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Prevalence of Extended Spectrum Beta lactamases (ESBLs) producing *Klebsiella pneumoniae* from Urine samples in Namakkal District

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Abstract

The aim and objective of the present study was to find out the antibiotic susceptibility patterns and to determine the prevalence of ESBL producing *Klebsiella pneumoniae* in Urine samples. Totally 150 Urine samples were collected from in and around the Namakkal district and identified by standard biochemical methods. Antibiotic sensitivity testing was carried out using the Kirby Bauer's disc diffusion method. The *Klebsiella pneumoniae* showed resistances to third generation Cephalosporins were subjected to phenotypic confirmatory test for ESBL production. Among the 150 samples, 70 (47%) isolates were identified as a *Klebsiella pneumoniae* and 30 (43%) isolates were found to be positive for ESBL production by combination disc method and Double Disc Synergy Test (DDST). In Antibiotic sensitivity test, 70 isolates of *Klebsiella pneumoniae* were subjected in that 100 % resistance was shown to Ampicillin, Cefotaxime, and tetracycline. Of Amikacin (89%), Chloramphenicol (79%), Co-trimoxazole (17%), Ciprofloxacin (7%) and sensitive to Ciprofloxacin(93%), Co-trimoxazole (83%), Chloramphenicol(21%), and Amikacin (11%). The prevalence of ESBL producing *Klebsiella pneumoniae* was found to be high in routine screening of ESBLs when compared with other bacteria in laboratory identification.

Key words: *Klebsiella pneumoniae*, ESBLs, DDST, Cephalosporins, Multidrug resistance

Introduction

A urinary tract infection (UTI) (also known as acute cystitis or bladder infection) is an infection that affects part of the urinary tract. Urinary Tract Infection (UTI) is classified into disease categorized by the site of infection: cystitis [the bladder], pyelonephritis [the kidney], and Bacteraemia [the bacteria]. Every year's 6-8 million cases of uncomplicated UTI occur in the United States and 130-175 million cases globally [Mohammed *et al.*, 2014]. Urinary tract infections occur more commonly in women than men, with half of women having at least one infection at some point in their lives. Recurrences are common. Risk factors include female anatomy, sexual intercourse and family history.*Klebsiella pneumoniae* are gram negative bacilli belonging to family enterobacteriaceae. *E. coli* mainly causes urinary tract infection, diarrhoea, pyogenic infection, sepsis and causes UTI, pneumonia, other pyogenic infectionsandrarely diarrhea (Ananthanarayan et al., 2005).

The increase in the rates of antibiotic resistance is becoming a major cause for concern in isolates of the *Enterobacteriaceae* family (Dongeun Yong *et al.,* 2009). Resistance to anti-infective agents is worldwide, both in developed and developing countries. Antimicrobials have been used successfully for over 6 decades, but genes expressing resistance to them have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration. (Isturiz and Carbon, 2000).

Over the last year many new β-lactam antibiotic, specially designed to resist known β-lactamase have been developed.However almost invariably new β-lactamase have emerged to combat each new class of β-lactams.Plasmid-mediated, extended-spectrum β-lactamases (ESBLs) emerged in gram-negative bacilli in Europe in the 1980s. ESBLs, so named because of their extended spectrum of activity, confer resistance to third- and fourth-generation cephalosporins (eg, ceftriaxone, cefotaxime, ceftazidime, cefepime and cefpirome) and monobactams (eg, aztreonam), in addition to the earlier generation cephalosporins and penicillins. ESBLs are inhibited in vitro by β-lactamase inhibitors such as clavulanic acid and tazobactam. Our aim was to get acquaintance with the predominant classes of ESBLs, which are prevailing in *Klebsiella*

pneumoniae in Namakkal area. We also investigated the antibiotic sensitivity pattern of these MDR *Klebsiella pneumoniae* meticulously to understand the general drug resistance pattern of them.

Materials And Methods

Collection of Urine Samples

A total of 150 Urine samples were collected around the Namakkal district, over a period of March 2015 to August 2015. All the samples were collected according to the CLSI guidelines and care should be taken within two hours the samples were transported to laboratory with ice pack were included in the study

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Processing of the Samples

A loopful of Urine samples were streaked on Nutrient and MacConkey agar. The suspected colony was once again streaked on CLED and Blood agar for the confirmation to study their cultural characteristics. A single isolated colony was considered for further studies and identification was done by using standard procedures. Gram's staining, morphological and cultural, biochemical test was performed (collee *et al.,* 1996).

