PREVALENCE AND ANTIBIOTIC SENSITIVITY OF *Escherichia coli* AND *Klebsiella pneumoniae* FROM PATIENTS AND ANIMALS IN BASRAH PROVINCE.

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ABSTRACT

During the period of seven months from October 2016 to May 2017, 299 samples were collected, 152(51%) human samples, of which 69 (45.4%) were from urine and 83 (54.6%) were from children suffering from diarrhea in hospitals in Al-Basra governorate. 147 (49.2%) samples were from fecal of animals, of which 82 (55.8%) samples were from buffalo and 65(44.2%) were from cow .A total of 101 *E. coli* serotype O157: isolates out of 299 were suspected *E. coli* analyzed 52/101 (34.2%%) were from human 16 (10.5%) samples were from urine and 36 (23.7%) samples were from stool . and 49/101(33.3%) were from animal 33(22.4%) samples were from buffalo and 16 (10.9%) samples were from cow . On the other hand 68(22.7%) isolates out of 299 were suspected *K. pneumoniae* analyzed 41/68 (27%)were from human 28/41(18.4%) isolates were from urine samples and 13/41 (8.6%) isolates were from stool samples and 27/68 (18.4%) were from animal 16 (10.9%) samples were from buffalo and 11(7.5%) samples were from cow).All suspected isolates were subjected to testing biochemical. It was found that 10 out of 101 were 4 isolates of animal faeces (2 buffalo and 2 cows).six isolates of 52 isolates (4 of the children's stool samples and 2 of the urine samples) 19.5% non-fermented sorbitol (NSFEC).The isolates were tested against 14 different antibiotics

in the manner of spread of the disk to Kirby-Pour. All isolates were resistant to at least ten antibiotics, they showed the pattern of multiple resistance to antibiotics.Therefore, all these isolates were considered to be multidrug resistant.

INTRODUCTION

Antibiotic resistance is a major threat to public health in Gram-negative bacteria [1]. Resistance challenges the achievements of modern medicine, including advanced surgery and immunosuppressive treatment, which are dependent on effective antibiotics, many members of the Enterobacteriaceae family are common members of the gut microflora in humans and animals. For Escherichia coli, the intestine is its primary reservoir. It is recovered from the stools of almost all humans and animals, and is the most frequently isolated facultative anaerobe from the intestine of humans [2, 3]. *E. coli* is less frequently encountered in the environment, and their presence in water and food sources usually indicate faecal contamination[4]. Comparatively, *Klebsiella pneumoniae* is more ubiquitous, and frequently found in environmental reservoirs of water and soil, as well as in stool samples and nasopharynx from healthy individuals [5].

Escherichia coli is important inhabitants of the human and animal, Intestine is one of the most frequently found species of facultative anaerobes in this environment. Although they constitute less than one percent of the total microbiota, they are found in the faecal flora of almost all healthy adults [6].There are several toxin producing strains of *E. coli* causing diarrhoea, but most commonly these bacteria cause community onset UTIs [7,8], especially among women and adolescent girls [9].Other types of infections that can involve E. coli are meningitis, septicaemia, pneumonia, intra-abdominal and gynaecological infections etc [10].

*Klebsiella pneumoniae*is a non-motile Gram-negative bacterium an important bacteria belonging to the family Enterobacteriaceae that is ubiquitous in the environment and is often found as a commensal resident of the human gastrointestinal tract [11]. When inoculated parenterally, it has a remarkable ability to cause a wide range of human diseases, from urinary tract infections to pneumonia [12].

ESBLs are a group of enzymes that are common among Enterobacteriaceae [13]. They are the increasingly important cause of transferable multidrug resistance (MDR) in Gram-negative bacteria throughout the world. ESBLs also have the ability to hydrolyze third and fourth generation cephalosporins and monobactams. ESBL-producing strains are prevented by lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) [14].

