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Isolation and Identification of Klebsiella pneumoniae from Infants with Necrotizing Enterocolitis

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ABSTRACT

Sixty four clinical isolates were collected from children with Necrotizing Enterocolitis admitted to the Children's Protection Hospital at the Medical City in Baghdad. These isolates included (41) stool samples, (14) blood samples and (9) urine samples for the period from 29/1/2018 to 4/4/2018. All the samples were cultured on MacConkey agar and Blood agar for diagnosis. All isolates were identified depending on macroscopic, microscopic, and biochemical tests, with Vitek-2 compact system. Forty three isolates were obtained from all samples; (26) isolates as Klebsiella pneumoniae (60.46%), 8 isolates as Escherichia coli (18.60 %), 4 isolates as Pseudomonas aeruginosa (9.30%), 2 isolates as Klebsiella oxytoca (4.65%), 2 isolates as Enterobacter cloacae (4.65%) and 1 isolate as Proteus hauseri (2.32%).The results showed that K. pneumoniae was the predominant in the samples taken from infants infected with Necrotizing Enterocolitis .

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Introduction:

Necrotizing Enterocolitis (NEC) is a disease which infects the digestive system. It mainly occurs in preterm infants less than 37 weeks of age and less than 1500 gm of weight [1]. Some types of bacteria such as the anaerobic bacteria and gram-negative bacteria play an important role in the occurrence of NEC disease. Also, the disease occurs due to weak immunity, viral infections and incomplete intestinal maturity [2].

Klebsiella pneumoniae is a Gram-negative straight rod, arranged singly, in pairs or in short chains, surrounded by a capsule. It is lactose fermenting, facultative anaerobic, nonmobile, and has both a respiratory and a fermentative type of metabolism. *K.pneumoniae* is characterized as negative for oxidase, indol and methyl red, yet catalase positive [3,4].

K.pneumoniae is widely present in nature, soil, water, plants and it exists in the normal flora of the mouth, skin and intestine [5]. Moreover, it exists on the mammalian mucosal surfaces such as a humans, horses and pigs [6]. Among the main reasons for the spread of *K. pneumoniae* in hospitals presented is its stability in the gastrointestinal tract of patients in hospitals and intensive care units, the hands of workers in the health units, as well as medical devices which are reservoir for the bacteria [7]. The frequent use of medical devices which are in direct contact with body fluids among the patients who are in the hospital can increase the injuries resulting from *K.pneumoniae* making biofilms on those medical devices to promote antibiotic resistance [8]. *K.pneumoniae* is an opportunistic pathogen as it represents a major cause of nosocomial pneumonia, septicaemia and urinary tract infections especially in newborns, blood cancer

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patients, immunocompromised candidates and diabetics. Despite the use of appropriate antibiotic therapy, the morbidity and mortality due to *Klebsiella* bacteraemia and pneumonia are more than 50% [9,10]. Also, *K.pneumoniae* causes pyogenic liver abscess, meningitis and necrotizing fasciitis. *K.pneumoniae* utilizes many of its virulence factors such as capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition for survival and immunity evasion during infection [11]. *K.pneumoniae* has at least three types of fimbriae; type 1 fimbriae, type 3 fimbriae and Kp(a-g) fimbriae [12,13]. *K.pneumoniae* has at least 78 capsular serotypes (K antigen) and it prepares serotypes K1 and K2 which are the most virulent patterns [11]. *K.pneumoniae* bacteria is characterized by having 9 groups of O-antigen namely; (O1, O2, O2ac, O3, O4, O5, O7, O8 and O12). O1 is the most common serotype among clinical *K.pneumoniae* isolates [14], and it plays a role in the protection of *K.pneumoniae* against the arrival of the complement and thus the bacterial resistance towards the complement mediated killing process. K.pneumoniae possesses virulence factors such as iron acquisitions which are necessary for bacterial growth in the body of the organism. Thus, there are at least 12 iron absorption systems peculiar to *K.pneumoniae*. Moreover, *K.pneumoniae* produces a typical iron acquisition called enterobactin and has the highest iron affinity [11]. Biofilm is one of the virulent factors of *K.pneumoniae* which is represented by the assemblage and adhesion of bacterial cells on biotic and abiotic surfaces [15,16]. As a result of the fact that the inflammation of the intestines in infants premature is one of the medical problems experienced by most countries of the world and the risk of the disease of the lack of knowledge of pathogens and pathogenicity, so the study aimed to investigate the bacterial strains causing the inflammation of the intestines in infants or their relationship with patients as well as the absence of local bacteriological studies For recessive enteritis.

