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Treatment and Experimental Infection with *Klebsiella pneumoniae* in Rats

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Abstract

So many diseases are caused by *Klebsiella* species including urinary tract infections (UTIs), pneumonia, sepsis, diarrhea and bacteremia. Also *Klebsiella* is responsible for a significant number of community-acquired infectionssuch as pneumonia that results in severe injury in the lung and responsible for a high death ratein children. The intranasal and left lung route of *K. pneumonia*e infection causes pathological change in the lungs tissues due to acute and chronic injury. Conducted to detect the histological and immunological changes in experimental rat's lung infected by different routes with *K.pneumoniae*.

Twenty-sevenAlbino Swiss male rats (Rattus rattus) were infected with 0.2ml of *K. pneumonia* suspension in different routes. After seven days from the last injection *K.pneumonia*e, the Lung and spleen are examined for histopathology changes. The blood vessels congestion with dispersed lymphocytic cells, infiltration throughout pulmonary parenchyma parts, edema formation along with hemorrhages Early neutrophils distribute into the Broncho-alveolar space, were detected. An acute splenitis was present in rat's infection by left lung injection route after 10days, were observed in the Spleen tissue with mild white pulp within pulp hyperplasia.

What makes *K. pneumonia*e infections more difficult to treat is that they gradually became more virulent and antibiotic resistant through time.The early *K. pneumonia*e infection -induced secretion of tumor necrosis factor alpha as pro-inflammatory cytokines.The level of cytokines has been related to severity of pulmonary inflammatory process.TNF-αis important for the acute phase response as proinflammatory responses.

***Keywords**: Acute lung injury, acute splenitis and TNF-α .*

Introduction

K.pneumoniae is a gram-negative, non-motile, rod-shaped, lactose-fermenting, facultative anaerobic bacteria with a prominent polysaccharide capsule. Some strains of *K. pneumonia* have been implicated in bloody diarrhea, such cases became not easily treated as a result of the developing capability of K. *pneumonia* to resist the effects of various antibiotics [(!)].

Klebsiella known of their ability to produce enterotoxin that is heat stable and can be considered as a cause of their pathogenicity, the major virulence factors of *K. pneumonia* which play essential roles in pathogenesis include capsular polysaccharide, lipopolysaccharide, type 1 and type 3 fimbriae, and siderophores [(2)] .

Recently, *K.pneumonia*e are emerging worldwide as a Leading cause of nosocomial infections, including pneumonia, bacteremia, urinary tract infections and wound infections [(3)]. *K. pneumoniae* causes the Pneumonia disease which is recognized by an excessive macrophage and neutrophil infiltration associated with exacerbated inflammatory response, severe lung injury and high production of pro-inflammatory cytokines [(4)].

Local inflammation gives an advantage in such infections by avoiding pathogen spreading, long lasting hyper-inflammation usually comes together with death and chronic inflammatory disorders [(5)].

Other researchers determined that *Klebsiella pneumoniae* taken from bloody diarrhea has the ability to bind to cytoskeletal proteins and Henrietta lacks

cells, such as the actin that gathers at the bacterium-host contact point [6].

Antibiotic resistance of G- bacteria is related to raising death, long periods of hospitalization and hospital expenses [7].*Klebsiella pneumonia*e and other species may inhabit the pharynx, intestinal tract and the skin of humanand it also colonizes in urine, sterile wounds and might presents as normal flora in many organs such as the intestine, biliary tract and colon [8].

TNF-α(cachectin) has significant functionality as an antitumor, inflammation, anorexia, immune modulation, viral multiplication, cachexia, septic shock and platelets agglutination. Many factors stimulating the secretion of cachetin by macrophages such as bacterial infection and it can be produced by many other types of cell such as NK cells, $CD4^+$ lymphocytes, mast cells, neutrophils,eosinophils, and neurons [9].

Biofilm of *K. pneumoniae* that is found on Contaminated medical devices such as catheters is the major cause of infections in catheterized patients [10].*K. pneumonia* is a major cause of bacteraemia, and abscesses. These infections may be nosocomial, healthcare-related or community-acquired[11].

