

Pattern of Multi Drug Resistance with Biofilm Formation among *Klebsiellapneumonia* isolated from Fecal Samples of Diarrheal Iraqi Patients

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Abstract

Klebsiella pneumonia (*K. pneumonia*) is an opportunistic pathogen, Most of its cause of health care associated infections (HAIs) and possess high stages of antibiotic resistance. These microorganisms are great recognized for his or her capacity to provide biofilms. The cause of this take a look at is to decide the sample of antibiotic resistance and the capacity to provide biofilms of *K. pneumoniae* from fecal samples of diarrheal sufferers in a few Hilla hospitals. Bacteria collected was isolated from patients with diarrheal cases in some Halli hospitals form the period (September-October 2020). All of them were collected from both sex and differenced ages (<10- 60) years and it isolates were cultured and diagnosed by ordinary method as well as by the Vitek2 system also, Antibiotic sensitivity test (AST) were done using Vitek2 system and biofilm production detected by Congo-red agar method. From one hundred fecal samples, 75 (75%) *K. pneumoniae* isolates were obtained from 25 male (33.3%) and 50 females (66.6%) . All bacteria isolates were resistant to Ampicillin at (100%) and more sensitive to Ertapenem, Imipenem, Piperacillin/Tazobactam, Amikacin, Tigecycline at (100%). 50 (66.6%) MDR isolates were obtained from *K. pneumoniae* and 52 (69.3%) biofilms products isolates with 23 (30.6%) non-biofilms were produced. Significant difference in ($p < 0.05$). from the current study, Most of the *K. pneumonia* bacteria isolates have proven resistant to a wide range of tested antibiotics and are biofilm-producing. This was a strong correlation between MDR ability and Biofilm formation in *K pneumoniae*.

Key words: *K. pneumonia*, Multi drug resistance (MDR), Biofilm Production.

1. INTRODUCTION

The *Klebsiella* genus is one of the earliest members of the Enterobacteriaceae family, first reported in 1885 via way of means of the German microbiologist Edwin Klebs (1836- 1911) have been first-ever *Klebsiella* strain was a capsulated bacillus [1]. Bacteria is a gram- negative, lactose fermenting, non-sporulation, non-motile, oxidase negative with a striking capsule of polysaccharides [2]. It was present anywhere in nature, which people colonize the skin, gastrointestinal tract, or pharynx, urine, sterile wound, also in lots of elements of the biliary tract, nostril also mouth is probably taken into consideration as ordinary flora [3]. Also, it's opportunistic pathogen related to each community-received also nosocomial infections, inflicting pneumonia, abscess, bacteremia, also urinary tract infections [2]. Recently, different microorganisms [*K. pneumoniae* like] evolve multi- drug-resistant, It has attracted increasing interest globally as an infectious microorganism due to the most recent upward push within a wide range of excessive bacterial infections antibiotic resistance, The problem of preparing powerful therapies is also

