ORIGINAL ARTICLE

Evaluation of Multi-Drug Resistance in Biofilm Forming *Klebsiella Pneumoniae* Isolated from Urinary Catheter Tips in Patients from ICU and CCU in DHQ Teaching Hospital Gujranwala

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ABSTRACT

Objectives: To identify the isolates by means of different identification tests, to assess the frequency of *Klebsiella pneumoniae*, biofilm formation ability and antibiotic susceptibility of biofilm forming isolates.

Study Design: Cross-sectional/Observational study

Place and Duration: Conducted at Microbiology Laboratory, Gujranwala Medical College / D.H.Q Teaching Hospital Gujranwala Pathology Lab from August 2020 to October 2020.

Methodology: A total of 250 urine catheter tips samples were collected from patients admitted in ICU and CCU. This study includes the methodology in which the study organism was inoculated and confirmed by standard microbiological methods including culture inoculation, staining, biochemical tests, biofilm formation tests and antibiotic sensitivity. Qualitative method has been used to see biofilm formation in *Klebsiella pneumoniae* isolates. 10 different CLSI recommended necessary disc of antibiotics were used to observe the sensitivity and resistance of *Klebsiella pneumoniae* by Kirby-Bauer disc diffusion method.

Results: There were 170 (68%) males and 80 (32%) females. Majority of patients 160 (64%) were ages 46 to 66 years. 75 samples were confirmed by morphological confirmation, biochemical tests and microscopic analysis with those of *Klebsiella pneumoniae.* From 75 confirmed isolates of *Klebsiella pneumoniae,* 17 shows strong biofilm formation, 33 shows weak biofilm formation and 25 shows no biofilm formation. Total number of biofilm forming *Klebsiella pneumoniae* are 50 out of 75 confirmed isolates which are 66.66**%** respectively. Out of 50 biofilm forming *Klebsiella pneumoniae* isolates 41 shows multi-drug resistance (MDR) which are 82% respectively. Out of 10 drugs that were used Amikacin, Tazocin and Sulzone were found to be most sensitive against *Klebsiella pneumoniae* while Augmentin and Ciprofloxacin were found to be most resistant against *Klebsiella pneumoniae.*

Conclusion: This study shows that higher percentage of extracted organism shows biofilm formation and from these biofilm forming isolates maximum shows multi-drug resistance. Further advances in the prevention of nosocomial infections will require new approaches to control infections.

Keywords: Urinary catheter tips, *Klebsiella pneumoniae*, Biofilm formation, Antibiotic susceptibility, Multi-drug resistance.

INTRODUCTION

The opportunistic Gram-negative pathogen *K. pneumoniae* develops a variety of nosocomial and community-acquired infections and usually infects patients with permanent medical devices, particularly urinary catheters, in which the organism may grow like a biofilm. The growing rate of antibiotic resistance acquired by *K. pneumoniae* strains has caused this multi-drug resistant pathogen to spread worldwide, mainly at hospital level. This scenario is compounded by the fact that intrinsic resistance to antimicrobial agents increases dramatically as the strains of *Klebsiella pneumoniae* develop as biofilm. In 1882, Friedlander isolated *K. pneumoniae* from the lungs of patients who suffered from pneumonia. In 1886, this encapsulated bacteria which originally called the bacillus of Friedlander's, renamed as *Klebsiella*. It was later identified as a saprophytic microbe which not only colonizes the human gastrointestinal tract, skin and nasopharynx, but can also trigger urinary tract infections, osteomyelitis, and bacteremia (Podschun et al., 1998, Varaldo et al., 1988, Bagley et al., 1985). Capsular polysaccharides, type 1 and type 3 pili, adhesive and siderophores aggregating factors are the factors of virulence that play an important part in *Klebsiella pneumoniae* severity (Arakawa et al. 1995, Martino et al. 1995 and Gerlahet al. 1989).

For *Klebsiella* spp. virulence it is essential that capsules subunits are divided into 77 serological forms (Orskov et al., 1984 and Ehrenwot et al., 1956). The fibrillar structures that line the surface of bacteria is formed from material present in capsule (Amako et al., 1988), it safe bacteria from phagocytosis (Podschun et al., 1992) and prevent bacterial death due to bactericidal serum factors (William et al., 1983).