Materials and Method

The specimens were collected carefully ways to avoid contamination. The specimens were transported by sterile transport swabs to the bacteriological laboratory, and each specimen was inoculated on blood agar, MacConkey agar, then inoculated at 37°C under aerobic condition for 18-24 hrs.

Culture of Clinical specimens

The bacterial sample was obtained by taking a colony from MacConkey and blood agar and repeating it`s growth to gain a pure culture and was diagnosed according to it`s cultural and morphological characteristics,microscopic characteristics after Gram's stain, then further identified by biochemical tests [12]. The final identification was performed with automated VITEK-2 compact system using G-ve ID cards.

Animals Study:

Twenty-seven Albino Swiss male rats (*Rattus rattus*) were used as the experimental animals.there ages ranged between eight to ten weeks and 230-270 g in weight. Rats were divided into three groupsas following:

Group A: 3 rats treated with normal saline as control suspension.

Group B: 12 rats were infected with 0.2ml of *K. pneumonia* suspension in different routes. 3 rats infected orally, 3 rats infected intranasal, 3 rats intra-dermally and 3 rats infected inleft lung.

Group C:12 rats infected with *K. pneumonia*and divided in to four sub-groups to treated with antibiotics (Tetracycline, Ciprofloxacin, Amoxicillin, amoxicillin and ciprofloxacin).

Histopathological examination

Lung and spleen were removed after seven days from the last injection and fixed in 10% formalin in PBS for 24 h, dehydrated in ethanol, cleared with xylene, infiltrated and embedded in paraffin. the histological section is cut with rotary microtome. Without knowing the origin of the tissue slides, hematoxylin and eosin stained slides were scored by a pathologist for inflammatory parameters.The sections are conveyed into water bath (52C°) to plain the tissues then fixed on slides containing Glycerin-Albumin mixture, as a thin film and placed for drying in the oven at 40C° for 24 hrs. Harris hematoxylin and Eosin are used in the tissue section staining while Southgate's Mucicarmine and Periodic Acid Schiff-(PAS) stains are used to stain the polysaccharide capsule according to [13]. Then Paraffin- embedded sections from each specimen are cut into 3-5 μm thick, sections are placed on positively charged slides, and left over night to dry at room temperature, or put slides in oven 60°C for 60min for Dewaxing. according to [14].The Slides are deparaffinized by heating in an incubator at 37°Cover night.

Immunological Assay:

TNF-α, was measured in serum, plasma and other biological fluids of rats by Sandwich enzyme-linked immunosorbent assay.the ELISA kit are prepared according to the manufacturer's instructions.

Statistical Analysis

All statistical analysis was performed by using SPSS v.24 software program. Also, it was drawing histograms by Microsoft Excel 2013.

Results

Samples collection and examination:

140 clinical specimens were collected from stool, urine, burns, wounds and bone inflammation. 115 specimens are positively bacterial growth (18 specimens are *k. pneumonia*, 97 specimens are other types of bacteria) and 25 specimens were not growth. Only 10 *k. pneumonia* isolates were obtained from urine and 8 *k. pneumonia* isolates were obtained from stool. The initial identification of bacteria depended on some characteristics such as morphological colonies, Grams stain, and biochemical diagnosis.

Histopathologicalexaminationafter induce infection by *k. pneumonia* from different infection routes:

In present study, there was significantly ($p < 0.05$) between different infection routes (Intranasal, Left lung, Orally, and Intra-dermal),*K. pneumonia* causes acute inflammation in the rats lungs throughthe intranasal and left lung routes .See figures (1, and 2).

