

Seasonal Variation in *Klebsiella pneumoniae* Blood Stream Infection: A Five Year Study

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Abstract

Introduction: *Klebsiella pneumoniae* is a ubiquitous environmental organism and a common cause of serious gram-negative infections in humans. This study was conducted to examine the association between seasonal variation and the incidence rate of *Klebsiella pneumoniae* blood stream infection.

Material and methods: The retrospective study was conducted in the Department of Microbiology, JN Medical College AMU Aligarh for a period of 5 years from January 2011 to December 2015. Samples were received for blood culture in brain heart infusion broth. Cultures showing growth of *Klebsiella pneumoniae* were identified using standard biochemical procedures. Antimicrobial susceptibility testing was done on Mueller Hinton's agar by Kirby Bauer Disc diffusion method as per the CLSI guidelines. Detection of ESBL, *AmpC* and MBL production was done.

Results: 495 (30.0%) isolates were found to be positive for *Klebsiella pneumoniae* during the five year study period. Prevalence of *Klebsiella pneumoniae* isolates in blood stream infection for the warmest four months (July-September) was found to be 61.8% as compared to 38.2% in the remaining year (January-June and October-December). Maximum number of *Klebsiella pneumoniae* were isolated the month of August 86 (17.3%) followed by July 75 (15.1%) while minimum isolates were found in the month of February 14 (2.8%) and January 19 (3.8%). ESBL producing isolates were 44 (8.8%) and 318 (64.20%) isolates were *AmpC* producers. 36 (7.2%) isolates were found to be MBL producer.

Conclusion: Our study suggests that *K. pneumoniae* is an important pathogen in hospitals worldwide and that rates of *K. pneumoniae* blood stream infection vary seasonally.

Keywords: *Klebsiella pneumoniae*; Infection; *AmpC*; Antimicrobial susceptibility; Cultures

Introduction

Seasonality is defined as a periodic surge in disease incidence corresponding to seasons or other predefined calendar periods [1]. As compared with community-acquired infections, for which seasonal variations have been identified, little is known about such variations. Infections caused by Gram-negative bacilli and healthcare associated infections. Seasonal variation in rates of infection with certain Gram-negative organisms including *Acinetobacter* species, *Aeromonas hydrophila* and *Burkholderia pseudomallei* has been previously described [2,3]. In addition, seasonal variation in bloodstream infection (BSI) due to *Acinetobacter* spp. and *Klebsiella pneumoniae* has been reported in studies from tertiary care centres [4].

Klebsiella pneumoniae is a ubiquitous environmental organism and a common cause of serious gram-negative infections in humans, including bloodstream infection, ventilator associated pneumonia, and urinary tract infection [5]. Infections due to *K. pneumoniae* occur in both endemic and outbreak settings [6]. Isolates of *K. pneumoniae* are becoming increasingly resistant to antibiotics and subsequently may become even more difficult to treat. We conducted this study to examine the association between seasonal variation and the incidence

rate of *Klebsiella pneumoniae* BSI. The primary aim of the study was to compare the prevalence of *Klebsiella pneumoniae* BSI in the population between the warmest 4 months of the year (June through September) with that during the remainder of the months.

Material and Methods

The retrospective study was conducted in the Department of Microbiology, JN Medical College AMU Aligarh for a period of 5 years from January 2011 to December 2015. Samples were received for blood culture in brain heart infusion broth. Subcultures were done on 5% sheep Blood agar and Mac-Conkeys agar after 24 hours, 48 hours and 7 days of incubation at 37°C. Cultures showing growth were identified by standard biochemical procedures [7]. Cultures showing growth of *Klebsiella pneumoniae* from blood (collected after 48 hours of hospital admission) with signs and symptoms of BSI (fever>38°C) were included in the study.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on Mueller Hinton's agar by Kirby Bauer Disc diffusion method as per the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014) for the following antimicrobials-amikacin (30 µg), gentamycin (10 µg), cefoperazone (75 µg), ceftriaxone+sulbactam (25 µg/75 µg),

cefoperazone+sulbactam (75 µg/75 µg), levofloxacin (5 µg), piperacillin+tazobactam (100 µg/10 µg) and imipenem (10 µg).

Detection of extended spectrum beta lactamases

Screening of possible ESBL production was done by using ceftriaxone (30 µg) and cefoperazone (75 µg). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone sulbactam [8].

Detection of inducible and derepressed *AmpC* beta lactamase

Detection of *AmpC* beta lactamase was done on isolates resistant to ceftriaxone (30 µg), cefixime (15 µg), cefoperazone (75 µg) and cefoperazone sulbactam (75/75 µg). Induction of *AmpC* synthesis was based on the disc approximation assay using imipenem as inducer [8].