The type 3 pili (Livrelli et al., 1996) (also referred to as fimbria), are non-flagellar filamentous fimbriae1 adhesins, mostly located on a bacterial surface and consisting of sub-units of polymer globular proteins (pilins) (Jones et al.,

1983). These adhesins are called mannose-sensitive haemagglutinins (MSHA) or mannose-resistant haemagglutinins (MRHA) due to their capacity to agglutinate erythrocytes and whether the reaction is inhibited by D-mannose (Ottow et al., 1975). Fimbriae type 1 is an operon (fim), containing all the genes required for the fimbriae's structure and assembly which is carried out along chaperone-usher pathway (Kline et al., 2010). Fimbriae type 1 of *K. pneumoniae* is managed by phase changes similar to *E. coli* fimbriae type 1(Rosen et al., 2008 and Struve et al., 2008). The operon was discovered in 100 pecent of the 69 isolates of *K. pneumoniae* by Alcántar-Curiel and his colleagues. 96 percent of these strains have been detected with type 1 fimbriae (Alcántar-Curiel et al. 2013). In comparison, type 3 fimbriae are encoded by the MRK operon and should also be assembled using a chaperone usher route with a chromosome or plasmid transmitted gene cluster of the MRK (Burmolle et al., 2008).

In the pre-antibiotic period, due to extreme pathogenic property of *K. pneumoniae* it was considered as main agent that causes for community acquired infections, especially among alcoholics and diabetics. While infection pneumonia has become rare as a result of this pathogen whereas in recent years, new manifestations related to *K. pneumoniae* infections have occurred regularly, including abscesses of the liver complicated by endophthalmitis, multiple metastatic infections (Ong et al., 2010). Specific serotypes of both extremely virulent strains have been found for K1 (Fung et al. 2002) and urinary tract infections (Laupland et al., 2007).

MATERIALS AND METHODS:

All the research work was conducted in Microbiology Laboratory, Gujranwala Medical College / D.H.Q Teaching Hospital Gujranwala Pathology Lab. A total of 250 urine catheter tips samples were collected from patients admitted in ICU and CCU of D.H.Q Teaching Hospital Gujranwala in a time span of 3 months from August 2020 to October 2020. Samples were preserved in sterile specimen containers and were taken in cold packages under aseptic conditions for quality assessment of microorganisms within an hour of collection. Samples were assessed for Total plate count. Samples were homogenized with Butterfield's phosphate buffer (pH 7.2). Every sample was mixed with 90 ml of Butterfield's phosphate buffer. 1 ml aliquot volumes were transferred to Petri dishes with plate count agar and mixed with medium. After inoculation of samples, they were incubated at 37°C for 48 hours and the colonies became visible inside and on the surface of medium after 48 hours. Colonies were counted by using colony counter (Gallenkamp, England). Counts were expressed as colony forming unit per gram of homogenate sample (cfu g-1). Various morphological attributes of the colonies were pragmatic and recorded. Separate colonies were isolated and purified by numerous sub-culturing. Pure culture was preserved on slants at 4°C for further tests. The bacterial isolates were identified based on standard microbiological methods. Cultural characteristics, Gram staining and biochemical tests (Urease test, Citrate test) were carried out as preliminary tests as given in Monica Cheesbrough/District Laboratory Practice in Tropical Countries-2nd edition (Part2) 2006.

RESULTS

Total 250 clinical samples were taken from ICU and CCU of DHQ Teaching hospital Gujranwala. Samples from patients of both gender and age groups were collected for the study. Data is present in table1.

Table 1: Total number of samples collected and their gender wise distribution

Category	No. of samples
Male	170
Female	80
Total	250

During the period of three months from August 2020 to October 2020 samples were collected, distinguished between males and females (170 and 80) with percentage of incidences as 68% and 32% respectively.