MATERIAL AND METHODS

Collection of Samples:

In the present study, isolation of *E. coli* and *K.pneumoniae* was carried out in the period from October 2016 to May 2017. A total of 299 samples were collected from stool and fecal samples as: 147 buffalo & Cow samples (feces) were collected in sterile plastic containersfrom different area in Basrah City (Abu al-Khasib, Al-Qurna, Garmat-Ali, Al-Zubair), and 152 stool & urine samples were collected from children and women suffering from diarrhea of both genera from (Aben-Gazwan Hospital, Al-Fayhaa Hospital ,Al-Shafa Hospital, General Basrah Hospital and Al-Sadr Hospital in Basrah city.

Culturing of *E. coli*

Loopful of each samples were inoculated in 5 ml of trypticase Soy broth (TSB-V) supplemented with (1.5 g) bile salt and incubated at 37°C for 18-24h [16]. A loopfull of bacterial growth was streaked on Eosin-Methylene Blue agar (EMB) and MacConkey agar then incubated overnight for *E. coli* strains. Typical colonies on MacConkey agar and EMB were streaked on sorbitol MacConkey agar supplemented with cefixime (0.05mg/L) and potassium tellurite (2.5mg/L) and incubated for additional overnight to identify on NSFEC[15].

Culturing of *Klebsiella spp.*

Loopful of each samples were inoculated on to MacConkey agar plates and incubated overnight at 37C˚. *Klebsiella* spp. isolates were identified by fermention lactose and form mucoid colonies on MacConkey agar [17 and 18].

Identification of *E. coli* and*K.pneumonia*

All suspected colonies on MacConkey agar and EMB were streaked on the nutrient agar plates, to allow the isolated colonies to grow. The inculcated plates were stored in the incubator at 37C˚for 24h. The pure colonies acquired was used for primary identification, biochemical tests (gram staining, indole test, simmon's citrate test and triple sugar iron test[17] (Hi-Media, India)). Bacterial identities were confirmed with a Vitek 2 GN ID card using the Vitek 2 System [19] (bioMérieux, Marcy I'Etoile, France).

Antimicrobial Sensitivity Testing

The antimicrobial test of all positive isolates were performed according to [20] by disc-diffusion method using 14 different antibiotic discs in the following concentrations :cefixime (5 µg), ampicillin (10 µg), amikacin (30µg), cefotaxime (30µg), ciprofloxacin (30µg), gentamicin (10µg), ceftriaxone (300 µg), ceftazidime (5µg), imipenem (20µg), ticrcin (30 µg),penicillin G (10 µg),cefaclor(30 µg), cefopazone(75 µg) and tetracycline (30 µg), (Bioanalyse) [20, 21], using Mueller-Hinton (MH) agar plates (Difco Laboratories, Detroit, USA) were overlaid with each of the *E. coli* strains inoculum (turbidity equivalent to that of a 0.5 McFarland Standard[21]. Inhibition zone diameters were measured after 24 - 48 h incubation.

RESULTS

According to the results of culturing on EMB and MacConkey agar there were 169isolates out of 299 tested samples. 152 were from human (44 (28.9%) samples were from urea and 108(71.1%) samples were from feces) and 147 were from animals (82 (55.8%) samples were from buffalo and 65 (44.2%) samples were from cow feces),Table (1).

Additionally, 101 isolates out of 169 were suspected *E. coli* analyzed 52/101 (51.5%) were from human (16 (30.8%)samples were from urea and 36(69.2%) samples were from stool) and 49/101 (48.5%) were from animal. On the other hand 68 isolates out of 169 were suspected *K. pneumoniae* analyzed 41/68 (60.3%) were from human (28/41 (68.3%) isolates were from urea samples and 13/41 (31.7%)

isolates were from stool samples) and 27/68 (39.7%) were from animal, Table (2 and 3).

Frequency of NSFEC Isolates in *E. coli*.

The percentage of frequency of NSF*E. coli* was based on the fermentation of the sorbitol in SMACTable (4).

Table (1) Number and percentage of samples collected from animals and human.

Sample Source		No. of samples			
		Total No.of samples	(%)	Total No. of samples	(%)
Animal	Buffalo	147	(49.2)	82	(55.8)
	Cow			65	(44.2)
Human	Urea	152	(50.8)	69	(45.4)
	Stool			83	(54.6)
Total		299	(100)	299	(100)

There was no significant differences between number of samples (P>0.05). There was significant (P<0.05) for % of samples.

Table (2) Prevalence and percentage of *E. coli* isolated from animals and human.

