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VIRULENCE PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BLOOD CULTURE ISOLATES OF KLEBSIELLA PNEUMONIAE AT TERTIARY CARE CENTER, INDIA

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ABSTRACT

Introduction: Microbial invasion of the blood stream constitutes one of the most serious conditions in the infectious disease. *Klebsiella pneumoniae* is among the most common cause of nosocomial gram-negative bacteremia. It is responsible for 4-15% of septicemia in adults and 3-20% of neonatal septicemia (both early and late onset) especially in premature infants and ICU, thereby increasing the disease burden, morbidity and mortality. **Aims and objectives:** 1) To study various virulence determinants of *Klebsiella pneumoniae.* 2) To compare the virulence factors with the antibiogram. **Methods:** A prospective study was conducted over a period of 1 year (Oct, 15 to Sep, 16) in the department of microbiology, PGIMS, Rohtak. Total blood cultures received were 15,141. Out of which 110 *Klebsiella pneumoniae* were isolated & were subjected for various virulence factors determination like capsule, hyperviscosity (mucoid), biofilm production, siderophore production as per standard microbiological procedures. Antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method with various antimicrobial agents as per CLSI guidelines. The virulence factors were compared with their antimicrobial susceptibility pattern. **Results:** Eighty four (76%) isolates were capsulated. String test was positive in 79 (72%) of the isolates, biofilm production was seen in 73 (66%) and siderophore production was seen in 6(5%). The least effective drugs were gentamicin and amoxy-clav (11% & 15% respectively) whereas meropenem (81%) and imipenem (84%) were the most effective antimicrobial agents. It was seen that the number of virulence factors produced were more in resistant isolates. **Discussion and Conclusion:** Therefore, this study shows the importance of determining various virulence factors and delineates correlation of virulent strains with their antibiotic susceptibility patterns in blood culture isolates of *Klebsiella pneumoniae,* this will aid in better management of patients, thereby reducing morbidity as well as mortality.

KEYWORDS: *Klebsiella pneumoniae,* virulence factors, blood culture.

INTRODUCTION

Microbial invasion of the blood stream constitutes one of the most serious condition in the infectious disease[1] *K. pneumoniae* is among the most common cause of nosocomial gram negative bacteremia second only to *E. coli* encountered worldwide. It is responsible for 4-15% of septicemia in adults and 3-20% of neonatal septicemia (both early and late onset) especially in premature infants and Intensive Care Unit (ICU). It accounts for 8% of endemic hospital infections and 3% of epidemic outbreaks.[2,3] *K. pneumoniae* possess various virulence factors like hyperviscosity, capsule, siderophore, and biofilm which contributes to its pathogenicity making it resistant to many antibiotics This lead to increase in infections with multidrug resistant *K. pneumoniae* which is responsible for increase in morbidity and mortality.

Limited studies are available in Indian literature regarding determination of virulence factors and the antibiotic susceptibility patterns of blood culture isolates of *K. pneumoniae.* Antimicrobial therapy largely depends on local antimicrobial data. Therefore, this study was designed to determine various virulence factors of *K. pneumoniae* and delineates association of virulent strains with their pattern of antibiotic susceptibility in the setting of a tertiary care hospital so that the results could be helpful in better management of *K. pneumoniae* blood stream infections thereby reducing morbidity as well as mortality.

MATERIALS AND METHODS

An investigational laboratory based study was conducted in the department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak over a period of one year (October, 15 to September, 16). A total of 15141 samples were

received in the department during this period. Out of these, 2301(15.2%) samples were culture positive. Two hundred thirty three (10.12%) *K. pneumoniae* isolates were recovered on processing of cultures by the conventional methods i.e. by studying the colony morphology on the culture plates, gram staining, and various biochemical reactions. One hundred ten *K. pneumoniae* isolates were processed further for the purpose of this study and confirmed by automated identification and antibiotic susceptibility testing system (BD phoenix). Various virulence factors such as hyperviscosity, capsule, biofilm and siderophore production were detected. The antibiotic susceptibility testing was performed as per Clinical and Laboratory Standard Institute Guidelines (CLSI).[4]

Detection of virulence factors

a) Hyperviscosity: Mucoid or non-mucoid colonies of *K. pneumoniae* was determined by string test.

