IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 4, Issue 5 (Sep. – Oct. 2018), PP 01-05
www.iosrjournals.org

A Study on The Enterobacteriaceae Pathogen Klebsiella Pneumoniae Isolated From Sewage And Drinking Water Environment

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Abstract: *The* ***Enterobacteriaceae*** *are a large family of Gram-negative bacteria. This family is the only representative in the order* ***Enterobacteriales*** *of the class Gammaproteobacteria in the phylum Proteobacteria. Enterobacteriaceae includes, along with many harmless symbionts, many of the more familiar pathogens, such as Salmonella, Escherichia coli, Yersinia pestis, Klebsiella, and Shigella.* ***Klebsiella*** *is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. Klebsiella species are found everywhere in nature. This is thought to be due to distinct sublineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects and other animals including humans. They can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections. Klebsiella species have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies. The majority of human Klebsiella infections are caused by K. pneumoniae, followed by K. oxytoca. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer, and most infections involve contamination of an invasive medical device. The present study is aimed at studying the Serotype of this Enterobacteriaceae pathogens isolated from sewage and drinking water environments.*

Keywords : *Enterobacteriaceae, Klebsiella, Serotype, Sewage and Drinking Water Environments.*

Date of Submission: 15-09-2018 Date of acceptance: 30-09-2018

I. Introduction

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines. (**Ayling Smith, B. and Pitt, T.L., 1990**). It is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae and is closely related to *K.oxytoca* from which it is distinguished by being indole-negative and by its ability to grow on both melezitose and 3-hydroxybutyrate. It naturally occurs in the soil and about 30% of strains can fix nitrogen in anaerobic condition (**Bagley *et.al.,* 1978)**. As a free-living diazotroph, its nitrogen fixation system has been much studied (**Fung *et.al.,* 2002)**. *K.pneumoniae* can cause bacterial pneumonia, typically due to aspiration by alcoholics; though it is more commonly implicated in hospital-acquired urinary tract and wound infections, particularly in immune-compromised individuals (**Kabha *et.al.,* 1997)**. Patients with *Klebsiella pneumonia* tend to cough up characteristic sputum that is said to resemble "red-currant jelly". *Klebsiella* ranks second to *E.coli* for urinary tract infections in older persons. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy and rhinoscleroma. Feces are the most significant source of patient infection, followed by contact with contaminated instruments. Members of the *Klebsiella* genus typically express 2 types of antigens on their surface. The first, O antigen is a lipopolysaccharide of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. Both contribute to pathogenicity and form the basis for subtyping (**Leung *et.al.,* 1997**). As a general rule, *Klebsiella* infections tend to occur in people with a weakened immune system formed due to improper diet patterns. Many of these infections are obtained when a person is in the hospital for some reason. The most common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia. *Klebsiella pneumonia* tends to affect people with underlying disease, such as alcoholism, diabetes and chronic lung disease (**Mizuta *et.al.,* 1983 and Orskov, I. and Orskov, F. 1984)**.

Klebsiella possesses a chromosomal class a beta-lactamase giving it resistance to amplicillin. Many strains have acquired an extended-spectrum beta-lactamase with additional resistance to carbenicillin, amoxicillin, beta-lactamase, and increasingly to ceftazidime (**Orskov, I. and Fife-Asbury, M.A. 1977).** The bacteria remain largely susceptible to aminoglycosides and cephalosporins. Varying degrees of inhibition of the beta-lactamase with clavulanic acid have been reported (**Podschun, R. and Ullman, U. 1998).** Infections due to multidrug-resistant Gram-negative pathogens in the ICU have invoked the re emergence of colistin, an antibiotic that had rarely been used for decades (**Rennie, R.P. and Duncan, I.B.R. 1974).** However, colistin-resistant strains of *K.pneumoniae* have been reported in Greek ICUs (**Seidler *et. al.,* 1975**).