Materials and Methods:

Sixty four clinical samples were collected from children infected with Necrotizing Enterocolitis admitted to the Children's Protection Hospital at the Medical City in Baghdad. The clinical specimens included (41) stool samples, (14) Blood samples and (9) urine samples for the period from January, 2018 to April, 2018.

All specimens were cultured on MacConkey agar and Blood agar then incubated aerobically for 24 hrs. at 37 ºC. Bacterial isolates identification was carried out by macroscopic, microscopic and biochemical tests which included oxidase test, catalase, indol, methyl red, Voges-proskauer, citrate utilization, sugar test on kligler iron agar and urease test with Vitek-2 compact system GN-card.

Results and Discussion:

Sixty four clinical isolates were collected from children infected with Necrotizing Enterocolitis and admitted to the Children's Protection Hospital at the Medical City in Baghdad. These isolates included (41) stool samples, (14) blood samples and (9) urine samples for the period from 29/1/2018 to 4/4/2018. All the samples were cultured on MacConkey agar and Blood agar for diagnosis. All bacterial isolates grown on Blood agar showed grayish or white colonies whereas bacterial isolates grown on MacConkey agar showed pink colonies, indicating that they are lactose fermenting colonies. Other isolates showed pale colonies, indicating non fermented to lactose. Under microscopic test, bacterial isolates reacted negatively and positively with Gram stain, the majority were garm-negative bacilli and some isolates were gram-positive.

Results of the biochemical tests of all bacterial isolates have shown that the predominant bacteria was *Klebsiella pneumoniae*. *Klebsiella pneumoniae* gave negative results to oxidase test, indol and methyl red, and positive results to catalase test, citrate, voges-proskauer and urease, and glucose fermented. It did not create H2S on kligler iron agar medium. *Escherichia coli* gave negative results to oxidase test, citrate, voges-proskuer and urease and positive results to catalase test, indol, methyl red and did not create H2S. On the other hand, *Pseudomonas aeruginosa* showed negative results to indol test, and voges-proskauer, positive results to oxidase test, catalase, citrate, variable results to methyl red, did not create H2S and was variable to urease test. *Klebsiella oxytoca* gave positive results to catalase test, indol, citrate, voges-proskauer and urease, and negative results to oxidase test and methyl red and glucose fermented and did not create H2S on KIA medium. In addition, *Enterobacter cloacae* showed positive results to catalase test, citrate, voges-proskauer, variable results to urease test, and negative results to oxidase test and indol and methyl red and glucose fermented and did not create H2S on KIA medium. *Proteus hauseri* showed negative results to oxidase test , citrate and voges-proskauer, and positive results to catalase test , indol, methyl red and urease, glucose fermented and did not create H2S on KIA medium. Vitek-2 system was used to diagnose the bacterial isolates taken from stool, urine and blood for infants less than two years infected with Necrotizing Enterocolitis. It provides 64 biochemical tests necessary to diagnose bacterial isolates. Culture characteristics of colonies and microscopic properties of bacterial cells were identified and then diagnosed using biochemical tests and confirmed with Vitek-2 system. 43 isolates were identified as gram-negative bacteria out of 64 samples (stool , blood and urine) from infants. Some of the remaining samples were Gram positive and another part did not show growth on blood agar and MacConkey agar. Using Vitek-2 system for diagnosis, it appeared that the number of isolates of *K. pneumoniae* were 26 (60.46%), *E.coli* 8 (18.60%), *P.aeruginosa* 4(9.30%), *K. oxytoca* 2 (4.65%), *E.cloacae* 2 (4.65 %) and *Proteus hauseri* 1 (2.32%), as shown in figure 1. Results showed that *K.pneumoniae* are the predominant species in the samples taken from infants infected with Necrotizing Enterocolitis. The number of isolates of *K. pneumoniae* bacteria taken from the stool samples were 23 isolates (88.46%), 2 isolates from blood (7.69%) and one isolate from urine (3.84%).