developing. It is now the second most unusual location for the cause of gram-poor bacteremia and also the first-order pathogen in contamination a hospital receives, especially in immunocompromised patients[4]. Bacteria generate special enzymes that deactivate particular also goal elements of antibiotic. Beta-lactam are commonly the focutilized one by means of produced enzymes, even as a few goal different drug classes, as well as fluoroquinolones, aminoglycosides, trimethoprim, also sulfamethoxazole. These enzymes together with Metallo-beta-lactamases, oxacillinases, prolonged range beta-lactamases, also *K. pneumonia* carbapenemases, also not the same special enzyme. These enzymes stand coded on the *K. pneumonia* plasmids[5]. *Klebsellia* has a few virulence elements together with tablet polysaccharide, lipopolysaccharide, kind 1 also kind three fimbriae, outside tissue proteins, also factors for iron acquisitions also nitrogen gas supply usage. *K. pneumoniae* utilized those virulence elements for be alive also to stay away from the impregnable machine at some point of contamination in addition to biofilm formation itself [6, 7]. *K. pneumoniae* can produce a heavy sheet of extracellular biofilm that helps the microscopic engagement to dwelling or non-dwelling surfaces, protective antibiotics penetration, also lowering its effects [8]. The antibiotic surrender of developed microscopic biofilm is 10–1,000 instances that of planktonic microorganisms, also microorganism in biofilms can withstand also phagocytosis, making them very tough to eliminate [9]. *K. pneumoniae* also *K. oxytoca*, each commensal of the human gastrointestinal tract, every so often reason diarrhea in people. Some of those diarrhea genes encode the thermos table or thermo labile toxin [10]. However, the position of those pollutants within the pathogenesis of *Klebsiella*-related diarrhea has now no longer been clarified. There have been no sequences detected on this isolate that has been homologous to genes of input pathogenic *E. coli* traces that code for thermolabile also thermostable pollutants, that are produced via way of means of a few *K. pneumoniae* traces [11, 12]. Around the world, antimicrobial resistance in the latest healthcare representing a developing issue. Several pathogenic microorganism traces are Multidrug-resistant (MDR), which are quite simply turning into widespread, that forming an excessive chance to patients. Today, the *K. pneumoniae* was taken into consideration the maximum famous species of microorganisms that generate health care problems [13]. Aims this study a look turned into to pick out the connection between antibiotic resistance styles also the biofilm-formation ability of *K. pneumoniae* fecal isolates from diarrheal humans.

2 . MATERIALS AND METHODS

2.1. Collection of sample

One hundred stool specimens had been gathered in disposable, smooth screw-capped, commercially to be had packing containers utilized for this purpose. All the specimens had been processed right away or utilized Carry Blair shipping media if not on time for 1-2 hours after their series after which cultured. The current examine diarrheal isolates of *K. pneumoniae* from diarrhea sufferers to each intercourse at distinct age group from AL-Hilla Teaching, AL- Qasim General Hospital, also Al-Hashimiya General Hospital from September-October 2020.

2.2. Isolation of microorganism

Fecal samples had been cultured in enrichment media (heart infusion broth) had been incubated at 37C for 24hrs. then the bacterial growth was cultured to automatically

media on (Nutrient also MacConkey agar) had been incubating at 37 °C for 24hrs. The positive growth culture was counted according to count bacteria, with biochemical check, staining, also microscopic exam had been done. Bacterial isolates recognized via way of means of the automatic gadget VITEK 2 to attain the very last diagnostic. The identity with VITEK 2 consists of an ID-GN card for gram-poor microorganisms.

2.3. Biofilm Formation Assay

1- Congo Red agar Test. Brain Heart Infusion Broth, agar supplemented with 50gm/l sucrose also 8gm/l Congo red had been organized consistent with [14]. Then study the end result as following: if the microorganism shaped black colonies with a dry crystalline consistency that changed into implying it biofilm manufacturer isolates whilst if it shaped crimson colonies that changed into implying the non-biofilm manufacturer isolates [15].

2- Tissue culture plate Test (TCP): (additionally referred to as semi-quantitative microtiter plate check (biofilm assay) defined via way of means of [16] changed into maximum extensively utilized also changed into taken into consideration as preferred check for detection of biofilm formation as follow:

1- Isolates from purified agar plates were added to TSB containing 1% glucose, then incubated at 37 °C for 18 hours, then diluted in a ratio of 1:100 with pure TSB.

2- sterile, polystyrene, 96 pcs. The posterior biological tissue wells are filled with 150 µl of purified culture medium and a fine soup is used as a function to check the binding of unknown substances. Each subdivision turned into three vaccinations. After incubation for 4 hours at 37 °C

3. After growth, gently touch the plate to remove all the contents. Wash the springs 4 times with phosphate saline (PBS pH 7.2) to remove airborne bacteria.