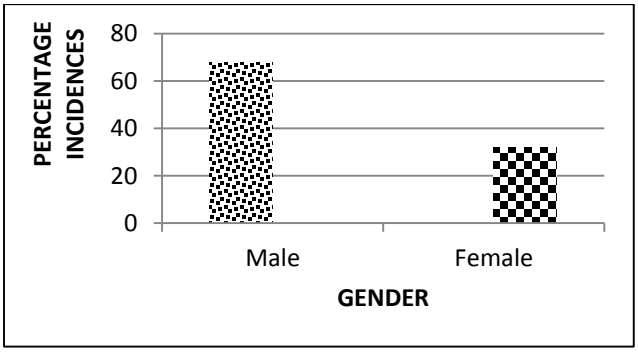

Figure1: Indicated percentage of male and female patients

Table:2 Age wise distribution of patients

Category	No. of samples
25-45 Years	90
46-66 Years	160

It represents collection of total number of samples from age groups of 25 to 45 years and 46 to 66 years which were 90 and 160 respectively.

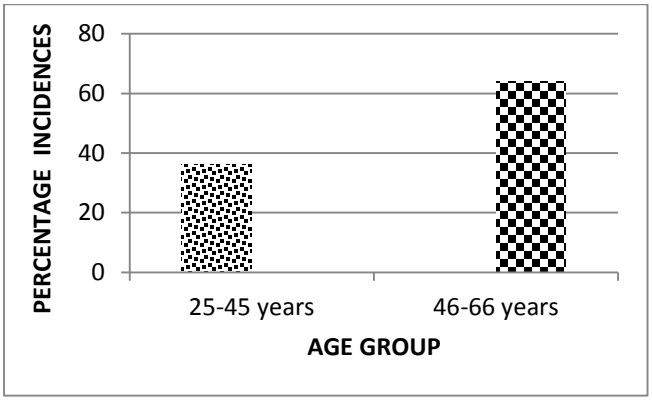

Figure 2: Age wise distribution of patients according to frequency

Figure 2 represents frequency distribution of patients according to age which were divided into two groups i.e.; 25 to 45 years in which low percentage (36%) of patients shown and higher percentage (64%) of patients shown in 46-66 years of patients.

From 250 samples, 75 samples were confirmed by

morphological confirmation, biochemical tests and microscopic analysis with those of *Klebsiella pneumoniae*. The gender wise distribution of positive samples is described in table 3.

Table:3 Total number of positive strains according to gender

Category	No. of samples
Male	47
Female	28
Total	75

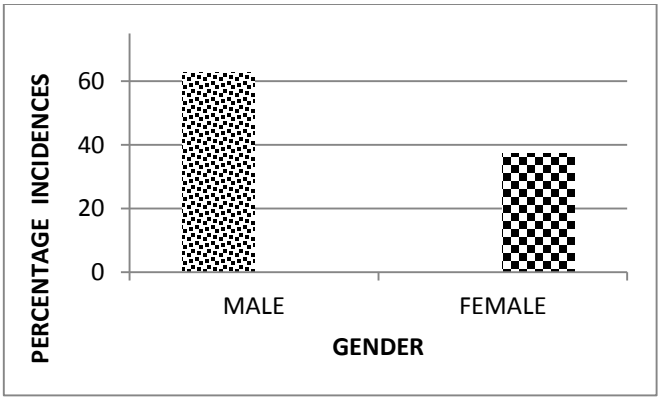

Figure 3: Show percentage of positive strains according to gender which were 62.66% of male and 37.33% of female respectively

On the basis of qualitative analysis different results are shown by biofilm forming isolates. According to the results of biofilm formation ability, the isolates were distinguished into three categories i.e.; strong biofilm formers, weak biofilm formers and no biofilm formers. From 75 confirmed isolates of *Klebsiella pneumoniae,* 17 show strong biofilm formation, 33 show weak biofilm formation and 25 show no biofilm formation.