II. Materials And Methodology

By plate count method 1 ml of the sample was prepared and transferred to 9 ml of saline and was maintained as master dilution. From this (10^{-1} to 10^{-6}) dilutions were prepared and 1 ml of sample was poured to cool sterilized agar count plate and incubated at 37°C for 24 hours. Colony was counted by colony counter. Morphological study was achieved by microscopic observation of Grams staining, Motility test, Catalase test and Oxidase test. A small portion of suspected colony was streaked on medias such as Nutrient Agar, MacConkey Agar and Eosin Methylene Blue Agar. Biochemical tests were performed using Standard Protocol. Following this serological typing was done. Depression plates were taken and were marked as A, B and C. In A depression plate it was marked as negative control in which phenolized saline suspension was added. In B depression plate it was marked as test in which phenolized saline suspension and antiserum of respective organism was added and in C depression plate it was marked as positive control which contain phenolized saline suspension of known organism and antiserum.

III. Result

The total number of positive and negative samples obtained from sewage and drinking water for *Klebsiella pneumoniae* was found to be 8. In identification of bacterial isolate of morphological characteristics by Grams staining and motility for *Klebsiella pneumoniae*, it was found to be lactose fermenting rods and non - motile. Cultural characteristics of *Klebsiella pneumoniae* on Nutrient Agar formed small colonies whereas on MacConkey Agar formed pink colour mucoid colonies and on Blood Agar Non Haemolytic colonies. Biochemical Test, Antibiotic Sensitivity Test and Serotype study results indicate the prevalence of *Klebsiella pneumonia* to be moderate in the Sewage and Drinking water samples collected (**Tables 1 – 4 and Figures 1 – 5**).

Table 1 : Biochemical Test for *Klebsiella pneumoniae*

S. No.	Biochemical Tests	Results
1.	Catalase	Positive
2.	Oxidase	Negative
3.	**TSI Test** Butt Slant Gas H_2S	 Acid Acid Positive Negative
4.	Indole	Negative
5.	Methyl Red	Negative
6.	Voges Proskauer	Positive
7.	Citrate	Positive

Table 2 : Antibiotic Sensitivity Test for *Klebsiella pneumoniae*

S. No.	Name of antibiotics	Zone of inhibition in mm	Interpretation
1.	Amikacin (AI)	20 mm	Sensitive
2.	Chloramphenicol (C)	18 mm	Sensitive
3.	Co – trimoxazole (CT)	17 mm	Sensitive
4.	Tetracycline (T)	13 mm	Resistant
5.	Gentamycin (G)	19 mm	Sensitive
6.	Ceftriaxone (CTR)	21 mm	Sensitive
7.	Cephotaxime (CTX)	20 mm	Sensitive
8.	Norfloxacin (NX)	18 mm	Sensitive
9.	Meropenem (MR)	10 mm	Resistant
10.	Imipenem (I)	18 mm	Sensitive

Zone of inhibition
Below 10 mm – least active
Between 11-25 mm – active
Above 26 mm – very active

Table 3 : Serotyping of *Klebsiella pneumoniae* from Sewage Sample

S. No.	Antiserum	Isolates	Result
1.	K1	**3**	Positive
2.	K2	**2**	Positive
3.	K7	**1,4**	Positive
4.	K21	**5**	Positive

Table 4 : Serotyping of *Klebsiella pneumoniae* from Drinking Water Sample

S.No.	Antiserum	Isolates	Result
1.	K1	**2**	Positive
2.	K2	**1**	Positive
3.	K7	**3**	Positive
4.	K21	**3**	Positive

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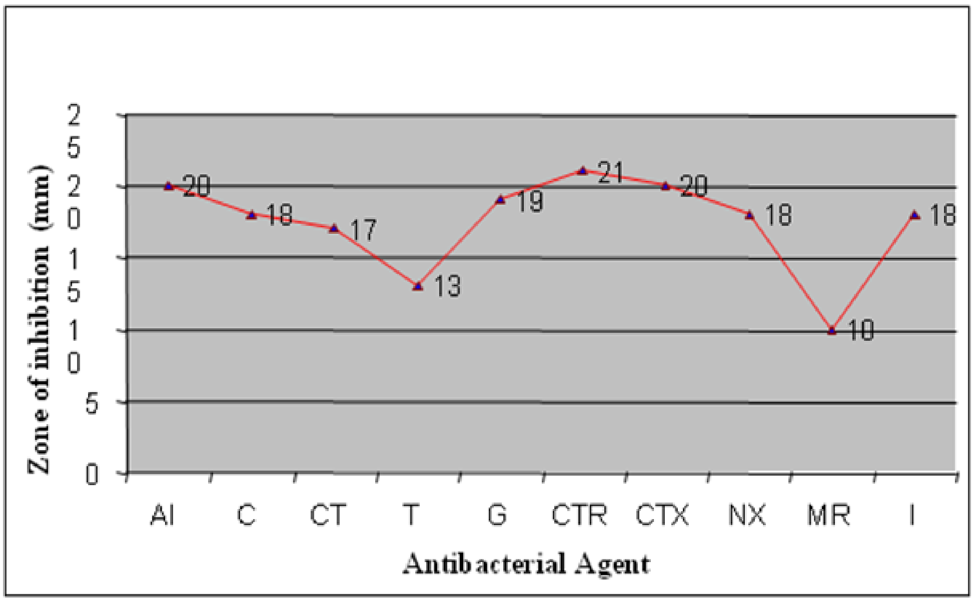
Figure 1 : Antibacterial Activity of *Klebsiella pneumoniae*