Antibiotic Sensitivity Test

The resistance to one or more third generation antibiotics (ceftriaxone, and cefexime, etc.,) prompted us to detect ESBL producers, the common mechanism of beta lactam resistance. Antibiotic disc Ampicilin (10 mcg/disc), Co-trimoxazole (25mcg/disc), Gentamycin (10 mcg /disc), Ciprofloxacin (5 mcg /disc), Cefotaxime (30 mcg/disc), Ceftriaxone (30mcg/ disc), Cefixime (5 mcg/ disc), Amoxicillin (10mcg/disc), Chloramphenicol (30 mcg/ disc), Amikacin (30 mcg/ disc), Nalidixic acid (30 mcg/disc), norfloxacin (10mcg/disc), tetracycline (30 mcg/disc) (Renuka Rampure *et al.,* 2013).

Detection of ESBL by Phenotypic Methods

Combination disc method

A standardized suspension of the isolate was plated onto Mueller Hinton agar using the antimicrobial discs Ceftazidime (CAZ) (30µg) and Ceftazidime/clavulanic acid (CAC) (30/10µg). After incubation, the zone of inhibition around each of the discs is measured. An increase of zone Diameter ≥ 5mm for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone indicates positive for ESBL production (CLSI, 2010).

Detection of ESBL of Double disc synergy test (DDST)

The isolated organisms were inoculated onto peptone water and incubated at 37°C for 4-6 hours. The turbidity of growth was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Muller-Hinton agar plate by lawn culture. A disc containing amoxyclav (amoxicillin + clavulanicacid) was placed at the center of the plate. Ceftazidime, cefotaxime, ceftriaxone, aztreonam, Cefpodoxime were placed with the inter disc distance (edge to edge) of 15 mm from the amoxyclav disc. The plates were incubated at 37°C for overnight. Enhancement of zone of inhibition towards amoxyclav by any one of these drugs such as ceftazidime, cefotaxime, ceftriaxone, aztreonam or Cefpodoxime was considered as a positive result. (Eshwar, et *al.*, 2011).

ESBL – screening test

The *Klebsiella pneumoniae* were screened for possible ESBL production using ceftazidime, cefotaxime, aztreonam, ceftriaxone and Cefpodoxime disks. The isolated organisms were inoculated onto peptone water and incubated at 37°C for 4-6 hours. The turbidity of growth was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Muller Hinton agar plate by lawn culture. The above five discs were placed in a gap of 20 mm each. These plates were incubated at 37°C for overnight. Then the reading was taken. The zone around the disks were measured and interpreted as sensitive and resistant. The isolates which showed resistance to any one of these drugs were considered as screen positive. (Eshwar Singh, R *et al.*, 2011).

Results and Discussion

The chapter deals with analysis and results of primary data collected through a study conducted extended spectrum of β- lactamase in gram negative clinical isolates in Namakkal area. The study is based on the sample of 150 subjects. The data regarding this study and other background variables, such as age, resistance and enzyme production, etc., were elicited from the isolates over the area. The collected samples and information were put into several statistical analyses to extensively explicate the prevalence of extended β- lactamase products among the isolates over the study area.

Isolation and Identification of *Klebsiella pneumoniae*

The collected 150 urine samples were streaked on Nutrient and MacConkey agar. The suspected colony was identified according to the Bergey's manual of determinative bacteriology, the isolates identified positively by Voges proskauer, Citrate utilization, Catalase test, TSI agar test and also Colony morphology of *Klebsiella pneumoniae* in different medium which was shown in **(Table 1).** Among the 150 samples, *Klebsiella pneumoniae* was found in 70 (47%) samples.

Table -1:Incidence growth of organism among the study subjects

S.No.	Growth of organism	Bacterial isolates	
		Number	Percent
1	*Klebsiella pneumonia*	70	47 %
2	Other gram negative	50	33 %
3	No growth	30	20 %
Total		**150**	**100 %**

The above **Table-1** depicts that more than half 70(47%) samples were found to be *Klebsiella pneumoniae.* Nearly half of the samples 50(33%) were evidenced to be other gram negative organism and the rest of the samples 30(20%) were shown no growth.