Detection of metallo-beta-lactamases

Detection of MBL was done by Hodge test and Double Disc synergy test using EDTA. The method was as described by Lee et al. [9].

Results

A total of 18,604 samples were received for blood culture during the five year period. 1648 (9.0%) samples were found positive on culture. Out of these samples, 495 (30.0%) isolates were found to be positive for *Klebsiella pneumoniae* during the five year study period. The other pathogens isolated during this period were *Staphylococcus aureus* (295, 17.9%), coagulase negative *Staphylococcus* species (200, 12.1%), *Enterococcus faecalis* (35, 2.1%), *Pseudomonas* species (157, 9.5%), *Acinetobacter* species (120, 7.2%), *Citrobacter* species (156, 9.4%), *Escherichia coli* (123, 7.4%), and *Salmonella typhi* (67, 4.1%) (Figure 1). Out of these 495 isolates positive for *Klebsiella pneumoniae*, 212 (42.8%) strains were isolated in the year 2015, 107 (21.6%) in 2014, 80 (16.1%) in 2013, 53 (10.7%) in 2012 and 43 (8.6%) in 2011 (Figure 2). There was a steep rise in the *Klebsiella pneumoniae* isolates from 8.68% in the year 2011 to 42.8% in the year 2015. Majority of the isolates were from paediatric age group.

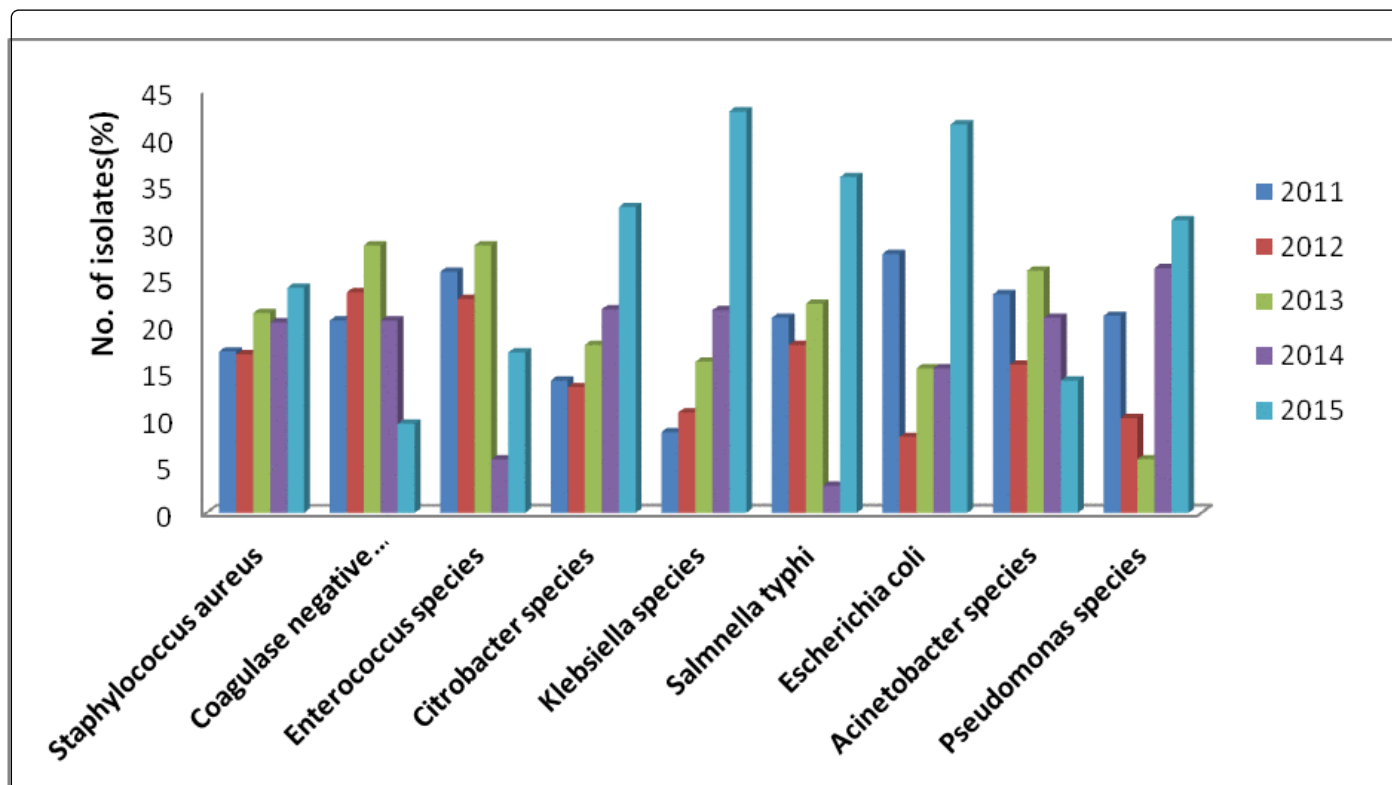


Figure 1: Distribution of bacteria isolated from blood culture for the period of five years.