AI - Amikacin, C - Chloramphenicol, CT - Co-trimoxazole,
T - Tetracycline, G - Gentamycin, CTR - Ceftriaxome,
NX - Norfloxacin, MR - Meropenem, I – Imipenem

Figure 2 : Serotyping of *Klebsiella pneumoniae* from Sewage Sample

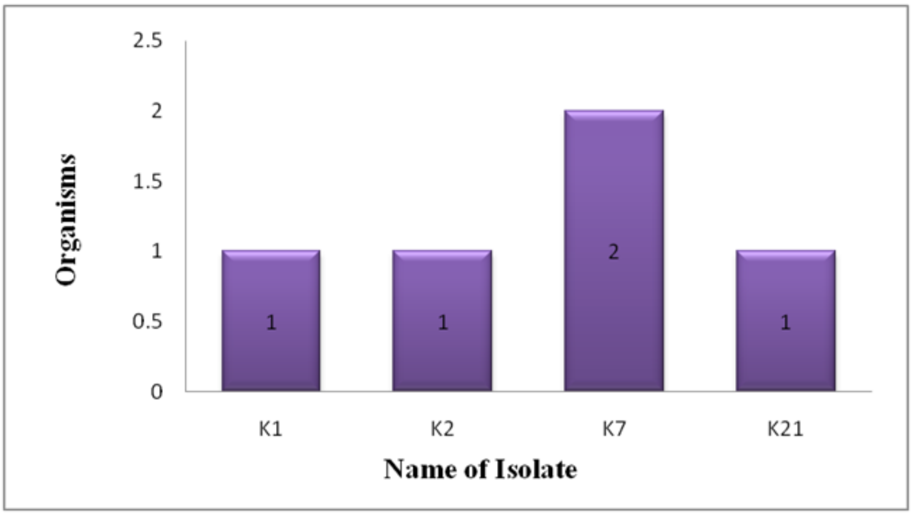


Figure 3 : Serotypying of *Klebsiella pneumoniae* from Water Sample

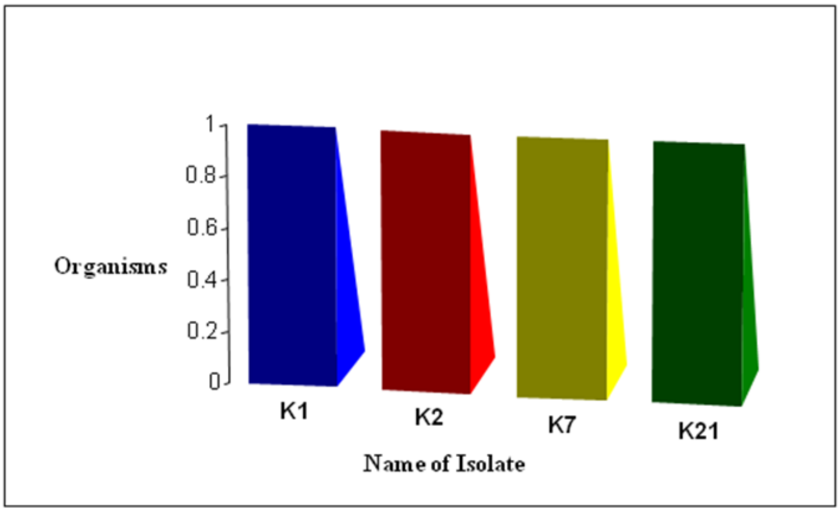
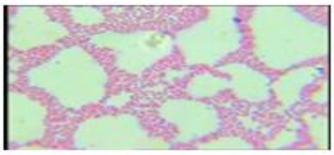