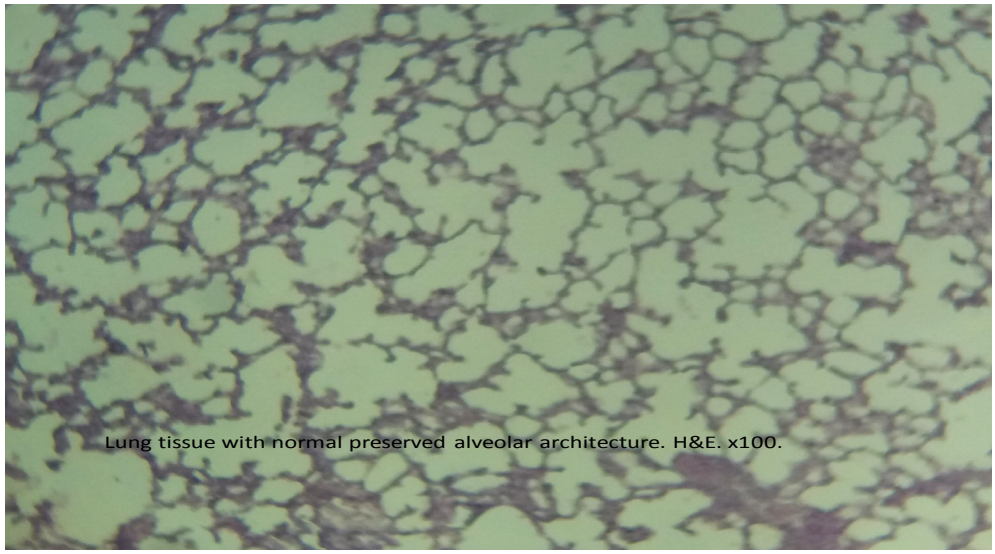

Fig. 1: Normal lung tissue in control group 40X

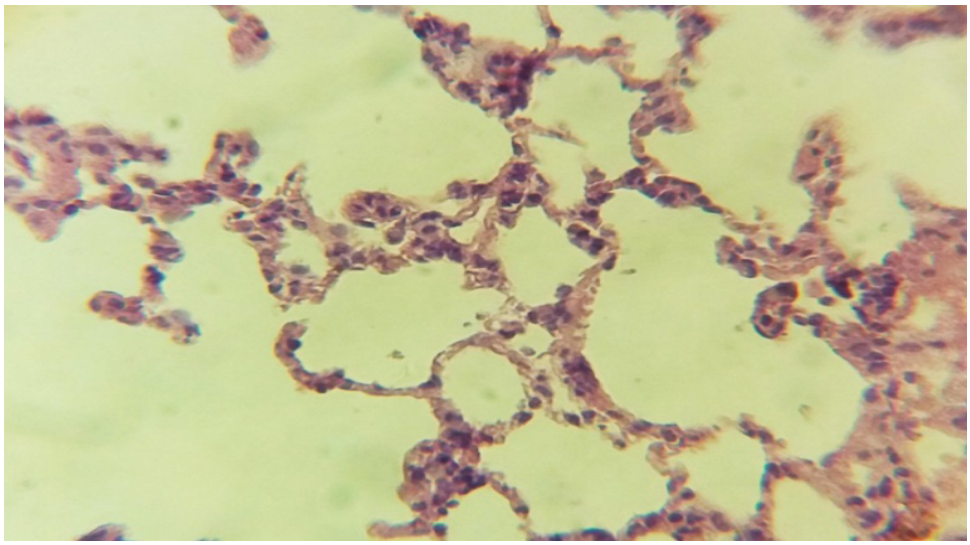

Fig. 2: Lung tissue with normal preserved alveolar architecture and alveolar wall thickness after orallyinfection. H&E. 40X.

The microscopic examination of lungs showed normal histological features (normal alveoli and interstitial parenchyma portion's) in control group, , whereas infected group by *K. pneumonia* , the lung sections showed pathological changes in the rats lung, which is characterized by blood vessels congestion with dispersed lymphocytic cells, infiltration throughout pulmonary parenchyma part's, lymphocytes are distribute in the pulmonary tissue, and lymphoid aggregates, edema formation along with hemorrhages, congestion

and severe lymphocyte and macrophage infiltration were detected. Histological examination exposed that at 10 days afterinjection of *K. pneumonia*, the rats had moderate to severe multifocal bronchopneumonia characterized by a cellular infiltrate composed almost exclusively of neutrophils and a few macrophages. These cells filled alveolar and bronchiolar spaces of affected areas. Alveolar spaces at the margin of a lesion were filled with eosinophilic proteinaceous edema fluid.