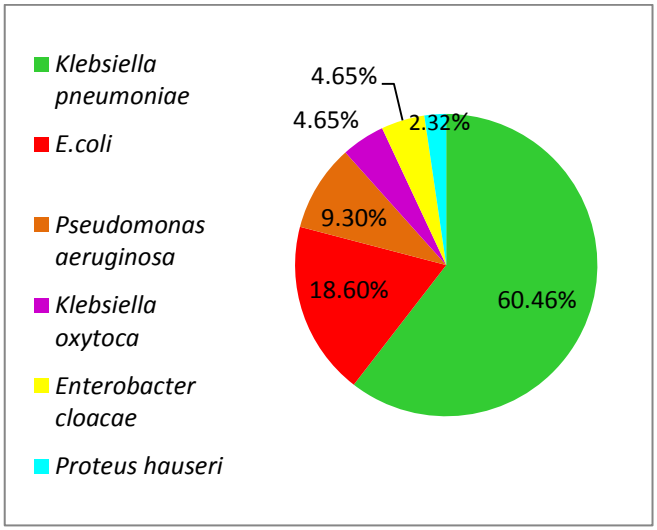

Figure (1) percentages of bacterial isolates causingNecrotizing Enterocolitis in infants.

Grishin *et al* (2013), pointed out that *K. pneumoniae* bacteria recorded the highest infection percentage with Necrotizing Enterocolitis disease in infants. Other bacterial species causing less percentages of Necrotizing Enterocolitis infections were *P.aeruginosa*, *Acinetobacter* and *Cronobacter sakazakii* [17]. However, Warner *et al* (2016), pointed out that genera the Gram-negative bacillus bacteria such as *Klebsiella*, *E.coli* and *Enterobacter* caused Necrotizing Enterocolitis with variable percentages

[18], whereas Raveh-Sadka *et al* (2015), indicated that *K.oxytoca* caused Necrotizing Enterocolitis with different percentages in infants [19]. Of the causes of the(NEC) disease, Completion of pregnancy, Colonization of bacteria, Lack of breast milk and Intestinal nutrition[2]

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عزل وتشخيص بكتريا *Klebsiella pneumoniae* المسببة لالتهاب الامعاء التنخري عند الاطفال الرضع

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الخلاصة

جمعت (64) عينة سريرية من مستشفى حماية الأطفال بمدينة الطب ببغداد لأطفال مصابين بالتهاب الأمعاء التنخري تضمنت (41) عينة من الخروج Stool و (14) عينة من الدم Blood و 9 عينات من البول Urine للفترة من 2018/1/29 إلى2018/4/4 . زرعت جميع العينات على وسط أكار الماكونكي MacConkey agar ووسط أكار الدم Blood agar .

شخصت العزلات بالفحوصات المجهرية والمزرعية والكيموحيوية ، فضلاً عن التشخيص باستعمال جهاز Vitek-2 إذ أمكن الحصول على (43) عزلة، ومن خلال التشخيص بنظام الفايتك تبين إن (26) عزلة تعود لبكتريا *Klebsiella pneumoniae* بنسبة 60.46% و(8) عزلات *Escherichia coli* بنسبة 18.60% و (4) عزلات *Pseudomonas aeruginosa* بنسبة 9.30% وعزلتين *Klebsiella oxytoca*بنسبة 4.65% و عزلتين *Enterobacter cloacae* بنسبة 4.65% وعزلة واحدة لبكتريا *Proteus hauseri* بنسبة 2.32% .

ومن خلال النتائج أظهرت بكتريا *K. pneumoniae* هي النوع السائد في العينات المأخوذة من الأطفال الرضع المصابين بالتهاب الأمعاء التنخري ، وكانت عدد عزلات بكتريا *K.pneumoniae* المعزولة من عينات الخروج (23) عزلة بنسبة 88.46% وعزلتين بنسبة 7.69% من الدم وعزلة واحدة من البول بنسبة 3.84% .