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Identification of *shigella* sp. causing children bloody diarrhea in Baghdad

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Abstract

There is a confusion among doctors about the distinguishing between *Shigella daisentry* and *Shigella flyxnerii* because the similarity of symptoms, thus the big goal of this study was to identify the species of shigella in children bloody diarrhea using diagnosis system included Api2o E., Mini APi and device, Rapid ID32 E and a Vitekcompoet-2; study their antibiotics susceptibility. Sixty-bloody diarrhea specimens were collected from infected children less than two years old in Baghdad city. Different Bacterial suspected species were isolated and the results showed that higher ratio for *Shigella flxynerii*. It was noticed that infection with diarrhea was increased during summer months. The isolated bacteria confirmed to *Sh. flexineri* By ABT-G., API and Vitek Compact -2. the isolates of diagnosed *Sh. flexinerri* were susceptible to Amikacin with 17 mm of inhibition zone, ciprofloxian and Amikacin 17mm (18mm). While the bacteria showed resistance towards antibiotics ampicillin, tetracycline and chloramphenicol.

Keywords: *Shigella flyxneri*, Food contamination, Antibiotic.

Introduction

Diarrhea is one of the most important health problems in the world of what caused the depletion of important nutrients and the result for the deterioration of the health status of the individual and then the death of untreated cases, especially children (kantakai *et al*., 2009; Musher and Mushor, 2004). The diarrhea can be divided either by stool nature (watery or bloody) or incubation period of disease (acute or Chronic) (Christina et al, 2002; WHO, 2007; Lima, 2007). One of the most dangerous causing of bloody diarrhea is the species *Shigella flexnerii* that belong to the gastrointestinal family; their members are gram negative bacilli, non-motile, non-spore forming and not forming a capsule. Shigella produces two types of toxin the heat resistant Endotoxin which is the main virulence factor (Zilbauer *et al.,* 2007) and Exotoxin a characteristic of the cell cytotoxin. Another toxins may produce, nervous Neurotoxin and intestinal Enterotoxin(shigella toxin) which consists of two types of particles alpha and beta that associated with glycolipid host cell in the large intestine and then injected molecule alpha in the cell by the receptor to endocytosis to stimulate the manufacture of proteins through reduce Activity of ribosome 60s that cause to cell death. Also,The toxin enterotoxin stimulates the secretion of fluids Inside the intestines gap without cause damage to the mucous membrane for the gut (Braun, 2002) . Featuring *Shigella* the existence of a system which represents a virulent factor called (T3SS) T3-secretion system that encodes by genes located at a bacterial plasmid which has the ability to transmit from cell to cell. Also, other terms of these proteins working on the membrane and membrane digestion analysis and colonizes the region and move within the host cells directly and such a process now called Paracytophage. These bacteria transmitted to humans through contaminated food and water and that the injury occur a small number of bacteria (10-100) Hives Cafe to infection. (Niyogi, 2005).

The big concern come to some isolates that secrete toxin enteronervious which is similar to neurotoxin of bacteria *E.coli* O.157: H7 .The danger of these toxins when they enters and associated with neutrophils down to the glomerular cells and then hemolysis of kidney and finally central nervous system failure occurs. Intestinal toxin sensitive to heat (Heat Lablile enterotoxin) which is a high molecular weight protein influenced by the temperature of 56 ° C for half an hour and lead to the accumulation of fluid in the small intestine, causing diarrhea and cholera-like toxin (Finkelstein, 1988). The neurotoxin causes fever and abdominal spasms and contractions down to paralysis and fainting (Cherla, 2003),

Material and Methods

Specimens: Sixty diarrhea samples were collected from Children 's Hospital Alaluea and Zaafarneh and Imam Ali from 1-6 to 8-30 at 2016. Stool samples were collected in plastic containers. Many observations were recognized for each sample includes texture, blood existence , color and odor of stool and Microscopic examination.

Isolation of bacteria: successful isolation of bacteria was achieved by mixing one part of each stool sample with 9 part of peptone water to provide colony forming private's enrichment for any few numbers of bacteria. Cultures were incubated at 37 c for 48 hrs. A loop full of broth supplemented with iodine solution to discourage gram positive bacteria were incubated at 37 c for 24 hrs. Mean while a drop of culture was streaked over XLD agar (Xylosis lysine chocolate agar and incubated for 18 hr. The red colonies were picked from the agar and maintained for confirmation by further diagnostic tests. While pale colonies were neglected because they might refer to another *shigella* or *salmonella* (ISO, 2007).