4. Biofilms formed by adherent 'sessile' organisms in plate were fixed by placing in oven at 37°C for 30min

5. All sources are stained with 5-crystal violet (0.1% w/v). The dyeing process is also done by washing thoroughly with water extracted from the water, which is kept drying. 6-150 µl acetone/ethanol (20:80, v/v) binding bond. It gives a blue melting. Optical Quantity (O.) 630nm had been recorded also the effects had been interpreted consistent with a table (1).

Table 1: Interpretation of biofilm formation Mathur et al (2006).

Mean of OD value at 630nm	Adherence	Biofilm formation
<0.120	non	Non
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

2.4. Antibiotic testing by VITEK-2 Compact

Antibiotic trying out become accomplished with the automatic VITEK-2 compact gadget primarily based totally on MIC method dedication through the

usage of also AST-N222 playing cards gram-negative. These playing cards contained the subsequent Antibiotics, Ampicillin, Piperacillin/ tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, Trimethoprim/sulfamethoxazole.

2.5. Statistically study

Frequencies and percentages were used to explain the variables in this study. Associations among the antibiotic sensitivity sample also the biofilm generating potential of *K. pneumoniae* had been examined through Chi-rectangular checks the usage of (SPSS VER.16). The effects had been offered as incidence ratios with a 95% self-belief interim. Statistically importance become set if p-value .

3. Results and Discussion

3.1. Identification of *Klebsiella pneumoniae*

From September-October 2020, 75(75%) *K. pneumoniae* isolates have been tested from 100 general fecal bacterial isolates also 25% isolates of different Enterobacteriaceae (10% showed *Escherichia coli*, 10% showed *Raoultella ornithinolytica* also 5% showed *Citrobacter sedlakii*) Figure (1) display those results.

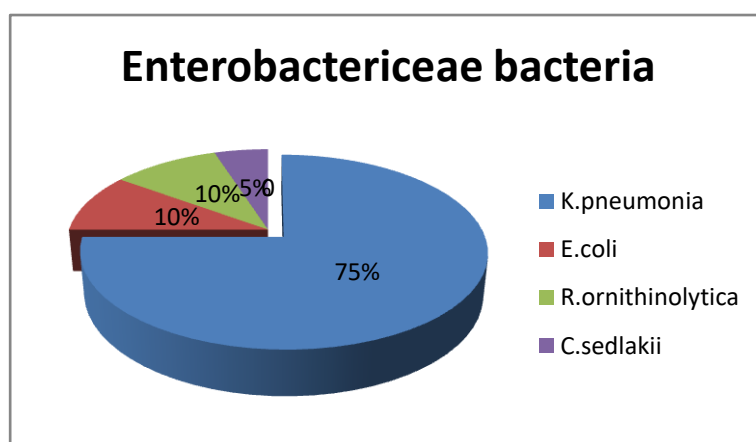


Figure (1): Number also Percentage of Enterobacteriaceae isolates from Human Diarrhea

These results, the percentage of *K. pneumoniae* separates becomes 75% of the overall fecal samples bacterial isolates. This end result becomes in line with [17] who located that approximately 65% of inpatients with diarrhea are precipitated through *K. pneumoniae*, also this outcome different from [18]. How based 22% *K. pneumoniae* lines precipitated diarrheal in sufferers. It is one of the maximum not unusual place for Gram-terrible pathogen located in human's nasopharynx also within the intestinal tract [19]. Also due to the fact this microorganism have lipopolysaccharide represents a critical also critical issue in bacterial pathogenicity, especially *K. pneumoniae*, as it's milestones of the superficial compositions of

microorganism that assist it to withstand phagocytosis, also it's miles characterized through its capability to set off the supplement factor [19]. Virulence of *K. pneumoniae* is related to the presence of capsule also pili, to the manufacturing of lipopolysaccharides also siderophores, to allantoin utilization, also to iron uptake systems, efflux pumps, also kind VI secretion systems [20]. Also, it have the pili is taken into consideration essential to the virulence of *Klebsiella*, because it protects the bacterium from phagocytosis also forestalls the microorganism through bactericidal serum factors [19]. Also, amongst this outcomes microorganism isolates had been accumulated from special long-time sufferers however the maximum isolates had been on the age <10 [21]. *K. pneumoniae* is located with inside the intestinal flora of healthful individuals, however generally in small numbers [22].