In antibiotic disk diffusion, totally 8 various concentrations of antibiotics were used against *Klebsiella pneumoniae*. Ampicillin (100%), Cefotaxime (100%) and Tetracycline (100%) antibiotics were highly resistant to *Klebsiella pneumonia*.The moderate resistant to Co-trimoxazole (17%), Ciprofloxacin (7%), Ciprofloxacin (10%), Chloramphenicol (79%) and

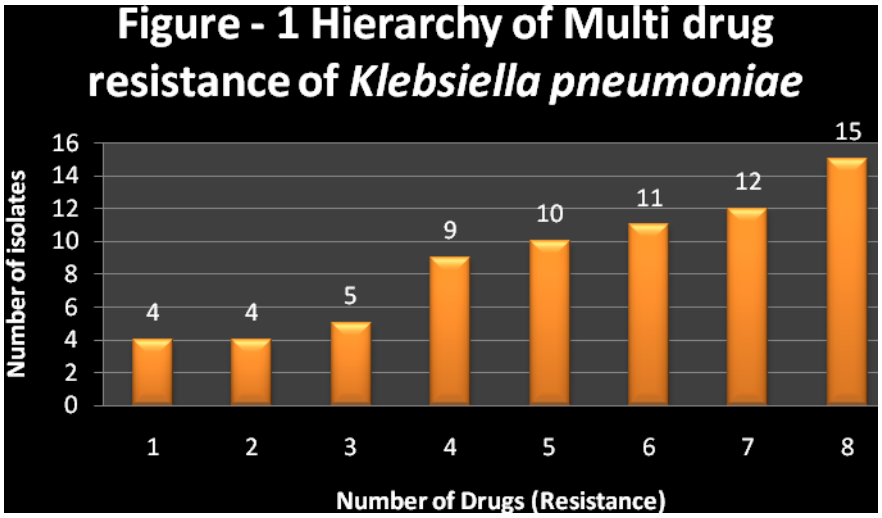
Amikacin (89%). Antibiotics Co-trimoxazole (83%), Ciprofloxacin (93%) were highly sensitive to these isolates, and showed very low sensitive against Chloramphenicol (21%), Amikacin (11%) **(Table-2)**.

Table: 2 Resistance/ Sensitivity Patterns Of *Klebsiella Pneumoniae* in Urine Sample

S.NO	Antimicrobial agent	Conc.	Resistance No.	Percentage (%)	Sensitive No.	Percentage (%)
1	Ampicillin (AMP)	10 mcg	70	100 %	-	-
2	Co-trimoxazole (COT)	25 mcg	12	17 %	58	83 %
3	Ciprofloxacin(CIP)	5 mcg	5	7 %	65	93%
4	Cefotaxime(CTX)	30 mcg	70	100 %	-	-
5	Ceftriaxone (CTR)	30 mcg	70	10 %	-	-
6	Chloramphenicol (C)	30 mcg	55	79 %	15	21 %
7	Amikacin (AK))	10mcg	62	89 %	8	11 %
8	Tetracycline (TE)	10 mcg	70	100 %	-	-

Table 3 Hierarchy of Multiple Drug Resistance of *Klebsiella pneumoniae*

S.NO	Number of drugs resistance	Bacterial isolates	
		Number	Percent
1	8	15	21.42
2.	7	12	17.14
3.	6	11	15.74
4.	5	10	14.28
5.	4	9	12.85
6.	3	5	7.00
7.	2	4	5.71
8.	1	4	5.71
	Total	**70**	**100**