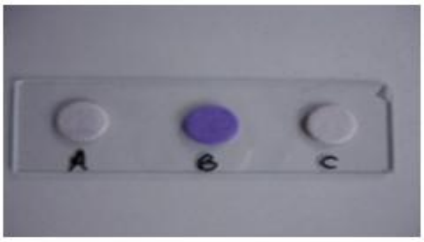
Figure 4 : Biochemical Test Results for *Klebsiella pneumoniae*



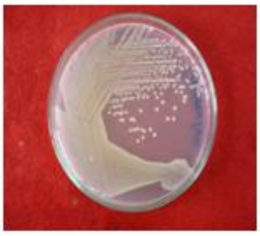
Gram Negative Rod

Positive **Negative**

Catalase Test

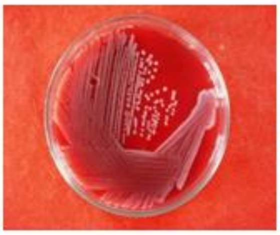


Oxidase Test
A = Negative Control
B = Positive Control
C = Test Sample



K.pneumoniae on Nutrient Agar

K.pneumoniae on MacConkey Agar

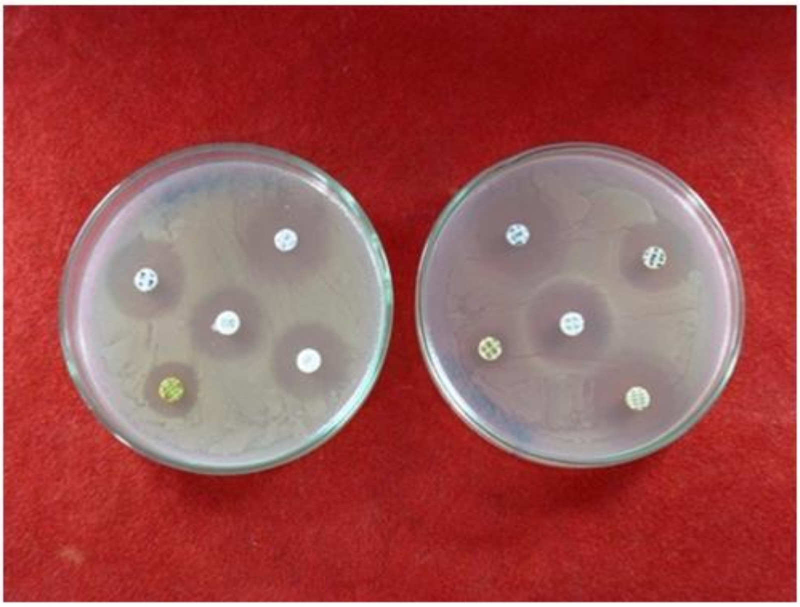


K..pneumoniae on Blood Agar

IMVIC test for *K.pneumoniae*

A = Indole
B = Methyl Red
C = Voges Proskaur
D = Citrate
E = TSI

. **Figure 5 : Antibiotic sensitivity test for *Klebsiella pneumoniae***



IV. Conclusion

Klebsiella, a Gram-negative bacteria (bacillus) causes different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections (SSI), and meningitis. This organism is found in the human intestines and is usually excreted in human stool. In healthcare settings, these infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators or intravenous catheters, and patients who are taking long courses of certain antibiotics are most at risk for infections. Some of these strains have become highly resistant to antibiotics. This occurs when bacteria such as *Klebsiella pneumoniae* produce an enzyme known as a carbapenemase (referred to as KPC-producing organisms). This species belongs to the family Enterobacteriaceae, a normal flora of the gastrointestinal tract that can become carbapenem-resistant (CRE stands for carbapenem-resistant Enterobacteriacea). Unfortunately; carbapenems are often the last line of defense against Gram-negative infections that are resistant to other antibiotics. Thus the present study was aimed at studying this Enterobacteriacea pathogen which revealed high prevalence pattern in the water samples collected from polluted environments.

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G.Archana " A Study On The Enterobacteriaceae Pathogen Klebsiella Pneumoniae Isolated From Sewage And Drinking Water Environment." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.5 (2018): 01-05.