Original Article

Frequency and Antibiotic Susceptibility Pattern of *Klebsiella* Species in Blood Culture of Paediatric Population

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ABSTRACT

Objective: To isolate *Klebsiella* species in the blood culture of pediatric patients, evaluate its antimicrobial susceptibility, and determine the frequency of extended spectrum beta-lactamase (ESBL) among *Klebsiella* species.

Methodology: It was a descriptive cross-sectional study conducted at the Department of Microbiology, Children Hospital & Institute of Child Health, Lahore. A total of 2000 blood samples were enrolled in the study by convenience sampling technique. The blood samples received from various wards of the hospital were immediately processed in the laboratory by a semi-quantitative method as per standard protocol after the ethical approval of the committee. After the isolation & identification of the organisms, all the bacterial isolates were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method.

Results: Out of 2000 study samples, 193 yielded *Klebsiella* species. Among *Klebsiella* species, *Klebsiella pneumoniae* (*K. pneumoniae*) caused more common bloodstream infections (BSIs) (87.6%) than *Klebsiella oxytoca* (*K. oxytoca*) (12.4%). Antimicrobial susceptibility testing revealed that *K. pneumoniae* and *K. oxytoca* were susceptible to chloramphenicol.

Conclusion: The *Klebsiella* species are among the significant pathogens for bloodstream infections in pediatric patients. The isolated *Klebsiella* species include *Klebsiella pneumonia*e and *K. oxytoca* with a predominance of *K. pneumoiae.* A significant antibiotic resistance (>70%) is exhibited by the isolated *Klebsiella* species against tested cephalosporin, fluoroquinolones, and aminoglycosides.

Keywords: *Klebsiella pneumoniae. Blood culture. Multidrug resistance.*

INTRODUCTION

Klebsiella are gram-negative bacilli commonly found in the environment and human microbiota. They can cause a broad range of infections, including bloodstream infections. It is associated with high morbidity and mortality rates. Blood culture is an essential diagnostic tool for identifying and isolating bacteria or fungi causing bloodstream infections.[1] By analyzing blood cultures, healthcare providers can determine the causative organisms and their antibiotic susceptibility, enabling targeted treatment strategies. Isolating *Klebsiella* species in blood cultures and identifying the susceptibility patterns provide valuable insights into these pathogens' prevalence, distribution, and antibiotic resistance patterns. The emergence of antibiotic resistance among *Klebsiella* species has become a global concern.[2] The ability of these bacteria to acquire and transfer resistance genes is leading to the development of multidrug resistance strains. This poses significant challenges in managing *Klebsiella* bloodstream infections as treatment options become limited and the risk of treatment failure increases.[3]

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Unfortunately, *Klebsiella pneumoniae* is an emerging microorganism among members of *Enterobacteriaceae* which produces AmpC β-lactamases. The increased incidence of antibiotic resistance among bacteria in resource-poor settings is due to the empirical use of antibiotics. To counter the antimicrobial properties of antibiotics, bacteria are equipped with effective mechanisms. Extended spectrum beta-lactamase is plasmid-mediated and confers broad resistance against penicillin, cephalosporin, and monobactam, except carbapenems.[4] Extended spectrum beta-lactamase producing *Klebsiella pneumoniae* can resist the third generation cephalosporin like cefotaxime, ceftriaxone, and ceftazidime. This is the predominant cause of childhood infections and presents notable challenges such as adverse outcomes, treatment failure, and high mortality and morbidity. Detection of ESBL is important because there are extremely limited antibiotic options for treating ESBL-producing organisms.[5] In several parts of the world, the prevalence of ESBL-producing *Klebsiella pneumoniae* strains ranges from 5-25% in hospitals. *Klebsiella* species are opportunistic pathogens and commonly cause nosocomial infections in immunocompromised patients.[6] There is no sufficient data about this bacterial infection in children of Pakistan. *Klebsiella* species frequency determination and their antibiotic susceptibility pattern in blood cultures is crucial for guiding appropriate antibiotic therapy and infection control measures. This study was designed to assess the prevalence and current antibiotic resistance pattern of the *Klebsiella* species in pediatric patients with bloodstream infections.