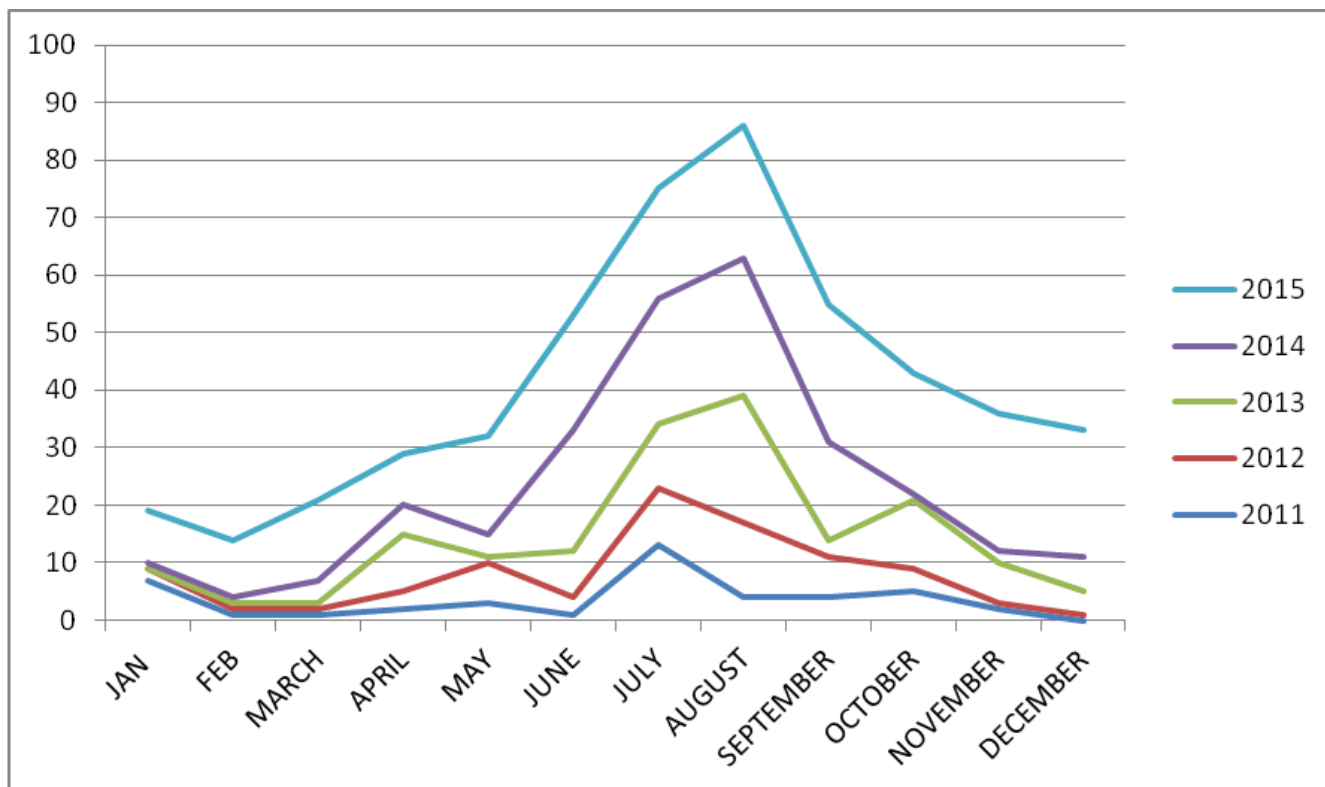


Figure 2: Monthwise distribution of *Klebsiella pneumoniae* in blood culture for the period of 5 years.

Prevalence of *Klebsiella pneumoniae* isolates in blood stream infection for the warmest four months (July-September) was found to be 61.8% as compared to 38.2% in the remaining year (January-June and October-December). Maximum number of *Klebsiella pneumoniae*

were isolated the month of August 86 (17.3%) followed by July 75 (15.1%) while minimum isolates were found in the month of February 14 (2.8%) and January 19 (3.8%) (Table 1).