The comparison between types of cytokines with different infection routes

The comparison between different cytokines types with route infections showing TNF- α, IL6 and IL10 levels was different increases in *K. pneumonia* dose 10^5 CFU/ml in comparison to infection routes and slightly significant increased other level compared to control group, selection left lung injection and intranasal routs continuously research and experiments working.as showed in Table (1)

Table (1): Levels of pro-inflammatory TNF-α

Mean±SD TNF-α					
Control(n=3)	**Intranasal**(n=3)	**Left lung**(n=3)	**Orally**(n=3)	**Intra-dermal**(n=3)	**P- Value**
120	**189.4±1.21 ***	**1184.7±0.52 ***	**55.1±0.87**	**120.3±1.21**	**0.01**

***Significant differences = p<0.05**

Histopathological observations after infection *by K. pneumonia* from different infection routes and treatment by different antibiotics:

A histological study showed that the intranasal and intraperitoneal routes of infection with*K. pneumonia* have caused pathological changes in the lung tissues, which is characterized by congestion of blood vessels with dispersed lymphocytic cells, infiltration throughout pulmonary parenchymal components, lymphocytes are distribute in the pulmonary tissue with blood vessels blocking, and lymphoid masses(Figures 3 – 4).

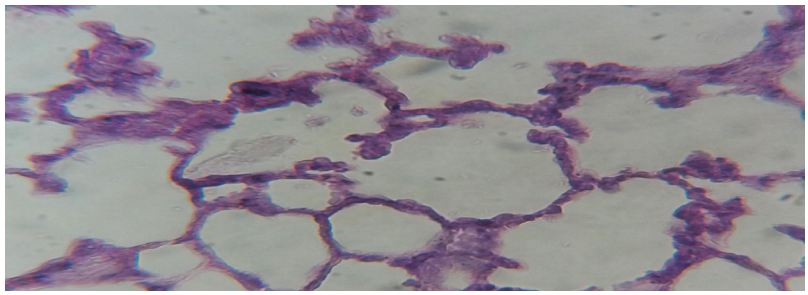

Fig. (3)Rat lungs after intra nasal introduction of predatory bacteria K.penumoniae & Lung treated with Amoxicillin. (Lung tissue with normal alveolar wall thickness and architecture and moderatecongestion of blood vessels. H&E. 40X).

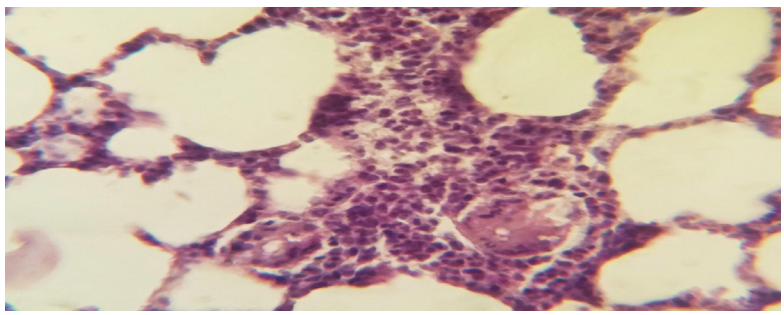

Fig. (4). Rat lungs after left lung introduction of predatory bacteria *K.penumonia&* Lung treated with Ciprofloxacin . (Lung tissue with normal alveolar wall thickness and architecture, with a focal area of acute inflammatory cell infiltration around pulmonary venules (arrow). H&E. 40X.)

Effect of antibiotics on Pro-inflammatory cytokines production.

-TNF-α (pg/ml):

The results in the Table (2) showed decreased considerablevariations p<0.05 in TNF-α concentration of treated rats with different antibiotics when compared with Infected rats, therefore the results indicated to decrease significant in Treated ratsgroups with different antibiotics CIP, AX, TET, AX+CIP when compared between them.

Table (2): levels of TNF-α cytokine before and after treatment

Groups	Infected rats N=12	Treated rats N=27			
		AX	CIP	TET	AX+CIP
Left lung injection	1185* **±	757 * **±	80 * **±	667* **±	1103* **±
	0.52	1.73	0.70	0.36	1.4
	0.31	1.00	0.41	0.22	0.80
Intranasal cavity injection	156* ** ±	54 * **±	94.2 * **±	62 * **±	1040.23 * **±
	0.90	0.17	0.61	1.62	0.97
Collected from – blood	0.52	0.10	0.35	0.93	0.56
Intranasal cavity injection - Collected from secretion	184 * **±	825 * **±	714.1 * **±	604.* **±	1011 * **±
	1.73	18.95	7.01	6.08	11.10
	1.00	10.95	4.00	3.51	6.41
Control	120 ± 0.00 Mean ± Std. Deviation 2.8 ± 0.00 Mean ± Std. Error				
LSD	10.031				