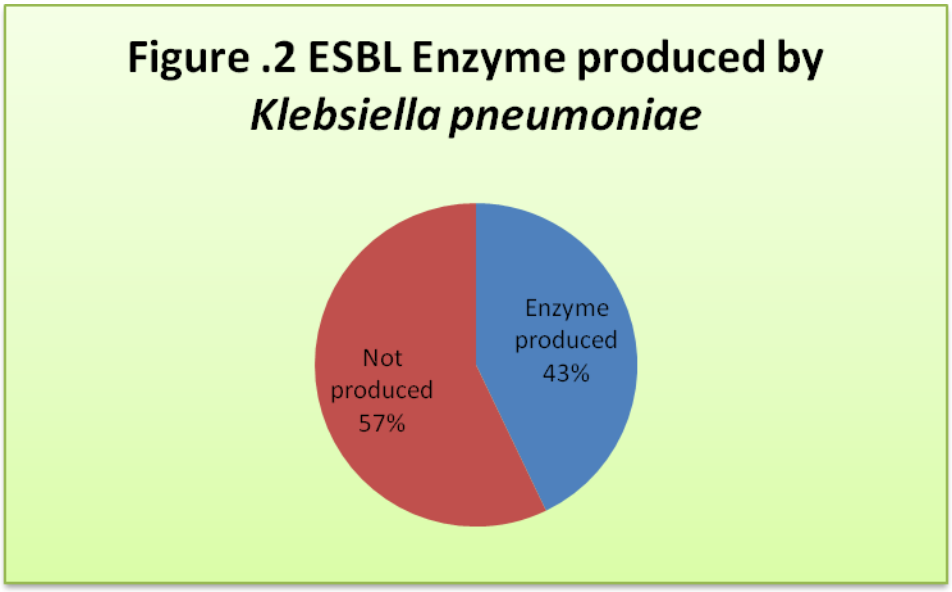
The above Table 3 and Figure -1 depicts that, out of the 70 *Klebsiella pneumoniae*, (4) 5.71% shows resistant to 1 drugs, (4) 5.71% shows resistant to 2 drugs, (5) 7% shows resistant to 3 drugs, (9) 12.85% shows resistance to 4 drugs, (10) 14.28 % shows resistant to 5 drugs, (11) 15.74% shows resistant to 6 drugs, (12) 17.14% shows resistant to 7drugs, (15) 21.42% shows resistant to 8 drugs.

Detection of ESBL Enzyme

According to the clinical and Laboratory standard Institute (CLSI) published guidelines for performing an extended spectrum of the ß lactamases confirmatory test. In *Klebsiella pneumonia* isolate produced extended spectrum lactamases of beta lactamases activity and it was observed by using various screening test. In combination disc method, Ceftazidime (30 mg) and Ceftazidime clavulnate (30/ 10 mg) were placed on the Muller Hinton Agar plates. The zone of inhibition around the Ceftazidime disk was expanded by the Cepftazidime clavunate on *Klebsiella pneumoniae*. The zone of inhibition was measured as greater than 5mm between two antibiotics such as cephotaxime and cephotaxime clavunate were detected as an extended spectrum of beta lactamases produced. In Double Disc Synergy Test (DDST), zone of inhibition around the disk such as Ceftazidime, Cefotaxime, Ceftriaxone, Aztreonam and Cefpodoxime was increased towards the disk containing amoxicillin clavulanic acid as an indicative of extended spectrum beta lactamases produced by *Klebsiella pneumoniae.* In this study, the Beta-lactamase enzymes were produced by two methods. Totally, 70 isolates of Klebsiella pneumoniae were subjected to detect the beta-lactamase enzymes among that only 30 isolates of Klebsiella pneumoniae showed enzyme activity in both the methods which were shown in the **Table 4**, **Figure 2**.

Table: 4 ESBL Enzyme Production by *Klebsiella pneumoniae*

S.NO	*Klebsiella pneumoniae*	Enzyme activity by Combination disc and DDST method	
		Number	Percent
1	Enzyme produced	30	43 %
2.	Not produced	40	57 %
Total		**70**	**100 %**