METHODOLOGY

It was a descriptive cross-sectional study conducted at the Department of Microbiology, Children Hospital & Institute of Child Health, Lahore. All blood samples collected from patients suspected to have bloodstream infections with age less than 10 years were included. The study was conducted from January to May 2021. Informed consent was taken from all the patients. The study was approved by the institutional review board of the institution. Convenience sampling technique was used. The unlabelled or mislabelled samples were excluded from the study. Three milliliters of venous blood were drawn and collected into the conventional blood culture bottles using the proper aseptic technique to prevent contamination. Samples were processed in the laboratory for culture, identification of organisms, and antibiotic sensitivity patterns. Samples were incubated at 37°C aerobically at room temperature for 18 to 24 hours. The bottle with signs of hemolysis, turbidity, production of gas, and pellicle formation was cultured on blood and MacConkey agar. The *Klebsiella* species on blood agar appear as small translucent, mucoid, raised, and non-hemolytic. It is lactose-fermenter and produces pink mucoid colonies on MacConkey agar. Biochemical tests i.e. citrate utilization test, triple sugar iron test, urease test, and indole test were used for further identification. Antimicrobial susceptibility was performed by the modified Kirby-Bauer disc diffusion method and interpreted according to Clinical & Laboratory Standards Institute (CLSI) guidelines 2020. A zone of inhibition or clear zone was observed. Extended spectrum beta-lactamase screening was performed using the clavulanate inhibition test method.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) version 24 was used to analyze the collected data. Categorical variables were expressed in frequency and percentage.

RESULTS

Out of a total of 2000 samples, 193 had positive bacterial growth (*Klebsiella* species) and 1807 either exhibited no growth or yielded growth of bacteria other than *Klebsiella*. The majority of positive blood cultures were of male patients [106(54.9%)]. Out of 106, 90(53.25%) were *Klebsiella pneumoniae* and 16(66.67%) were *Klebsiella oxytoca*. Eighty seven (45.1%) female patients showed positive growth in blood culture, from which 79(46.75%) were *Klebsiella pneumoniae* and 8(33.33%) were *Klebsiella oxytoca*. Out of 193 *Klebsiella* isolates, 169(87.6%) were *Klebsiella pneumoniae* and 24(12.4%) were *Klebsiella oxytoca* based on the indole test. The *Klebsiella oxytoca* was indole positive, whereas *Klebsiella pneumoniae* was indole negative. From 193 *Klebsiella* isolates, 71(36.7%) isolates of *Klebsiella pneumoniae* and 15(7.7%) isolates of *Klebsiella oxytoca* were ESBL positive (Table 1). Frequency of *Klebsiella* species isolated among paitents of different age groups is shown in Figure 1. The antibiotic susceptibility pattern of *Klebsiella* species showed the highest sensitivity towards chloramphenicol (Table 2).

DISCUSSION

Bloodstream infection is characterized by positive blood cultures and systemic signs of infection. It may either be primary or secondary to a documented source. *Klebsiella pneumoniae* accounts for more than 70% of all catheter associated bloodstream infections.[7] In this study, the susceptibility pattern and frequency of *Klebsiella* species causing bloodstream infections were evaluated.

