susceptibility pattern of ESBL producing and non- ESBL producing E .coli

S.N.	Antibiotics	*E. coli* ESBL positive isolate (%)	*E. coli* ESBL negative isolate (%)
1.	Amikacin (AK)	210 (60.35%)	139 (80.81%)
2.	Amoxicillin+Clavulanic acid (AC)	113 (32.5%)	105 (61.05%)
3.	Cefotaxime (CE)	01 (0.29%)	169 (98.26%)
4.	Ceftazidime (CA)	00 (0.00%)	170 (98.84%)
5.	Ceftriaxone (CI)	03 (0.86%)	167 (97.09%)
6.	Cotrimoxazole (CO)	39 (11.21%)	46 (26.74%)
7.	Doxycycline hydrochloride (DO)	70 (20.11%)	55 (31.97%)
8.	Gatifloxacin (GF)	179 (51.44%)	122 (70.93%)
9.	Imipenem (I)	348 (100%)	172 (100%)
10.	Meropenem (MR)	348 (100%)	172 (100%)
11.	Nitrofurantoin (NF)*	150 (69.12%)	108 (85.71%)
12.	Norfloxacin (NX)*	02 (0.92%)	32 (25.40%)

* Norfloxacin and nitrofurantoin were tested against urinary isolates only.

Table.5 Antimicrobial Susceptibility pattern of ESBL producing and non-ESBL producing K. pneumonia

S. N.	Antibiotics	*K. pneumoniae* ESBL positive isolate (%)	*K. pneumoniae* ESBL negative isolate (%)
1.	Amikacin (AK)	62 (55.86%)	55 (79.71%)
2.	Amoxicillin+Clavulanic acid (AC)	03 (2.70%)	33 (47.83%)
3.	Cefotaxime (CE)	01 (0.90%)	68 (98.55%)
4.	Ceftazidime (CA)	02 (1.80%)	67 (97.10%)
5.	Ceftriaxone (CI)	05 (4.50%)	65 (94.20%)
6.	Cotrimoxazole (CO)	06 (5.41%)	25 (36.23%)
7.	Doxycycline hydrochloride (DO)	30 (27.03%)	30 (43.47%)
8.	Gatifloxacin (GF)	70 (63.06%)	57 (82.60%)
9.	Imipenem (I)	111 (100%)	69 (100%)
10.	Meropenem (MR)	111 (100%)	69 (100%)
11.	Nitrofurantoin (NF)*	24 (66.67%)	34 (87.18%)
12.	Norfloxacin (NX)*	03 (8.33%)	18 (46.15%)

*Norfloxacin and nitrofurantoin were tested against urinary isolates only.

Table.6 Association of risk factors with ESBL production

S.N.	Risk factors	ESBL positive isolate (%)	ESBL negative isolate (%)
1.	Prior exposure to antibiotics	93 (20.26%)	06 (2.49%)
2.	ICU stay	21 (4.58%)	00 (0.00%)
3.	Severe illness	144 (31.37%)	17 (7.05%)
4.	Invasive medical devices (urinary/arterial catheterization, central venous lines)	68 (14.81%)	15 (6.22%)
5.	Intubation & Mechanical ventilation	09 (1.96%)	00 (0.00%)
6.	Recent surgery	74 (16.12%)	13 (5.39%)
7.	Old age (>60 year)	112 (24.4%)	59 (24.48%)
8.	Poor nutritional status	31 (6.75%)	5 (2.07%)
9.	Urinary tract pathology (Polycystic kidney disease, Renal/Bladder calculi, Stricture urethra)	08 (1.74%)	02(0.83%)

Lautenbach (2001) *et al*., observed that the prior antibiotic use and longer duration of hospital stay were the risk factor for ESBL-producing E. coli or K. pneumoniae infection.

As ESBL-positive isolates occur in large number of patients and show false susceptibility to expanded-spectrum cephalosporins in standard disk diffusion test, therefore care should be taken by not giving cephalosporins and aztreonam, regardless of the routine susceptibility test results.

In conclusion, *Escherichia coli* (66.92%) were found to be more extended spectrum β-lactamase producer than *Klebsiella pneumoniae* (61.67%), this may be peculiar to Indian subcontinent. The antimicrobial resistance was significantely higher in ESBL producer than non-ESBL producer.Among Extended spectrum β- lactamase producing isolates imipenem and meropenem was most sensitive followed by nitrofurantoin, amikacin and gatifloxacin. Least sensitive antibiotic were cephalosporins (cefotaxime, ceftazidime, ceftriaxone). The risk factors involved in acquisition of ESBL production in the present study were:- severe illness, prior exposure to antibiotics, recent surgery, invasive medical device (urinary catheterization), poor nutritional status, ICU stay, intubation and mechanical ventilation respectively. Therefore clinician should take adequate measures in treating the patients with risk factors.

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How to cite this article:

Bhagirath Ram Bishnoi, Abhishek Binnani and Priyanka Soni Gupta. 2016. Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-Lactamse Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated at a Tertiary Care Institute in North West Region of Rajasthan, India. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 912-923.
doi: http://dx.doi.org/10.20546/ijcmas.2016.507.103