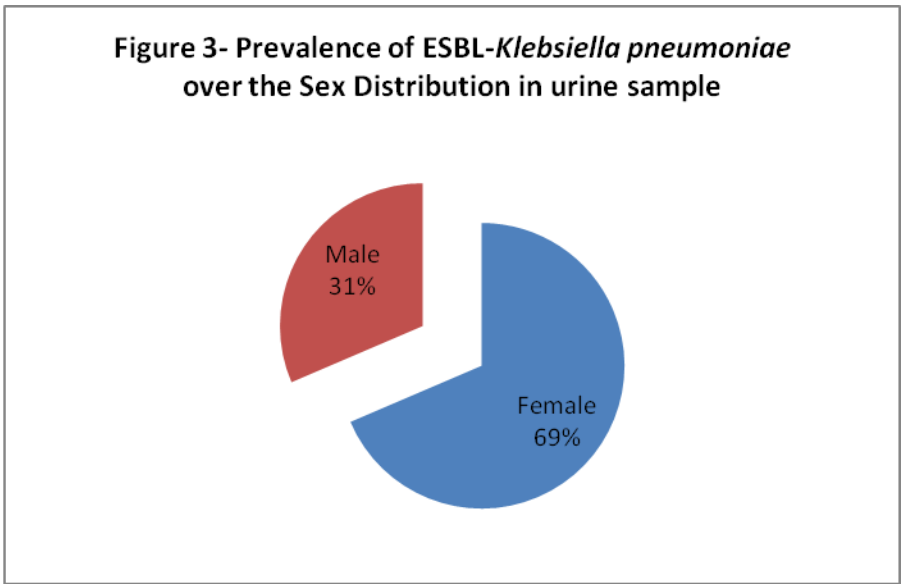


Prevalence of ESBL over the Sex and Age

Sex wise distribution of ESBL producers are revealed that the prevalence was more among the females as compared to males.

Table: 5 Prevalence of ESBL over the Sex Distribution

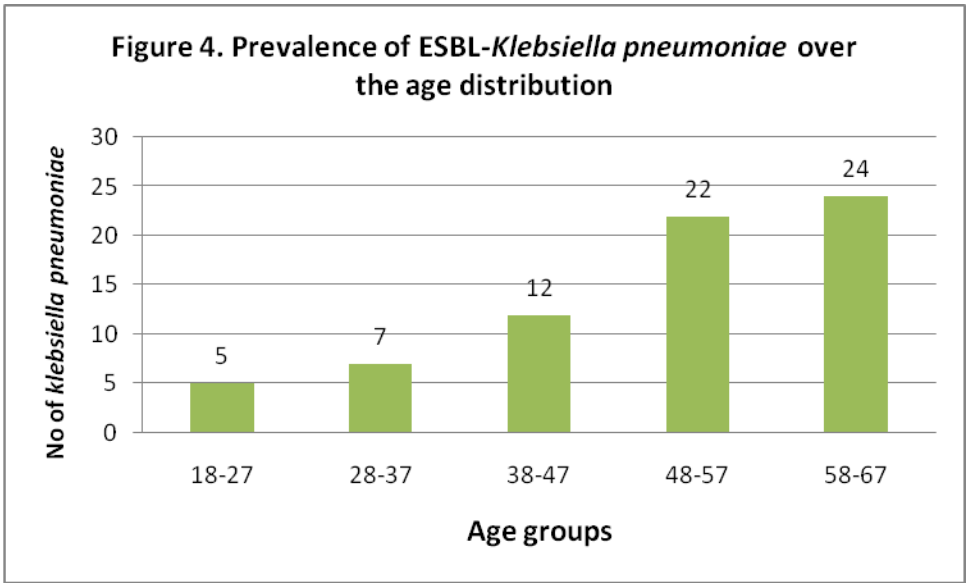
S.No.	Sex	Sample	Number	Percent
1	Female	Urine	48	69 %
2	Male	Urine	22	31 %
Total			**70**	**100 %**



The above **table 5 and figure 3** shows that, among the total of 70 isolates, 48 (69%) from female and 22 (31 %) from the male.

Table: 6 Prevalence of ESBL over the Age Distribution

S.No.	Age group	*Klebsiella pneumoniae*	% of isolates
1	18-27	5	7 %
2	28-37	7	10 %
3	38-47	12	17 %
4	48-57	22	32 %
5	58-67	24	34 %
Total		**70**	**100 %**



The prevalence of ESBL-KP over the age group distribution were studied and presented in **Table-6, figure 4**. The study subjects were classified into 5 age groups of the 70 urine samples. More number of *Klebsiella pneumoniae* was found in the age group between 48-57 (32%) and 58-67 (34%) followed by 38-47 (17 %), 28-37 (10%). The least growth was shown by the age group 18-27 and respectively.