Samples Source		No.of Isolates	(%)of Samples	(%) of Isolates	No of Isolates	(%) of Samples	(%) of Isolates	(%) of Samples
Animal (147)	Buffalo	49	(33.3)	(48.5)	33	(22.4)	(67.3)	(32.7)
	Cow				16	(10.9)	(32.7)	(15.8)
Human (152)	Urea	52	(34.2)	(51.5)	16	(10.5)	(30.8)	(15.8)
	Stool				36	(23.7)	(69.2)	(35.7)
Total (299)		101	(33.8)	(100)	101	(100)	(100)	(100)
Chi-Squares			NS	NS	102.92 (0.01)	52.91347685 (0.01)	387.9844 (0.001)	123.6328 (0.001)

Table (3) Prevalence and percentage of *K. pneumonia* isolated from animals and human.

Samples Source		No. of Isolates	(%)of Samples	(%) of Isolates	No of Isolates	(%) of Samples	(%) of Isolates	(%) of Samples
Animal (147)	Buffalo	27	18.4	(39.7)	16	(10.9)	(59.2)	(23.5)
	Cow				11	(7.5)	(40.7)	(16.2)
Human (152)	Urea	41	27	(60.3)	28	(18.4)	(68.3)	(41.2)
	Stool				13	(8.6)	(31.7)	(35.7)
Total (299)		68	(100)	(100)	68	(100)	(100)	(100)
Chi-Squares						25.29412 (0.01)	1.859637 (NS)	323.8319 (0.001)

Table(4) Percentage of non sorbitol fermenting *E.coli* isolated from different sources.

Samples	No. of isolates	Non-sorbitol fermenter (NSF)	(%)
Human	52	6	11.3
Animal	49	4	8.2
Total	101	10	19.5
Chi-Squares			0.48 (NS)

Antimicrobial Susceptibility Test

The disc diffusion test was used to examine the antimicrobial sensitivity of the isolates of EHEC O157:H7 and*K. pneumonia*. The finding all the *E. coli* O157:H7 isolates (100%) were sensitive to Ceftazidime, while (100%) of the isolates were resistance to imipenem, cefopepazone ,gentamycin, ampicillin, cefotoxime, and 75% of *E. coli* O157:H7 strains were resistance to ciprofloxacin, cefaclor, while 75% of the isolates were sensitive to tetracycline, cefixame. Some of them were (90%) were resistance to pencillinG and ticirin, and (50%) isolate were resistance to ceftriaxone Figure (1), Table (5).