Enterobacteriaceae considered as the maximum place pathogens remoted from fecal samples in growing countries. Underlying situations including malnutrition, loss of secure water, insufficient sanitation also of diarrhea spreading in growing countries [23]. Because of that, *K. pneumoniae* had been isolated in excessive numbers from the small bowel of people with acute diarrhea [24] also malnourished youngsters who've persistent diarrhea [25].

All the fecal samples had been collected from 25 males (33.3%) also 50 females (66.6%). Most of *K. pneumoniae* obtained from sufferers with diarrheal instances of various a long time (table 2).

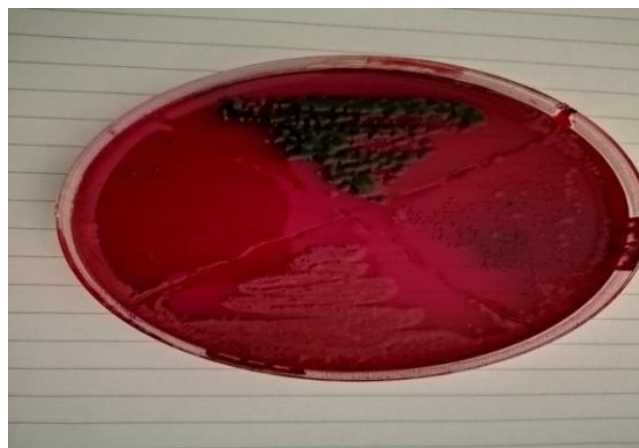
Table (2): Demographic characteristics of patients with *K. pneumonia* infections

Patient profile	Status	No . of <i>K.pneumoniae</i> (n=75)	No .of <i>K. pneumonia</i> (%)	X ²	P value
Age group (Years)	<10	30	40%	16.66	0*
	10-20	0	0		
	20-30	10	13.3%		
	30-40	15	20%		
	40-50	10	13.3%		
	50-60	10	13.3%		
Gender	Male	25	33.3%	46.8	0*
	Female	50	66.6%		

X² : chi-square test, * significant difference (P<0.05)

3.2. Biofilm Formation Discovery

Among the 75 *K.pneumoniae* isolates tested, Appeared on Congo Red agar 52(69.3%) isolates as biofilm production also 23(30.6%) isolates that have been now no longer biofilm producers (Fig 2) suggests those results. This end result similar with [26]. The ability to form biofilm appears differently from one isolation to another due to the fact there are numerous elements such as physical and chemical factors. From the characteristics of *K. pneumoniae*, the physical interaction among Plug, form of the floor in which biofilm suspends, temperature, pH, etc. [27].



(Figure 2) *K.pneumoniae* biofilm formation on Congo Red Agar

Those results , among the fifty two (69.3%) *K.pneumoniae* biofilm maker show result by tissue culture plate 35(46.6%) separates as solid, 15(20%) separates as moderate 2(2.6%) separates as weak biofilm makers (Table 3). This consequence remained in line with Nirwati et al., 2019. it had been rumored that out of the 167 *K. pneumoniae* tested, forty five isolates (26.95%) were known as high or medium biofilms also fifty separates (29.94%) were weak biofilm makers. The ability to create biofilms was totally different for every isolate as a result of many factors typically have an effect on the capacitance appreciate the physical also property of *K. pneumoniae*, the physical interface between machineries, the kind of external on that the biofilms adhere, temperature, pH, etc. [27].

Table 3 Biofilm manufacturing capability of *K.pneumoniae* isolates by Tissue Plate Culture.

