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Prevalence of ESBL producing *Klebsiella pneumoniae* from blood culture isolates in a tertiary care hospital.

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I. Introduction

Bloodstream infections (BSIs) are defined as the presence of viable infectious microorganisms in the bloodstream causing clinical illness [(1)] They are among the leading causes of mortality and morbidity worldwide [(2).] The term bloodstream infection and bacteraemia are synonymously used which generally refer to the significant growth of a microorganism in a blood culture obtained from the patient with clinical signs of infection [(3).]

Wide range of organisms have been isolated in BSIs, which include *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter spp*., and *Pseudomonas aeruginosa* among Gram-negative bacteria and coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, enterococci, and alpha-hemolytic streptococci among Gram-positive bacteria. The predominance of organisms varies from region to region with different antimicrobial resistance profile. [(4, 5, 6)]

Klebsiella pneumoniae (KP) is one of the leading causes of nosocomial infections seen worldwide, causing pneumonia, bloodstream infections, urinary tract infections, surgical site or wound infections and meningitis [(7).] β-Lactam antimicrobial agents represent the most common treatment for bacterial infections and continue to be the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria worldwide. The persistent exposure of bacterial strains to a multitude of β-lactams has induced dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the newly developed β-lactam antibiotics. These enzymes are known as extended-spectrum β-lactamases (ESBLs) [(8, 9, 10).] Treatment of these multiple drug-resistant organisms is a therapeutic challenge.

This study was conducted to determine sensitivity of K.pneumoniae to various 3rd generation cephalosporins and to know the prevalence of ESBL producing K. Pneumoniae strains in blood samples.

II. Material And Methods

A retrospective study conducted in the department of microbiology, S V Medical College, Tirupati over a period of one year from January 2018 to December 2018. Total number of Klebsiella pneumoniae isolates included in this study was 126, which were isolated from blood samples.

Blood for culture was collected from clinically suspected bacteraemia cases under strict aseptic precautions. A volume of 5–10 ml from adults and 2–3 ml from paediatric patients were obtained for culture. The same was inoculated into conventional paediatric and adult blood culture bottles containing brain–heart infusion broths (1:10 dilution). These were then continuously incubated aerobically at 37°C. After 18–24 h of incubation, a blind subculture was done to appropriate solid culture media irrespective of the turbidity status. The bottles were taken out and visually observed for turbidity every morning and then manually agitated for aeration. The bottles showing turbidity were subcultured on MacConkey agar, 5% sheep blood agar. All the negative bottles were incubated for 7 days and another blind subculture was done at the end of 7 days of incubation before reporting them as negative[(11).] Any growth obtained was processed and identified using Gram-staining, colony morphology, and standard biochemical tests. Antibiotic susceptibility testing was performed according to Kirby–Bauer's disc diffusion method and interpreted according to CLSI guidelines using antimicrobials for screening of ESBL *Klebsiella pneumoniae*: cefotaxime—30 μg; ceftazidime—30 μg; ceftriaxone—30 μg; amikacin—10 μg; amoxycillin—20 μg; gentamycin—30 μg; tetracycline—30 μg; imipenem—30 μg; ciprofloxacin—5 μg; Piperacillin tazobactum—100/10 μg HiMedia antibiotic discs.[(12)]

Screening of ESBL-Producing Strains for Klebsiella pneumoniae

Clinical and Laboratory Standards Institute [13] has developed screening tests for identifying the ESBL-producing *Klebsiella* pneumoniae. According to CLSI guidelines, strains showing zone of inhibition of ≤22mm for ceftazidime, ≤27mm for Cefotaxime, and ≤25mm for ceftriaxone were selected for conformational test of ESBL. Klebsiella pneumoniae ATCC 700603 strain was used as ESBL positive control.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL *[14]* ESBL production was confirmed among potential ESBL-producing isolates by phenotypic tests. Lawn culture of the organism was made and 3*rd*-generation cephalosporins ceftazidime (30 μg) disc and ceftazidime + clavulinic acid (30 μg + 10μg) disc was placed with 25mm apart. An increase of ≥5mm in zone of inhibition for ceftazidime+ clavulinic acid compared to ceftazidime was confirmed as ESBL producers.

III. Results

In the present study, 126 isolates of K. pneumoniae isolated from blood cultures were included. The antibiogram of K pneumoniae (Table-1) showed (126)100% susceptibility to Imipenem followed by Amikacin (117) 93%, and Piperacillin tazobactum (112) 89%. High resistance shown to Amoxycillin 102(81%) followed by ceftazidime 99(78%), Cefotaxime 96 (76%), ceftriaxone 94 (74.6%), Gentamicin 96 (76%) and Ciprofloxacin 87(69%).

Table—1

Antibiotic	Resistance (N=126)	Percentage	Susceptibility (N=126)	Percentage
Imipenem	00	00	126	100%
Amikacin	09	7%	117	93%
Piperacillin tazobactum	14	11%	112	89%
Ceftazidime	99	78%	27	22%
Cefotaxime	96	76%	28	24%
Ceftriaxone	94	74.6%	32	26%
Gentamicin	96	76%	28	24%
Ciprofloxacin	87	69	39	31
Amoxycillin	102	81	24	19

Out of 126 K pneumoniae isolates, 81(64.2%) were screened according to CLSI guidelines and selected for phenotypic confirmatory disc diffusion test. 44(54.3%) isolates were confirmed as ESBL producers by PCDDT.

IV. Discussion

Extended spectrum beta lactams are commonly included in the empirical antibiotic regimens for treatment of gram negative sepsis. The increasing use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of extended spectrum beta lactamase producing microorganisms [15].

In India, the percentage of ESBL producing Klebsiella pneumoniae ranges from 4%-83 %[16, 17] and high prevalence of ESBL-producing Klebsiella pneumoniae strains has been reported by various groups [18–21]. In this study, the prevalence of ESBL producing *K. pneumonia* in blood culture isolates was 54.3% which is in correlation with Khalid Abdalla Ali Abdel Rahim et al [22] and Vemula Sarojamma et al [23]

All the isolates showed susceptibility to Imipenem i.e. (100%) and 93% of susceptibility rates towards Amikacin which is similar to BabyPadmini et al [24] and A K Singh et al[25].

Klebsiella species has been an important source of transferable antibiotic resistance. In our study, the resistance to 3rd generation cephalosporins was transferred to the recipient strain along with resistance to Gentamicin and other beta lactam antibiotics. ESBL production is coded by genes that are prevalently located on large conjugative plasmids of 80-160 Kb in size [26]. We found such associated resistance with Gentamicin, ciprofloxacin. Other workers in India have reported such association were A K Singh et al [25].

V. Conclusion

The present study showed high prevalence of ESBL producing Klebsiella pneumoniae from blood cultures. The routine antimicrobial sensitivity tests may fail to detect ESBL mediated resistance against 3GC which may lead to treatment failure especially when cephalosporins are used. Since all the isolates were sensitive to Imipenem and there were large number of isolates from blood it can be the drug of choice for the treatment of infections due to ESBL producing K.pneumoniae strains in seriously ill patients.

Amikacin showed a good susceptibility rates and it may be recommended as a first-line of treatment of infections caused by ESBL-KP wherever suitable. Hence it is suggested that, routine detection of ESBL producing strains should be done to avoid misuse and overuse of antibiotics and to reverse the undesired effects of multidrug resistant and ESBL producing Klebsiella.

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