Discussion

The study showed an alarming incidence of ESBL-producers amongst *Enterobacteriaceae* isolates from intra-abdominal infections in both "community-acquired" and hospital-acquired settings, especially in case of *E. coli* and *K. pneumoniae* isolates. However, the observation of high prevalence of ESBL producers among the infected patients in society. A significant observation in the study was that the ESBL producing *Klebsiella pneumoniae* isolates were identified. In the present study investigated the emergence of *Klebsiella pneumoniae* producing extended spectrum Beta lactamase [ESBL] from urine sample in Namakkal area during a period of months from March 2015 to August 2015. Recent studies on ESBL production among the members of *Enterobacteriaceae* which were isolated from specimens, showed an increase in the occurrence of ESBL producers (Peterson *et al.*, (2005).

There are 150 urine samples were collected from Namakkal district.The patients those who are suffered from various diseases. The samples were processed in various media such as Nutrient sgar, Macconkey sgar, Blood and CLED agar. β - lactamases production by several Gram negative organism is perhaps the most important mechanism of resistant to penicillins and

cephalosporins (Chsulgdary *et al.*, 2004). Over the last 20 year many β lactam antibiotic have been developed which were specifically developed to be resistant to hydrolytic action of β lactamases. (Bradford. 2001). Though ESBL might be produced by several members of *Enterobacteriaceae* , the present syudy was restricted only to detect their presence in clinically significant *K. pneumonia* isolates. (Shukla *et al.*, (2004). Screened the isolates by using cefotaxime (ce). Ceftazidime (ca), ceftrixone (ci) and cefpodoxime (cep) disc found 88.3% of isolates which were resistant to one of the above mentioned third generation cephalosphorins and 72% were resistant to all the three drugs. All the isolates were resistant to ampicllin and amoxyclav in the present study (Rodrigues et al. 2004).

The PCDDT was the most sensitive in detecting ESBL then DDST. DDST detected 32.3% whereas PCDDT 78.21% of ESBL producer (Renuka Rampure *et al.,* 2013). The study obtained from clinical samples of *K.pneumoiae*, shows high antibiotic resistant. Worldwide resistant to antibiotic has increased, 100% isolates resistant to ampicilin. (Farhat Uiiah et al., 2009).ESBL detection by phenotypic methods showed little more difference among them. In our study, the ESBL comfirmed by two phenotypic methods was correlated with above previous results. In this study, an overall prevalence rate of 43% ESBL producers in all isolates tested. This is higher than an earlier report done at Zagazig University with a rate of 28% ESBL producing strains of *Klebsiella pneumoniae* (Ejikeugwu Chika, *et al.*, 2013).

Extended spectrum β lactamases [ESBL] organisms pose unique challenges to clinical microbiologist, clinical, infection control professional and antibacterial discovery scientists. Clinical outcomes data indicate that ESBLs are clinically significant and detected indicate the need for use of appropriate antibacterial agents. Unfortunately, the laboratory detection of ESBLs can be complex and at times, misleading. First, these stains are difficulty in their detection by the current clinical methods, many of these strains have been reported to be susceptible to widely used and control broad spectrum β lactam. Secondly, ESBLs constitute a serious threat to current β lactam therapy. Treatment of ESBL infection is difficult as the CLSI recommends that all extended spectrum cephalosporin be resistant in ESBL producers. Thirdly, institutional outbreaks are increasing because of selective due to the heavy use of extended spectrum cephalosporin's and due to lapses to explore the current importance of ESBL producing *Enterobacteriaceae*.

Conclusion

The ESBL producing Enterobacteriaceae family was increased in the few decades and its risk factors were also continuously increasing in the different epidemiological region in the world. If we cannot control or prevent the ESBL means due to the mutant problem leading to increase the severity of the infection and sometime may cause the death. Otherwise the infected person will not cure completely from the disease and its make the way to cause the other unwanted problems (co-infection) in the patients to increase the illness of the infected person. From our findings the epidemiological study on the prevalence of ESBL in Namakkal region will helpful to know the spreading of ESBL in particular region at the same time it's given the knowledge to how to use the antibiotic in clinical field (infected patient) and should improve the hygienic condition in hospitals. Although beta lactam antibiotics are still the mainstay in treatment of numerous infections, agents effective against BLPB should be considered in the treatment of those who failed these agents. Since BLPB can spread within the community as well as the hospital efforts should be made to reduce the spread However, further studies are warranted to critically investigate these modalities.

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