Time Period	No. of Isolates					Total (N=495)
	2011 (n=43)	2012 (n=53)	2013 (n=80)	2014 (n=107)	2015 (n=212)	
June-September	22 (51.1%)	33 (62.2%)	42 (52.5%)	83 (77.5%)	126 (59.4%)	306 (61.8%)
Rest of the Year	21 (48.9%)	20 (37.8%)	38 (47.5%)	24 (22.5%)	86 (40.6%)	189 (38.2%)

Table 1: Prevalence of *Klebsiella pneumoniae* isolates in blood stream infection for the warmest 4 months as compared to remainder of the months.

On antimicrobial sensitivity testing maximum resistance (88.10%) was shown to the β -lactam group of antimicrobials. Aminoglycosides also had poor activity with resistance to 77.38% of the isolates. Flouroquinolones had better activity with resistance to only 39.39% of the isolates. Sensitivity to Levofloxacin remained uniform through all the five years while that of cephalosporins showed drastic fall through the years (23% in 2011 to 2.35% in 2105). There was no change in resistance pattern in the warmest four months and remainder of the year. 21 (4.24%) isolates were found to be multidrug resistant. ESBL producing isolates were 44 (8.8%) and 318 (64.20%) isolates were *AmpC* producers. 36 (7.2%) isolates were found to be MBL producer. There was an immense increase in resistance over the years. More than

fifty percent of the *AmpC* producers (162 out of 318) and MBL producers (25 out of 36) were isolated in the year 2015.

Discussion

Klebsiella pneumoniae causes serious, life-threatening infections in humans in endemic and epidemic settings. Our study is probably the first study in North India to describe a seasonal variation in the prevalence of *K. pneumoniae* blood stream infection in humans. In our study, the rate of *K. pneumoniae* BSI was 1.6 times higher during the 4 warmest months of the year as compared to the rest of the year. Our data suggest that rates of *K. pneumoniae* blood stream infection were associated with changes in temperature and humidity. The exact cause

for the observed higher rates of *K. pneumoniae* BSI during warm months remains elusive.

Several previously described characteristics of *K. pneumoniae* support our findings [4]. First, *K. pneumoniae* is the most heat tolerant of all enteric pathogens [10]. Second, the specific growth rate of *K. pneumoniae* is maximal at temperatures approaching 36.9°C [11]. Finally, *K. pneumoniae* survives better at higher humidity, as experimental models have shown that dehydration is an important factor in inactivating the organism [12].

K. pneumoniae is found naturally in 2 habitats: in environmental settings, such as water, sewage, soil, and plants, and on the mucosal surfaces of mammals. The density of *K. pneumoniae* in the environment (e.g., in freshwater ponds) is higher during warm months. Furthermore, *K. pneumoniae* mammary infections in cows are more common during summer [13]. Thus, it is reasonable to hypothesize that humans also have higher levels of colonization with environmental *K. pneumoniae* during warm months. These environmental *K. pneumoniae* isolates are just as virulent as clinical isolates from hospitals and can produce important virulence factors [14,15]. Furthermore, intestinal colonization typically precedes infection in humans, and hospitalized patients with *K. pneumoniae* colonization are at 4-fold higher risk of *K. pneumoniae* infection than non-carriers. Thus, increased colonization leading to infection may explain the observed increase in infection rate in summer [15].

Our study highlights an increase in the isolation of multidrug resistant *K. pneumoniae* in blood. In our study, 21 (4.24%) isolates were found to be multidrug resistant with 8.8% of isolates being ESBL producers, 64.20% *AmpC* producers and 7.2% MBL producer. Similar results were observed in other studies [6,16,17]. Our study showed a drastic rise in resistance over the years. Fifty one percent of the *AmpC* producers and sixty nine percent of the MBL producers were isolated in the year 2015. However there was no change in resistance pattern with seasonal variation. The increase in antibiotic resistance is due to several factors but the major cause appears to be excessive use of antibiotics [18]. Injudicious use of β -lactam group of antimicrobials, commonly used for treating *Klebsiella pneumoniae* infection over the years had led to development of resistant mutants to these drugs. However certain drugs like levofloxacin which were less commonly used maintain better sensitivity profile. One possible solution has been to call an "antibiotic holiday" for commonly used drugs in which a drug is retired for a while until the mutant strain of bacteria dies off and the antibiotic can be used again.

In summary, our study suggests that *K. pneumoniae* is an important pathogen in hospitals worldwide and that rates of *K. pneumoniae* blood stream infection vary seasonally. Our novel study should further stimulate hypothesis-generated investigations to evaluate further the impact of climate on infectious diseases. Furthermore, our study highlights that a sudden increase in the isolation of multidrug resistant pathogens should be taken as a challenge and prompt action should be taken. There is an urgent need for early detection of these isolates for better treatment outcomes. Rational and judicious use of all antimicrobial agents and infection control policies are of paramount importance in curtailing this growing epidemic.

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