Biochemical tests: were achieved for primary diagnosis of bacteria and they include: Oxidase test, Catalase, Methyl red Voges-Proskauer, Citrate utilization, Hydrogen sulfide (H_2S) formation and Urea test (Ploeg van der, 2010).

Serological confirmation: was conducted using O Antigen.

API 20 E system: was used to confirm intestinal bacteria through 20 biochemical test briefly, One isolated bacterial colony was picked, suspended by 0.85 % NaCl and the bacterial turbidity was equilibrated to McFarland 0.5, then distributed to 20 wells on plastic strip of API E 20 and strips were incubated for 18 hr at 37 C for result assessment.

Rapid ID 32 E and device Mini APi: A plastic strip contained 32 biochemical tests was developed for rapid diagnosis . Each well was filled with bacterial suspension (0.5 McFarland) incubated for only 4-5 hr at 37C and reagent were added mean while Mini API strips were put inside computer device with screen. Results output automatically confirming the bacterial genus and serotype with their accurate identity percentage.

Serology test: Antigen Antibodies reaction (*shigella flxynerii srogroup B*)

Antimicrobial susceptibility test: Modified Kirby-Bauer disc diffusion method was applied for antibiotic susceptibility test. Only 100µL of bacterial suspension (0.5 McFarland) was spread over Muller Hinton Agar; aseptically antibiotic discs were put over agar. Plates were incubated for 18 C hr at 37 C, the inhibition zones were measured in millimeters and results were recorded as resist, moderate resist and sensitive as indicated by standard antimicrobial susceptibility.

Results and Discussion

All isolates of *Sh. flyxnerri* showed antibiotic resistant to antibiotic tested in this study, they resisted amikacin, ampicillin, tetracycline, chloramphenicol and showed moderate resistance to cefotaxime and ciproflaxine as presented in table (1).

Table (1): Antibiotic standard

Antibiotic disc	Standard			Study result(mm)	Susceptibility test
	resistant	Moderate resist	sensitive		
Amikacin	≤ 15	16-18	≥ 19	15	R
Ampicillin	≤ 22	23-25	≥ 26	10	R
Cefotaxim	≤ 17	18-22	≥ 23	20	intermediate
Ciprofloxacin	≤ 14	15-20	≥ 21	19	intermediate
Chloramphenicol	≤ 22	23-25	≥ 26	15	R
Tetracycline	≤ 22	23-25	≥ 26	8	R

Table (2): Sensitivity test for bacteria *Shigella flyxnerii* All isolates gave the same results.Zoon Diameter of inhibition area

Tetracycline	Chloramphenicol	Ciprofloxacin	cefotaxin	Amp	Amik	Anibiotic
8 mm	15 mm	19 mm	20 mm	10 mm	15 mm	*Shigella flyxnerii*

The results of present study showed that the incidence of bacteria *Shigella flyxineri* was (25%), where concentrated in the summer months and agreed these results with the sentiments (Weir, E.

2002) that the increase in cases of diarrhea caused by bacteria *shagilla* during the summer, while indicated (2005 Elsevier Inc.) that the highest rate of infection with bacteria *shagilla* was in the spring, and dropped in a season in the winter in the city Mosmo. According to Baer, the control center reports on the disease the percentage total injury rate of 18.4% was considered prevalent cases (Baer *et al,* 1999) and the prevalence for 2003 (14,000) and 2004 (18,000) and reached the 20,000 injury in 2007 (CDC, 2009) and was control center estimates on Communicable diseases in the United States infected with bacteria between July to October 450000 injury (1999) and less of the injury was in January and February and March months, according to Kutlov the death of more than a million cases a year, especially in the warm months.(Kotloff *et al.,* 1999). (Nov 1991). According to a study it was consistent with the theme of anti profloxaci Find used in the treatment of cases of bacteria (Replogle, *et al* 2000) and showed bacteria resistant to anti ampicillin.

In a study in which the confirmed.(Taneja, 2007) resistant bacterial isolates were resistant to multiple antibiotics, such as tetracycline and Ampicillin and not detect the anti ciproflaxine and chloramphenicol and cefotaxine. A study carried out by Srinivasa (2009) that more than 70% were from bacterial isolates were resistant to anti Ampicillin and confirmed the same study bacteria Sense of Anti ciprofloxacin increased by 69.2% and anti cefotaxine 100%

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