Characteristics	Number(%)
Non-biofilm producer	23(30.6%)
Strong biofilm producer	35(46.6%)
Moderate biofilm producer	15(20%)
Weak biofilm producer	2(2.6%)
Total	75(100%)

3.3. Antibiotic susceptibility profiles of *K. pneumoniae*

Most of *K.pneumoniae* have been resistant to wide range variety of examined antibiotics. All *K. pneumoniae* strain more resistance against Ampicillin at (100%) also touchy to Ertapenem, Imipenem Piperacillin/Tazobactam, Amikacin, Tigecycline at (100%). As well as this bacteria resistance to Cefazolin (86.6%), Ceftriaxone, Ceftazidime, Cefepime, Trimethoprim/Sulfamethoxazole (60%) resistant respectively (Table 4) indicates those consequences. This document is supported through the observation performed by [21]. Who found were more resistances to Ampicillin [100%]. As nicely as we consequence comparable with [28] from wherein excess touchy to Piperacillin/Tazobactam, Imipenem also Amikacin. Exposure to antibiotics is the maximum crucial issue in antimicrobial resistance. Numerous factors including antibiotics used in hospital, in the community, or even in animal production,

agriculture, as well as the environment have led to the flourishing of antibiotic resistance. Given the reality that antibiotics may be sold unfastened without a prescription, also antibiotics are utilized excessively within the society environment, it's miles very in all likelihood that the heavy also extended use of antibiotics is the number one issue with inside the unfold of contamination also resistance to difficult-to-deal with antibiotics[29]. Among the antibacterial agents are circulating transmissible plasmids, which may also carry determinants of virulence. The capsule is usually referred to as a clear polysaccharide inside *K. pneumoniae* isolates. The phenotype of mucosal capsules is an important virulence factor for *K. pneumoniae*. The plasmid gene that regulates the mucosal phenotype (rmpA) has been found to give *K. pneumoniae* [6]. Which reported that 100% of *K. pneumoniae* was highly resistant, has been shown to ampicillin, the third generation of cephalosporin and aminoglycosides.

(Table 4) Antibiotic sensitivity profile of studied *K. pneumoniae*

Classes	Antibiotics	Resistance rate %	MIC	Sensitive rate %	MIC
B-Lactam	Ampicillin	100%	>16	0	0
	Piperacillin/Tazobactam	0	0	100%	≤ 4
	Cefazolin	86.6%	>32	13.3%	≤ 4
	Ceftriaxone	60%	>32	40%	≤ 1
Cephems	Cefoxitin	33.3%	>32	66.6%	≤ 4
	Ceftazidime	60%	8	40%	≤ 1
	Cefepime	60%	2	40%	≤ 1
Carbapenems	Ertapenem	0	0	100%	≤ 0.5
	Imipenem	0	0	100%	≤ 0.25
Aminoglycosides	Amikacin	0	0	100%	≤ 2
	Gentamicin	20%	>8	80%	≤ 1
Fluorquinolones	Ciprofloxacin	6.6%	0.5	93.3%	1
	Levofloxacin	6.6%	>4	93.3%	1
Glycylcycline	Tigecycline	0	0	100%	1
Nitro furans	Nitrofurantion	46.6%	128	6.6%	32
Sulfonamides	Trimethoprim/Sulfamethoxazole	60%	>160	40%	≤ 20
X ²	585.79				
P value	0*				

X² : chi-square test, * significant difference (P<0.05)

3.4. Relationship between Biofilm formation and Multi Drug Resistant

K. pneumoniae MDR isolates have been discovered in 50 (66.6%) isolates also 25 (33.3%) isolates have been non-MDR (Table 5). There became no tremendous affiliation between *K. pneumoniae* MDR also biofilm manufacturing ability primarily based totally at the statistically evaluation the use of chi-square tests. These antimicrobial-resistant micro organisms have turn out to be a global hassle also there may be nonetheless very confined facts concerning biofilm generating ability also antimicrobial resistance of *K. pneumoniae*. This observation confirmed that

antibiotic resistance become the biggest among *K. pneumoniae* so it's far a biofilm manufacturer from a non- biofilm product. This end result has been said in numerous research. A studies by [30, 31] highlights for excessive drug resistance *K. pneumoniae* the capacity to supply pulmonary biofilms is associated with antibiotics resistance profile. The general occurrence of MDR *K. pneumoniae* isolates on this observes become 50 (66.6%). Some preceding research support this excessive occurrence of MDR *K. pneumoniae* [32].