b) Capsule - Capsular antigen of the *K. pneumoniae* was detected by – India ink preparation

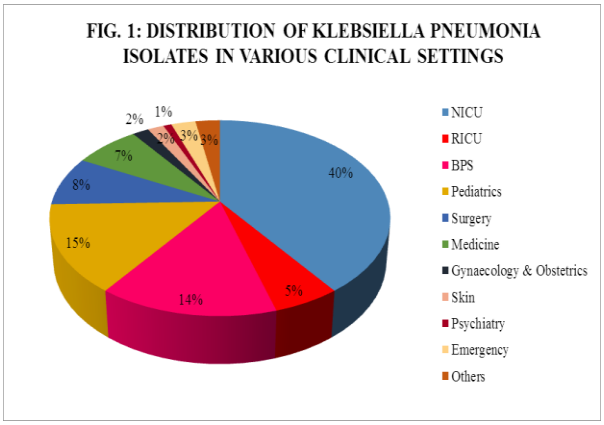
c) Biofilm production: Christensen Tissue culture plate method (TCP)[5]

d) Siderophore production – Csaky's assay was performed for the detection of siderophore production.

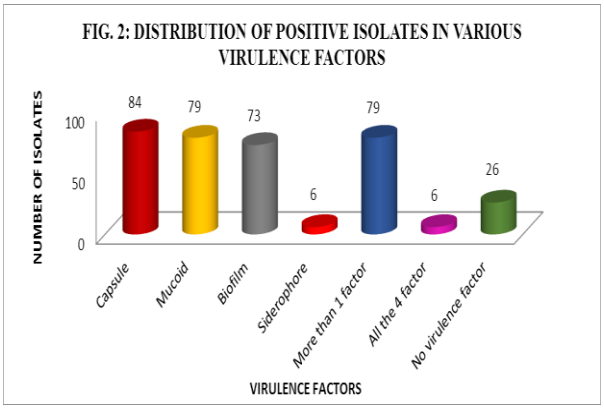
All the 110 isolates were subjected to antibiotic susceptibility testing using Kirby Bauer disc diffusion method. It was done on Mueller-Hinton agar plate.[4] The ATCC *Escherichia coli* 25922 was employed as standard control strain. The isolates were confirmed by automated identification and antibiotic susceptibility testing system (BD phoenix). Organism were defined as multi drug resistant if it was resistant to 3 or more antimicrobial categories.[6] Statistical association was tested using Chi-square and Z-test for proportions and statistical significance was considered when p <0.05. SPSS 20.0 software was used for analysis.

RESULTS

The age range in the study population in case of females was from newborn to 84 years with mean age 6.62±1.02. Blood stream *Klebsiella* infections were found to be more common in new born (54.54%). The ratio of M:F was 1.07:1. *Klebsiella* infection was highly prevalent in neonatal intensive care unit (NICU) patients 44 (40%) followed by burn and plastic surgery (BPS) and pediatrics ward i.e. 16 (14.54%) each (Fig. 1).



Of the established and identified four virulence factors of *K. pneumoniae*, majority were capsulated i.e. 84 (76%) followed by mucoid character 79 (72%) and biofilm production 73 (66%). Of these the least number encountered was 6 (5%) which corresponds to siderophore production. Most of the strains possessing these virulence factors were from ICU patients and the association of these virulence factors with the source of sample is depicted in figure 2. The study reveals that 79 (72%) isolates had more than 1 virulence factor and only 6 (5%) possessed all the four virulence factors.



The optical density of the biofilm producers were analyzed and the isolates were classified as highly positive, moderately positive and weak/non biofilm producers. Most of the isolates i.e. 49 (44.54%) were moderate biofilm producers followed by weak/non-biofilm producers i.e. 37 (33.63%).

Majority of the strains with virulence factors were isolated from various ICU's i.e. 52.38% of capsule forming, 51.89% of mucoid, 54.16% of biofilm producers and 66.66% of siderophore producers. The high and moderate biofilm producers were mostly associated with hospitalized patients i.e. ICU (91.6%) and wards (91.8%) as shown in table 1.

Table 1: Association between virulence factors and source of sample.