Table:4 Biofilm forming ability of *K. pneumoniae* isolates

Category	No. of samples
Strong	17
Weak	33
None	25

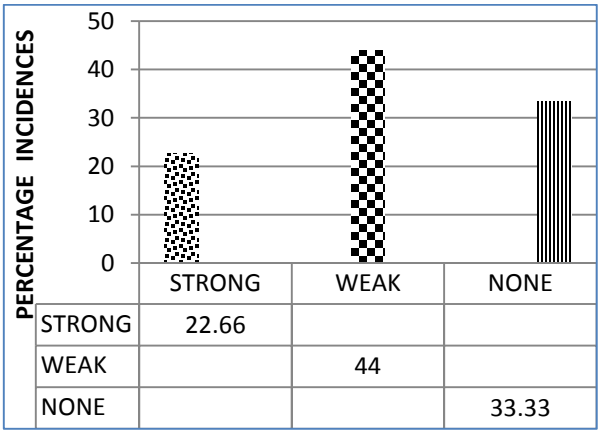

Figure 4: Categories of biofilm forming *K. pneumoniae*

Figure 4 show percentage of incidence of strong, weak and no biofilm formers as 22.66%, 44% and 33.33% respectively. Thus the total number of biofilm forming *Klebsiella pneumoniae* are 50 out of 75 confirmed isolates which are 66.66% respectively.

Table 5: Total number of biofilm forming *Klebsiella pneumoniae* found sensitive and resistant against drugs used

Antibiotic Used	Concentration	Sensitive	Resistant
Aztreonam (ATM)	30ug	22	28
Ciprofloxacin (CIP)	5ug	8	42
Nitrofurantoin (NIT)	300ug	20	30
Gentamicin (GEN)	10ug	35	15
Amikacin (AK)	30ug	44	6
Tazocin (TZP)	110ug	43	7
Fosfomycin (FOS)	200ug	21	29
Imipenem (IMP)	10ug	41	9
Sulzone (SCF)	20ug	42	8
Augmentin (AMC)	30ug	4	46

The antimicrobial susceptibility pattern against 50 biofilm forming *Klebsiella pneumoniae*, isolates were divided into two groups i.e.; sensitive and resistant. Out of 50 biofilm forming *Klebsiella pneumoniae* isolates 41 show multi-drug resistance (MDR) which are 82% respectively. Out of 10 drugs that were used Amikacin, Tazocin and Sulzone were found to be most sensitive against *Klebsiella pneumoniae* while Augmentin and Ciprofloxacin were found to be most resistant against *Klebsiella pneumoniae.*

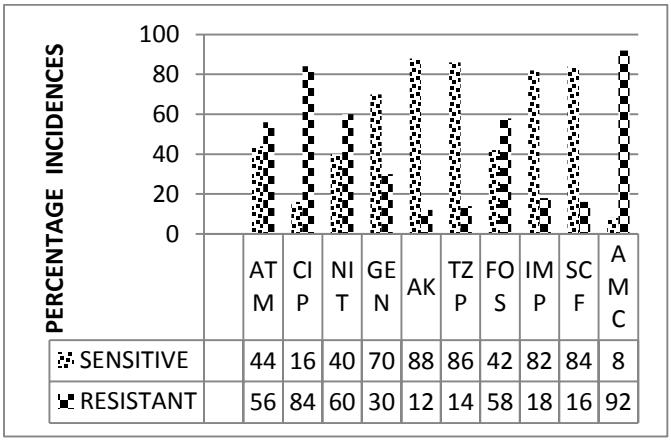

Figure 5: Comparison of antibiotics on basis of their susceptibility pattern

DISCUSSION

(P. Nauclér, M. Kalin, et al., 2018) showed that *Klebsiella pneumoniae* is the most common Gram-negative pathogen associated with the broadest range of infections, including urinary tract infections, second to *Escherichia coli* (UTI). Infections due to *Klebsiella pneumoniae* are associated with comorbidities, such as cancer, diabetes. High fatality rate of 18% to 49% has been identified with more recent research due to multi- drug resistant isolates. Population-based research of *Klebsiella pneumoniae* related infection 2000-2007 describes that low prevalence of antimicrobial resistance indicates a rise in disease load over the past decade with fatality rate of 19% and rate of death of BSI due to *E. coli* was 11% in the same demographic region 2000-2006. *Klebsiella pneumoniae* causes invasive infection which affects patients with high co-morbidity.

The prevalence of this pathogen has been markedly increased since 2001 which we concluded from this present study. Hospital-acquired infections have increased since in the last decade the patients were 19% and now in the present study 30% of the samples were *Klebsiella pneumoniae* positive. This proves that there is marked increase in the infection rate related to *K. pneumoniae* as compared to previous studies.