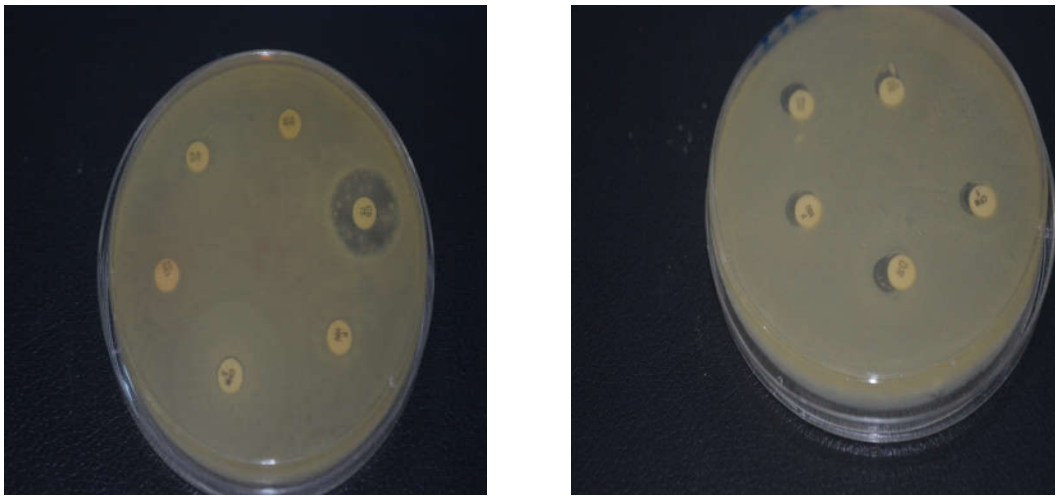

Figure (1) Antimicrobial susceptibility test

All *K. pneumoniae* isolates were (100%) were resistance toceftazidime and ticirin, but they were 100% sensitive to cefopepazone and impenem and 75% of the isolates were sensitive to cefaclor and ciprofloxacin, some of them 90% were resistance to amikacin, gentamycin and tetracicillin, while 90% of the isolates were sensitive to cefotoxime, and pencillin G, and 50% of the isolates were resistance to cefixame and ceftriaxone, and 75% of isolates were sensitive to ciprofloxacin Figure (1), Table (6).

Table (5) Antimicrobial susceptibility of *E. coli*O157:H7 toward 14 different antibiotics.

Type ofAntibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikain(AK)	90	0	10
Ampicillin(AMP)	0	0	100
Cefaclor (CF)	25	25	75
Cefixame (CFM)	75	0	25
Cefopepazone(CS)	0	0	100
Cefotoxime(CTX)	0	0	100
Ceftazidime(CAZ)	100	0	0
Ceftriaxone(CFX)	50	0	50
Ciprofloxacin(CIP)	0	25	75
Gentamycin(GM)	0	0	100
Impenem(IMP)	0	0	100
Pencillin G(P)	0	10	90
Tetracicillin (TE)	75	25	0
Ticrcin (TCC)	10	0	90

Table (6) Antimicrobial susceptibility of *K. pneumonia* toward 14 different antibiotics.

Type ofAntibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikain(AK)	10	0	90
Ampicillin(AMP)	50	25	25
Cefaclor (CF)	75	25	0
Cefixame (CFM)	50	0	50
Cefopepazone(CS)	100	0	0
Cefotoxime(CTX)	90	10	0
Ceftazidime(CAZ)	0	0	100
Ceftriaxone(CFX)	50	0	50
Ciprofloxacin(CIP)	75	25	0
Gentamycin(GM)	0	10	90
Impenem(IMP)	100	0	0
Pencillin G(P)	90	10	0
Tetracicillin (TE)	0	10	90
Ticrcin (TCC)	0	0	100

Sixty eight isolates out of 299 were suspected *K. pneumoniae* analyzed (22.7%)while [29]showed that 56 *K. pneumoniae*werefrom 155 isolates which was 36.2%.The disc diffusion test was used to examine the antimicrobial phenotypes of

the isolates of EHEC O157:H7 and *K. pneumonia*. The finding all the *E. coli* O157:H7 isolates were sensitive (100%) to ceftazidime. While (100%) resistance to ampicillin, cefopepazone, cefotoxime, gentamycin,and imipenem, 75% resistance to cefaclor,ciprofloxacin, while 75% of *E. coli* O157:H7 isolates were sensitive to cefixame andtetracycline,some of *E. coli* O157:H7 isolates (90%) were resistance to pencillin G and ticirin, and (50%) isolate were resistance to ceftriaxone.While in *K. pneumoniae* all isolates(100%) were resistance to ceftazipime and Ticirin, but were sensitive 100% to cefopepazone and impenem, and were 75% sensitive to cefaclor and Ciprofloxacin, some of *K. pneumoniae*isolates were 90% resistance to amikacin,gentamycin and tetracicillin, while 90% were sensitive to cefotoxime, pencillin G, 50 % of the isolates were resistance to cefixame and ceftriaxone.[30] is Agreement with results that revealed sensitivity of *E. coli* O157:H7 isolates to ciprofloxacin,imepenim.

On the other hand, it was found to be resistant to all other antibiotics, ranging from 90%. However, third-generation cephalosporins, ciprofloxacin and the other quinolones are not used by children due to their danger which cause damage to immature joints such as cefotaxime. This conclusion is also similar to those of [31] who showed all isolates of E.coli to be resistant (97.5%) to Amikacin.

E. coli isolates were more resistance to the antibiotics that used in the present study than *K. pneumonia* the interpretation of this may due to the containment of the *E. coli* to resistance gene more than *K. pneumonia*. The bacteria in the present study showed highly resistance to amoxicillin and gentamycin which were used highly in the hospital [32, 33].