(Table 5) Relationship between Multi Drug Resistant (MDR) with Biofilm Producing among *K. pneumoniae*.

Resistance of Antibiotic classes	Biofilm producer	
	Positive	Negative
≥ 3 classes	39(84%)	11(16%)
<3 classes	13(52%)	12(48%)
Total	52	23
X ²	5.29*	
P value	0.021	

The MDR pathway encountered by microbes constitutes a major task in infection, and therefore, it is essential to monitor as well as improve screening also with antibiotics through antibiotic stewardship programs. Several research have proven that remedy with a set of antibiotics can allow save you new resistance from rising traces wherein remedy screw-ups are typically observed in those who get hold of antibiotic remedy simple as soon as that still crucial for physicians also microbiologists collaborate to make it also emphasized strong pollution control [33]. *K. pneumoniae* isolates that confirmed resistance to 3 or extra specific training of antimicrobials have been categorized as multidrug-resistant (MDR) *K. pneumoniae* [34]. Biofilm formation is a be alive approach for micro organism also fungi to conform to their dwelling environment, specifically within the adverse environment. Under biofilm protection, microbial cells in biofilms grow to be tolerant also proof against antibiotics also impregnable responses, which will increase the problems of the medical remedy of biofilm contamination. Clinical also laboratory examinations confirmed a clean link among biofilm contamination also clinical foreign bodies or static devices. Clinical also experimental observations [35]. It confirmed that each one biofilm generating isolates supplied extra resistant styles as compared to the non-biofilm producers, however, no matter this end result, the protection mechanisms in biofilms vary from the ones accountable for traditional antibiotic resistance [36]. In biofilms, it's far assumed that the protecting masking of the adhesive biomaterial results in terrible antibiotic penetration, adaptive responses to stress, also the formation of everlasting cells a multilayered defense, which will increase the issue of eradication, specifically whilst blended with the resistant nature of the microorganism itself [37, 38]. It seems that antibiotics resistance as well as bacterial capacity to biofilm formation. It performs a critical function in the international spread of *K. pneumoniae*, and to date the clear link between these elements has not been diagnosed in detail either. This finding is supported by [39] This has been showing via way of means of numerous research in a few cases, the antibiotic remedy isn't sufficient to remove biofilm-forming infections. Also consequently, presently available contamination control antibiotics have grown to be also the

consequences have been evaluated crucial also pressing protocols for a hit remedy from biofilm-associated infections [40]. Generally, an allergy to antibiotics is vital to test. Collect medical specimens previous to antibiotics control is likewise a vital point. Many medical doctors individuals who prescribe antibiotics do now no longer absolutely apprehend if simplest their beside the point recipes may want to have an impact on the improvement of bacterial resistance. Initial settings Antimicrobial remedy primarily based totally on medical microbiology the end result will lessen choice stress on microorganisms in instances of contamination in hospitals. Thus, it's far from the high significance of each hospital antibiotic counseling or supervision application for all pharmacists also medical doctors at the maximum correct foundation microbiological data. In conjunction with this directive's consistent attempt in tracking hospitals also infection control, medical audits have to be achieved to combat the speedy improvement of antibiotic-resistant pathogens [36].

4. Conclusion

Most of the *K. pneumonia* bacteria isolates have proven resistant to a wide range of tested antibiotics and are biofilm-producing. This was a strong correlation between MDR ability and Biofilm formation in *K. pneumonia*.

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