Source	Capsule (%) (n=84)	Mucoid (%) (n=79)	Biofilm			Siderophore (%) (n=6)
			High (%) (n=24)	Moderate (%) (n=49)	Weak/Non-biofilm producer (%) (n=37)	
ICU	44(52.38)	41(51.89)	13(54.16)	27(55.10)	23(62.16)	4(66.66)
WARD	33(39.28)	31(39.24)	9(37.5)	18(36.73)	11(29.72)	2(33.33)
OPD	7(8.33)	7(8.86)	2(8.33)	4(8.163)	3(8.10)	-

The study reveals that these isolates had varying degree of susceptibility to the antimicrobials tested (Table 2). All the strains i.e. 100% were sensitive to colistin and polymyxin B. Most of these showed high susceptibility to carbapenems group of antibiotics in-vitro viz. imipenem (84%), meropenem (82%) and ertapenem (77%). Least susceptibility of the isolates was seen against followed by gentamicin (11%) and amoxicillin-clavulanic acid (15%). This shows *K. pneumoniae* have developed resistance to most commonly employed antibiotics namely fluoroquinolones, penicillin group, cephalosporins in this tertiary care study. It was observed that out of 110 isolates, 88.1% were resistant to ≥1 agent in ≥ 3 antimicrobial categories i.e. multidrug resistant.

Table 2: Antibiotic susceptibility pattern of the *K. pneumoniae* isolates.

Antibiotics	Sensitive Isolates (%)	Resistant Isolates (%)
Gentamicin	12(11)	98(89)
Amoxicillin-clavulanic acid	16(15)	94(85)
Co-trimoxazole	23(21)	87(79)
Piperacillin-tazobactam	26(24)	84(76)
Cefazolin	26(24)	84(76)
Cefoperazone	28(25)	82(75)
Ciprofloxacin	28(25)	82(75)
Ceftazidime	29(26)	81(74)
Cefuroxime	31(28)	79(72)
Levofloxacin	31(28)	79(72)
Cefepime	45(41)	65(59)
Amikacin	55(50)	55(50)
Ertapenem	85(77)	25(23)
Meropenem	89(81)	21(19)
Imipenem	92(84)	18(16)
Colistin	110(100)	0(0)
Polymyxin B	110(100)	0(0)

DISCUSSION

K. pneumoniae is a common pathogen associated with blood stream infections. Its pathogenicity is related to multitude of virulence factors and ability to acquire antibiotic resistance. The impact of *K. pneumoniae* BSI has far reaching consequences especially in the developing countries like India. The present study provides information regarding various virulence factors possessed by blood culture isolates of *K. pneumoniae* along with their antibiotic susceptibility pattern that plays a crucial role in the effective management of septicemic patients. The blood culture positivity rate in the present study was 10.2%. Similar results were shown in other studies done in other parts of India.[7,8] However, the rate of *K. pneumoniae* bacteremia was comparatively lower than the one reported by Thacker *et al*[9] i.e. 15.7%. The difference might be due to the fact that Thacker *et al* included all the patients from pediatric oncology ICU which has higher overall rate of BSI attributed to various risk factors like immunosuppressive therapy, long hospital stay, and higher rate of blood culture isolation as observed by various authors.[8,10]

The incidence of *K. pneumoniae* infections were found to be more common in new born (54.54%). Ramasubramanian *et al.*[10] showed similar results. There was no predilection for sex in the present study as males (52.7%) marginally outnumbered females (47.3%). Male to female ratio in the present study was 1.07:1.

In present study, out of the 110 isolates, *Klebsiella* infection was highly prevalent in neonatal intensive care unit (NICU) patients i.e. 44 (40%). The results were in accordance with an international prospective study conducted by Paterson *et al*[11] (43.5%). It has been shown in multivariate analysis that the admission to the ICU is an independent factor responsible for *K. pneumoniae* bacteremia. This could explain isolation of majority of *K. pneumoniae* strains from ICU patients in present study.