DISCUSSION

TSB was used as enrichment medium while MacConkey agar and EMB agar were used for selection and isolation purposes.In the present study 101 (33.8%)*E. coli* isolates were obtained from 299 samples. The percentage of frequency of *E. coli* isolated was 52 (51.5%) out of 152 stool and urine human samples (16 (30.8%) was urine samples and 36 (69.2%) was stool samples, while 49(48.5%) out of 147 animal samples (33(67.3%) out of 49 buffalo faces sample and 16 (32.7%) out of 49

Cow faces samples .The rate of *E. coli* isolated in the present study was 101 (33.8 %) out of 299 which agrees with the result obtained by [22, 23and 24] that found lower rates of *E. coli* isolates in stool 29%, 38.3% and 38%,respectively. Other studies such as [25] which was 59.4%, mentioned much higher rate of *E. coli* isolates in comparison with the rate in the present study. Based on the results, *E. coli* was the most frequent microorganism isolated from urine samples. Other investigators also reported that *E. coli* was the most commonly isolated aerobic microorganism from Urinery tract infections [26, 27].

The occurrence of NSFEC in human (stool and urine), and animal (buffalo and cow feaces) samples which detected by conventional microbiological methods were 11.5% and 8.2% respectively. The present occurrence of NSFEC in children stool sample was lower than the finding of [28 and 29] who detected57.5% and 73.9%, respectively.

On the other hand 68 isolates of *K. pneumoniae* out of 299 samples were isolated depending on cultural characteristics whereas only 20 isolates were considered as *K. pneumoniae* depending on VITEK 2 system investigation.

انتشار والحساسية تجاه المضادات الحيوية للإشريشيا القولونية و الكليبسيلا نيومونيا المعزوله من المرضى والحيوانات في محافظه البصرة

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الخلاصة

خلال فترة سبعة اشهر(اكتوبر2016 - مايو 2017) تم جمع 299عينة , كانت منها 152 (50.8%)عينة من الانسان (83 (54.6%)عينة براز الاطفال الذين يعانون من الاسهال و 69 (45.4%)عينة ادرار من المرضى و 147عينة من براز الحيوان كانت منهم 82(55.8%)عينة براز الجاموس و 65(44.2%)عينة براز الابقار (جمعت من مناطق ومستشفيات مختلفة في محافظه ألبصرة) . .

تم الحصول على 101عزلة من الاشرشيا القولونية المشكوك بها (33.3%)49 :عزلة من براز الحيوانات و(22.4%)33 من عينات الجاموس و 16(10.9%)من عينات البقر, و 52 (34.2%%)

عزله من عينات الانسان كانت منها 16(10.5%)عينة ادرار و 36(23.7)عينة براز ، اما بالنسبه للكلبسيلا فتم الحصول على 68(22.7%)عزله و فقط (41(60.3% عزلة كانت من عينات الانسان ، 28(18.4%) عزلة من عينات الادرار و (8.6 %) 13 عزلة من عينات البراز و (18.4%) 27 عزلة من براز الحيوان كانت منها (10.9 %) 16 عزلة من عينات الجاموس و (7.5%) 11(عزلة من عينات الابقار . جميع العزلات المشكوك بها اخضعت للاختبارات البيوكيميائية.

زرعت جميع عينات الاشريشيا القولونيه على وسط السوربيتول مكونكي أكارالمزود بالسفكسيم والبوتاسيوم تيلورايت لتحديد المستعمرات غير المخمرة لسكر السوربيتول كانت منها 52 عينة من الانسان و 49عينة حيوان . وجد ان 10من اصل 101 كانت منهم 4 عزلات من براز الحيوان 2من الجاموس و 2 من الابقار . و 6 عزلة من الانسان من اصل 52عزلة 4 من عينات براز الاطفال و 2 فقط من عينات الادرار كانت نسبة 19.7 % غير مخمرة للسوربيتول.

تم اختبار مقاومة العزلات تجاه 14 نوع من المضادات الحيوية بطريقة انتشار القرص ل كيربي بور وكانت جميع العزلات مقاومة ل عشرة منها ، اي انها أظهرت نمط ألمقاومة المتعددة للمضادات الحيوية .

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