Our results showed that 193 blood cultures yielded *Klebsiella* species. Out of 193, *Klebsiella pneumoniae* was 169(87.6%) and *Klebsiella oxytoca* was 24(12.4%). These results were comparable to the study conducted by Ali et al., which reported 84 isolates of *Klebsiella pneumoniae* and 4 isolates of *Klebsiella oxytoca* from 300 different clinical specimens.[8] Another study reported that 151 episodes of BSIs were identified per 100,000 population per year, the incidence of *Klebsiella pneumoniae* was 9.1, and *Klebsiella oxytoca* was 2.9.[9]

The study demonstrates that most of the positive cases were obtained from males, 106(54.9%) followed by female patients 87(45.1%). The study findings are in accordance with the study conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan where most positive cultures were from males 102(60%) followed by females 70(40%).[10]

Antibiotic-resistant strains are the most common cause of infection. The resistance is due to the prolonged use of antibiotics or the use of inappropriate antibiotics. The resistance of *Klebsiella* to the most commonly used antibiotics is due to ESBL production. Treatment approaches for these antibiotic-resistant organisms are limited because these organisms possess multidrug resistance phenotype.[11] In this study, 193 positive samples were collected from different wards and outpatient department patients. Seventy one (36.7%) isolates of *Klebsiella pneumoniae* and 15(7.7%) isolates of *Klebsiella oxytoca* were ESBL positive. Similar results were found in another study which showed that 28.2% (29/103) *Escherichia coli* and *Klebsiella* isolates were confirmed as ESBL producers by combination disc diffusion method out of 103 samples. Production of ESBL was 33.3% in *Klebsiella pneumonia*, 27.4% in *Escherichia coli,* and 16.7% in

Table 1: Frequency Distribution of the *Klebsiella* Species

Organisms	Frequency & Percentage	Male	Female	ESBL
Klebsiella pneumoniae	169(87.6%)	90(53.25%)	79(46.75%)	71(36.7%)
Klebsiella oxytoca	24(12.4%)	16(66.67%)	8(33.33%)	15(7.7%)