The pathogenicity of *Klebsiella* strains is due to the fact that it possess various virulence factors which not only play crucial role in causing invasive disease like BSI but also help bacteria to survive in unfavorable environments like in the presence of antibiotics, leading to persistent and drug resistant infections. The present study reveals that 79 (72%) isolates had one or the other of the established and identified four virulence factors of *K. pneumoniae.* Only 6 (5%) isolates possessed all the four virulence factors. The majority of the isolates were capsulated i.e. 84 (76%) followed by mucoid character 79 (72%) and biofilm production 73 (66%). Of these the least number encountered was 6 (5%) which corresponds to siderophore production. The results of the present study are similar to Fung *et al,*[12] Victor *et al,*[13] Podschun *et al.*[14] and Seifi *et al.*[15]

The biofilm play an important role in protecting *K. pneumoniae* from drug exposure. There is strong relationship between resistance to antibiotic and prevalence of biofilm formation i.e. biofilm forming

strains are generally more resistant to many antibiotics. The degree of biofilm formation is also directly proportional to degree of antibiotic resistance. Present study revealed that most of the isolates i.e. 49 (44.54%) were moderate biofilm producers while 37 (33.63%) were weak/non-biofilm producers and 24 (21.81%) isolates were highly positive for biofilm formation.

Presence of multiple virulence factor in an isolate of *K. pneumoniae* increases the virulence of these strains and are also associated with enhanced invasion and mortality as shown by many epidemiological studies.[16,17] Present study shows that 79 (72%) of 110 isolates were positive for presence of more than one virulence factor. The combination of all the four factors were seen in 6 (5%) isolates while combination of capsule, mucoid and biofilm was present in 67 (60.9%) isolates and that of capsule with mucoid character only was present in 6 (5%) isolates. In a similar study conducted on blood culture isolates of *K. pneumoniae* and *E. coli* by Vernet *et al*,[18] observed that aerobactin was always associated with mucoid phenotypes and the strain producing both virulence factors demonstrated higher lethality in mice intraperitoneal model. In a report of 73 *K. pneumoniae* isolates from liver abscess evaluated for the relationship between *rmpA* carriage and capsule serotype.[19]

Present study reveals that *K. pneumoniae* isolated from ICU patients tends to produce more virulence factors as compared to the ward and OPD. Isolates from ICU patients were capsule forming in 53%, mucoid in 52%, biofilm producers were 55% and 67% of siderophore producers. It was also observed that the highly and moderately positive biofilm producers were mostly associated with hospitalized patients i.e. ICU (91.6%) and wards (91.8%) which is in accordance with previous studies.

The antimicrobial profile of the *K. pneumoniae* isolated from blood culture reveals that these isolates had varying degree of susceptibility to the antimicrobials tested. All the strains i.e. 100% were sensitive to colistin and polymyxin B. Most of them showed high susceptibility to carbapenem group of antibiotics *in vitro* viz. imipenem (84%), meropenem (82%) and ertapenem (77%). Least susceptibility of the isolates was seen against cephalosporin group (20-30%), ciprofloxacin (25%), amoxicillin-clavulanic acid (15%) and gentamicin (11%). The fact that cephalosporins are one of the most common antibiotics used for inpatients as well as for outpatients could be the reason for such high resistance being observed in the developing countries. In present study, it was observed that the strains elaborating various virulence factors were more resistant to antibiotics. The association of antibiotic profile with capsule formation, mucoid phenotype and biofilm formation was tested by Chi-square test and found to be statistically significant (p value <0.05). The findings are similar to the study conducted by Gharrah *et al*,[20] who reported significant association virulence factors and ESBL producing *K. pneumoniae* compared to non-producing.

The carbapenem resistance in the present study accounted to 16% and all these strains were multidrug resistant. The greatest threat with MDR and carbapenem-resistant strains is that the infections caused by such strains are usually difficult to treat with limited antibiotic options available. With the shortage of newer drugs and increasing resistance, clinicians may soon face the condition of so-called pan-drug resistant infections.

CONCLUSION

This study shows an association between drug resistance and virulence factors. Also, increase in the prevalence of multidrug resistant *K. pneumoniae* strains emphasize the urgent need for increased alertness of clinicians and enhanced routine testing by laboratories to reduce failure of therapy and prevent dissemination of highly virulent and drug resistant strains of *K. pneumoniae*. Therefore, there is a need for rational use of antibiotics, formulation of hospital antibiotic policy and implementation of infection control practices for effective management and prevention of drug resistance.

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