***considerable variations = p<0.05 between control and study groups.**

**** Considerable variations = p<0.05 between them groups.**

Discussion

One of four scores was the lung pathology which was assigned according to their severity of inflammation as follows: 1- mild focal inflammation, 2-normal histology, 3- moderate to severe focal inflammation with areas of normal tissue, 4- severe inflammation to necrosis [15].

K. pneumoniae is avital cause of community-acquired and nosocomial infections, so gaining attention due to the high morbidity and mortality rates and the rising number of resistant strains to the antibiotic effects [16] [23].

Lungs considered as important organ of respiratory system, is susceptible to many agents smoking, genetics, and microbial infection that are responsible for numerous pathological conditions [17].

Pulmonary infection can cause a number of histological changes in the architecture of lung, as well as in the extrapulmonary organs as in the case of bacterial dissemination via blood stream, and these changes can impact the result of disease [18].

Pulmonary infection can cause a number of pathological changes in the lung, as well as in the extra-pulmonary organs as in the case of bacterial dissemination, and these changes can impact the outcome of disease. One of the greatest limitations to the study of microbial virulence factors is the availability of relevant animal models [19].

The present study, states thatTNF- α has considerable differences in levels between control and study groups and significant differences between these groups that are shown in table (1) In comparison with [20] who found significantly increased levels of TNF- **α** in groups of animals under acute stress and chronic mild stress due to bacterialLPS compared to non-stressed control groups.

TNF- **α** is a major pro-inflammatory cytokine mediate the development of many inflammatory lung diseases andits concentrationsignificantly higher 4 to6 fold after 6 and 24 hours respectively from infection [16]. Moreover, TNF- **α**mediate lung inflammatory diseases by enhances macrophage inflammatory protein, KC secretion, play an important role in the up-regulation of cellular adhesion molecules, vasodilatation and increasing the vascular permeability [21].

TNF-α, IL-1β and IL-6 main functions are to attract neutrophils to the site of inflammation,induce the acute phase response, i.e. starting inflammatory processes such as production of APPs in the liver.Stimulated macrophages by (lipopolysaccharide LPS) secret wide array of proinflammatory cytokines such as TNF- α, IL-1_, IL-6, and IL-8 [22].

Conclusions

Klebsiella pneumoniae infection and lung diseases are important topics regarding public health interest. Neutrophils are one type of the firstinnate immune cells recruitedto the site of infection to prevent bacterial colonization and clear bacteria.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1- Gerald collee; Andrew, G.;Fraser; Barrie ,P. Marmion and Anthony Simmons. Mackie & Mc Cartney:. Practical Medical Microbiology . 2012 ;14 th edition .

2- Podschun, R.; Pietsch, S.; Holler, C. and Ulmann, U. Incidence of Klebsiellaspp in surface waters and their expression of virulence factors . Appl. Environ. Micro. 2001 ;67: 3325 – 3327

3- Tawfick, M. M.; Hamed, S. M.; Darwich, H. M, and El-Mahallawy H. A. Phenotypic and Genotypic Diversity of Nosocomial Multi-Drug Resistant Klebsiella pneumoniae Isolated from Cancer Patients in Cairo, Egypt. Int.J.Curr.Microbiol.App. Sci, 2016 ;5(7): 931-943..

4- Zhang,P.;Summer, W.R.; Bagby, G.J.; Nelson, S. Innate immunity and pulmonary host defense. ImmunolRev; 2000 ; 173:39e51.

5- Medzhitov, R. Origin and physiological roles of inflammation . Nature; 2008 ; 454:428e35

6- Guerin, F.; LeBouguenec, C.; Gilquin, J.; Haddad, F. and Goldstein, F. W. Bloody diarrhea caused by Klebsiella pneumoniae: a new mechanism of bacterial virulence. Clin. Infect. Dis., 1998 ; 27: 648-649 .