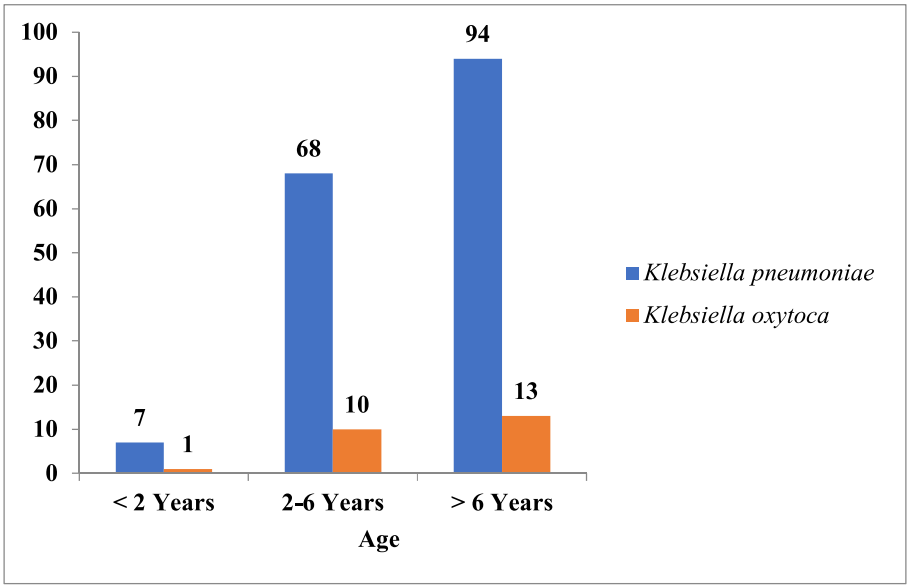

Figure 1: *Klebsiella* Species among Different Age Groups of Study Participants

Table 2: Antibiotic Susceptibility Pattern of *Klebsiella* Species

Antibiotics	Organisms			
	Klebsiella pneumoniae (n=169)		*Klebsiella oxytoca (n=24)*	
	Sensitive	Resistant	Sensitive	Resistant
Amikacin	41(24%)	128(76%)	4(17%)	20(83%)
Co-amoxiclav	19(11%)	150(89%)	4(17%)	20(83%)
Cefuroxime	5(3%)	164(97%)	4(17%)	20(83%)
Cefixime	5(3%)	164(97%)	3(13%)	21(87%)
Cefotaxime	22(13%)	147(87%)	4(17%)	20(83%)
Ceftazidime	15(9%)	154(91%)	4(17%)	20(83%)
Ceftriaxone	9(5%)	160(95%)	4(17%)	20(83%)
Cefepime	17(10%)	152(90%)	4(17%)	20(83%)
Ciprofloxacin	44(26%)	125(74%)	6(25%)	18(75%)
Levofloxacin	66(39%)	103(61%)	11(46%)	13(54%)
Moxifloxacin	54(32%)	115(68%)	7(29%)	17(71%)
Meropenem	39(23%)	130(77%)	4(17%)	20(83%)
Tobramycin	22(13%)	147(87%)	3(12%)	21(88%)
Co-trimoxazole	42(25%)	127(75%)	7(29%)	17(71%)
Chloramphenicol	81(48%)	88(52%)	13(54%)	11(46%)

Klebsiella oxytoca.[12] A study done by Dehshiri et al. showed that 62(31.3%) *Klebsiella pneumoniae* isolates have the gene phenotype of broad spectrum β-lactamase enzymes.[13]

Muller-Schulte and his colleagues conducted a study on clinical samples from a teaching hospital in Bouake. A total of 107 isolates were included and among all *K. pneumoniae* isolates, 90(84%) were ESBL producers.[14] A study was conducted in Iran to determine the antibiotic resistance pattern of *Klebsiella.* They

reported resistance to cephalexin, ceftriaxone, trimethoprim-sulfamethoxazole, and cefixime.[15] In the present study, *K. pneumoniae* and *K. oxytoca* demonstrated different susceptibility patterns to different antibiotics. Our data showed that 97% *of K. pneumoniae* showed resistance to cefuroxime and cefixime. Grundmann and his colleagues conducted a study on *Klebsiella pneumoniae* in a European Hospital where they studied 2703 clinical isolates, out of which 850(37%) *K. pneumoniae* were carbapenamase producing organisms.[16] In the current study, 54% *K. oxytoca* and 48% *K. pneumoniae* were sensitive to chloramphenicol. Regarding fluoroquinolones, *Klebsiella pneumoniae* showed 39% and 32% sensitivity to levofloxacin and moxifloxacin, respectively. *K. oxytoca* also showed significant resistance to tobramycin, cefixime, ceftriaxone, ciprofloxacin, co-trimoxazole, amikacin, and meropenem. *Klebsiella pneumoniae* is more prevalent than *K. oxytoca*. Multidrug resistance in *Enterobacteriaceae* is often the result of the acquisition of resistance genes by horizontal transfer. In addition, a large number of resistance genes are present on integrons carried by plasmids and transposons.[17]

CONCLUSION

The *Klebsiella* species are among the significant pathogens for bloodstream infections in pediatric patients. The isolated *Klebsiella* species includes *Klebsiella pneumonia*e and *K. oxytoca* with a predominance of *K. pneumoniae*. A significant antibiotic resistance (>70%) is exhibited by the isolated *Klebsiella* species against tested cephalosporins, fluoroquinolones, and aminoglycosides.

LIMITATIONS & RECOMMENDATIONS

The study was conducted on pediatric patients of a single tertiary care hospital. The sample size was relatively smaller as compared to the population of Pakistan. Furthermore, the study did not isolate strict anaerobic bacteria and fungi. Further multi-centered studies with large sample size are required to generalize the results.

The study recommends that all personnel handling blood samples should be taught the principles and practices of the aseptic technique. Good communication between the infection control unit and the hospital authority should exist. Patients with nosocomial infections should be separated from other patients to avoid cross-infection. Regular clinical meetings should be organized to discuss and review methods of handling clinical specimens. It is important to reduce the chances of infection in patients by cautious use of ventilators and intravenous catheters and prompt removal when they are not required.

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