According to Shiri, antibiotic resistance issues have grown into an albatross on the neck of physicians, veterinarians and infection prevention officers attempting to treat and avoid infection triggered by microorganisms. These species or superbugs return to new forms which virtually immune to all clinically applicable antimicrobials. *Klebsiella pneumoniae* is an important *Enterobacteriaceae* considered to be one of the opportunistic pathogens which cause large disease spectra and increasingly show antibiotic resistance. (Clement Yaw Effah et al. 2017) has studied the prevalence of drug resistance in *K. pneumoniae* which were Amikacin (40.8%), Aztreonam (73.3%), Ceftazide (75%), Cefotaxime (69.8%), Colistin (2.9%), Cefotaxime (79.2%), Cefepime (72.6%) and Imipenem (90%). Some of the resistance mediated genes found were TEMs (39.5%), SHV-11, (41.8%) and KPC-2 (14.6%). The most virulent factors of *K. pneumoniae* are: hypermucoviscous and mucus viscosity related genes, lipopolysaccharide biosynthesis genes, iron absorption and transportation genes and finally adhesive genes.

In addition to this extensive research, the work we have done in this respect shows that out of 10 drugs the multi-drug resistance in *Klebsiella pneumoniae* was Amikacin (12%), Augmentin (92%), Aztreonam (56%), Ciprofloxacin (84%), Fosfomycin (58%), Gentamicin (30%), Imipenem (18%), Nitrofurantoin (60%), Sulzone (10%) and Tazocin (14%). It can be concluded that the antibiotic resistant in *K.pneumoniae* is very clear and this presents danger that requires strong surveillance in order to mitigate this threat. It is very important to track and report improvements in antimicrobial-resistant isolates for public health departments.

(Claudia Vuotto et al. 2017) showed that *Klebsiella pneumoniae*, the Gram negative opportunistic pathogens, causes a range of community related and nosocomial infections and usually infects patients with indwelling medical devices, particularly urinary catheters, in which the microorganism can develop as a biofilm. Due to great increase in antibiotic resistance by *K. pneumoniae* strains, this multi-drug resistant pathogen spreads worldwide, mainly at the hospital level. This condition is exacerbated when it appears that the internal resistance to antimicrobial agents is greatly increased as *K. pneumoniae* grow as biofilms. The growing proof of *K. pneumoniae* as biofilms primarily on medical devices and the recent evidence supporting the association of such actions with the acquisition of antibiotic resistance should be further warned about the dangers of this pathogen in hospital settings.

75 samples showing positive results for the said pathogen are based on the results of this analysis. 50 of 75 isolates confirmed indicate a 66.66% biofilm formation. Of the 66.66%, 22.66% show strong formation of biofilms and 44% show weak formation of biofilms, which explains the increase in antibiotic resistance of *K. pneumoniae* due to biofilm formation. In this study it was also concluded that out of 50 biofilm forming *Klebsiella pneumoniae* isolates, 41 (82%) exhibit multi-drug resistance (MDR). Exploring these virulence factors and researching new mechanisms for their regulation may also be a significant method for countering nosocomial *K. pneumoniae* infections. In particular, the mode of biofilm growth makes bacteria more resistant to antibiotic therapy by up to 1,000 times. To date, some associations have been shown between antibiotic resistance and biofilm forming ability.

CONCLUSION

It is concluded from this study that unnecessary catheterization along with prolonged admission in hospital can lead to urinary tract infection. Most organisms causing urinary tract infection in hospitalized environment are Gram-negative microbes and it is observed that *Klebsiella pneumoniae* is one of the leading organisms causing urinary tract infection. It is also seen in this comprehensive study that most of the biofilm forming *Klebsiella pneumoniae* are multi-drug resistant (MDR). The unnecessary and inadequate use of antibiotics leads to the prevelance of MDR *Klebsiella pneumoniae* in the hospital settings. Augmentin and Ciprofloxacin were found to be the most resistant in most patients, hence they are ineffective in the treatment of patients suffering from urinary tract infection caused by *Klebsiella pneumoniae* therefore these drugs should be avoided. It is very important for public healthcare departments to monitor and report changes in biofilm forming antimicrobial-resistant isolates.

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