7- Schwaber, M. J.; Navon-Venezia, S.; Kaye, K. S.; Ben-Ami, R.;Schwartz, D. and Carmeli, Y. Clinical and economic impact of bacteremia with extended spectrum- β-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother, 2006; 50: 1257-1262.

8- Abdallah, M.; Benoliel, C.; Drider, D.; Dhulster, P.; Chihib, N.E. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Arch. Microbiol., 2014 ; 196, 453–472 .

9- Clark ,I. "How TNF was recognized as a key mechanism of disease". Cytokine Growth Factor Rev. 2007 ; 18 (3–4): 335–343 .

10- Cao, F.; Wang, X.; Wang, L.; Li, Z.; Che, J.;Wang, L.; Li, X.; Cao, Z.; Zhang, J.; Jin, L. and Xu, Y. Evaluation of the efficacy of a bacteriophage in the treatment of pneumonia induced by multidrug resistance Klebsiella pneumonia in mice. BioMed. Res. Int., 2015 ; 24 .

11- Aljanaby Ahmed Abduljabbar and Alhasani Alaa Hassan. Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. J. of microb.res. 2016 ; 4 (1):44-54.

12- MacFaddin, J.E. Individual Biochemical Tests for Identification of Medical Bacteria. 3th ed. Lippincott Williams Wilkins, London. 2000:57-424 .

13- Bancroft, J.D. and Stevens, A. The haematoxylin and eosin Theory and practice of histological techniques. 1996 ; 4th ed, Ch 6, pp.99–112. Churchill Livingstone, London, New York & Tokyo .

14- Zenclussen, A. C.; Lim, E.; Knoeller, S.; Knackstedt, M.; Hertwig, K.; Hagen, E., et al. Hemeoxygenases in pregnancy II: HO-2 is down regulated in human pathologic pregnancies. Am. J. Reprod. Immunol. 2003 ; 50, 66–76. 10.1034/j.1600-0897 .

15- Song Z. ; Kharazmi A. ; Wu H. et al., "Effects of ginseng treatment on neutrophil chemiluminescence and immunoglobulin G subclasses in a rat model of chronic Pseudomonas aeruginosa pneumonia," Clinical and Diagnostic Laboratory Immunology, 1998 ; vol. 5, no. 6, pp. 882–887, .

16- Tomislav,R;Vanja,V;andBrigita,T.Proinflammatory Cytokines in Anti-lipopolysaccharide Immunity Against Klebsiella Infections , Mediators of Inflammation •2005:2 (2005) 88–95 .

17- Ferdausi, T.; Haider, M.G.; Alam KJ, Baki MA and Hossain MM . Caprine lung diseases and causal bacteria. The Bangladesh Veterinarian 2008 ; 25 (1): 9-16

18- Chinchilli, V; Phelps, D; Floros, J. Sex

differences in the impact of ozone on survival and alveolar macrophage function of mice after Klebsiellapneumoniae infection. Respir Res; 2008 ; 9: 24 .

19- Khan, A. A. T. R.; Slifer, F. G.; Araujo, Y.; Suzuki, and Remington,J. S. Protection against lipopolysaccharide-induced death by fluoroquinolones. 2000

20- Manikowska, K.; Mikołajczyk M.;Mikołajczak P.;Bobkiewicz-Kozłowska T. The influence of mianserin on TNF-a, IL-6 and IL-10 serum levels in rats under chronic mild stress . Pharmacological Reports 2014 ; 66, 22–27 .

21- Sara,S.; Linlin, C.; David; O.; Gloria, P.; and Jacob, F. IL-1β Augments TNF-α–Mediated Inflammatory Responses from Lung Epithelial Cells. 2009 ; 29(5): 273–284.

22- Calandra, T., and J. D. Baumgartner. Antiendotoxin therapy, Clinical trials for the treatment of sepsis. Springer-Verlag, Berlin, Germany. 1995; p. 237–250. In W. J. Sibbald and J. L. Vincent (ed.),

23- Zahraa Hameed Oda Alquraishi1 ; Anaam Jawad Alabbasy & Aqeel A Alsadawi . Genotype and Phenotype Detection of E. coli Isolated from Children Suffering from Urinary Tract Infection. Journal of Global Pharmacy Technology. 2018; 10(1):38-45