Causes of Antibacterial Resistance in Some of Enteropathogens Isolated from Colon Cancer Patients in Al-Dewaniyah Governorate.

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Abstract:

Background: Bacterial infections associated with multidrug resistance have been implicated in high mortality and morbidity reported among cancer patients.

Objective: To detect the causes of β-lactamase resistance in *Escherichia coli* and *Klebsiella* spp. isolated from colon

Methods: Twenty -eight stool samples from colon cancer patients samples ,in addition to that, 25 samples were collected from control group (healthpersons). Samples were labeled and transported to the laboratory in portable container then streaked on MacConkey agar and incubated at 37˚C for 24 hours under aerobic condition. The antimicrobial susceptibility testing was done by the agar discs diffusion method. **Results**: out of 28 samples from colon cancer patients, the results showed that 26 (92.8 %) of stool were positive for culture. The study revealed that *Klebsiella* sp.a nd *E. coli* were the most common isolated species. The results showed that the vast majority of isolates were found to be resistant to ß-lactam antibiotics (ampicillin and amoxicillin) .

Conclusion and Recommendation: All of the tested *E. coli* and *Klebsiellae* isolates are multidrug resistant; therefore, such organisms represent a serious therapeutic challenge in cancer patients.

Introduction:

Colon cancer is a worldwide problem, with an annual incidence of approximately 1 million cases and an annual mortality of more than 500,000. The absolute number of cases will increase over the next two decades as a result of the aging and expansion of populations in both the developed and developing countries. Colon cancer is a common malignancy in developed countries and is the 3rd most common cancer in the United States which greater than 80% occur sporadically(1).

In spite of many potent and broad-spectrum antibiotics had been marketed in the past decades, bacterial infections still cause substantial mortality and morbidity among cancer

patients. This may be related to more immunosuppressant medications and aggressive diagnostic or therapeutic tools in clinical management of various cancers(2). The involvement of intestinal microflora in the pathogenesis of colon cancer has been hypothesized. Many cancers arise from sites of infection, chronic irritation, and inflammation(3). The strongest association of chronic inflammation with malignant diseases is found in inflammatory bowel diseases of colon(4) with a lifetime incidence of 10% (5,6).

Escherichia coli and *Klebsiella pneumoniae* that remain the prevalent causes of bacterial infections in cancer patients(7,8). With the increased use of β-lactams among cancer patients, an increase in bacteria resistance has developed. With the increased use of β-lactams among cancer patients, an increase in bacteria resistance has developed. In recent years Gram-negative organisms isolate from patients with cancer have been found to produce β-lactamases. Extended spectrum beta-lactamase resistances are now a problem among patients with chronic cases and carcinomas(9,10).

There are many studies concerned with the isolation and identification of microbes associated with tumor cells, the study of Bentzien *et al*.(11) who isolated microbes associated with tumor cells of colon cancer, While in Iraq, a number of studies are conducted to isolate and identify the bacteria associated with tumor cells (12,13,14).

In Iraq, a little information was available on the global prevalence of extended spectrum Beta lactamase (ESBL) producing isolates in cancer patients. Therefore, there is an increase demand to investigate the prevalence of ESBL-producing isolates in colon cancer patients. So,the aim of this study was to detect the causes of β-lactamase resistance in *Escherichia coli* and *Klebsiella* spp. isolated from colon and investigate the occurrence of ESBLs in *E. coli* and *Klebsiella* spp. isolates recovered from colon.

Material and methods:

A total of 28 stool samples from colon cancer patients, in addition to that, 25 samples from control group (healthy persons) were collected. Samples were labeled and transported to the laboratory in portable container, then streaked on MacConkey agar and incubated at 37˚C for 24 hours . Bacterial isolates were identified to the level of species using the traditional morphological and biochemical diagnostic tests, according to the methods of MacFaddin(15), then stored at maintenance medium until further tests. The antimicrobial susceptibility testing was done by the agar discs diffusion method as that

described by Bauer *et al*.(16) as follows : At least 3-5 well isolated colonies were suspended in 4-5 ml brain heart infusion. The broth culture was incubated at 37 C˚ for 8 hours. The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity optically comparable to the 0.5 McFarland standards tube (growth equivalentto1.5X10^{8}cell/ml). . Colonies of a young bacterial culture on MacConkey agar, were transferred to Eppendrof tubes containing 100 µl of penicillin G solution, and the tubes were incubated at 37˚C for 30 minutes. Then, 50 µl of starch solution was added and mixed well with the content of the tube. A portion of 20 µl of iodine solution was added to the tube which cause the appearance of dark blue color, rapid change of this color to white (within few second-2 minutes) indicated a positive result for Beta-lactamase production(17).

Extended spectrum β- lactamase production by β-lactam resistant isolates was initially screened by using disc diffusion of cefotaxime, ceftazidime, ceftriaxone, and aztreonam (30 µg each) placed on inoculated plates containing Muller-Hinton agar according to the CLSI recommendations(18). After 18 hr incubation at 37˚C, the diameters of the inhibition zones around the antibiotics were measured by caliper. The isolates which showed inhibition zone < 27 mm for cefotaxime, < 22 mm for ceftazidime, < 25 mm for ceftriaxone, and < 27 mm for aztreonam were suspected for ESBL production The results were analyzed statistically by Chi-square (X^2) test at the level of significant when P-value ≤ 0.01(19).

Results and Discussion:

Primary culturing on MacConkey agar revealed that out of 28 stool samples, the results showed that 26 (92.8 %) were positive for culture and 2 (7.1 %) of samples were negative , while control group showed no growth , all stool samples gave positive growth. The statistical analysis showed a significance differences (P ≤ 0.01) among tested samples. Morphological and biochemical characterization revealed that five bacterial types were identified belong to Gram negative bacteria (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterbacter* spp., *Pseudomonas aeruginosa*).

Since the present study focused on detection of *E. coli* and *Klebsiella* spp. rather than other microorganisms However, the frequency of other bacterial isolates listed in Table (1). Regarding to group control, out of 25 stool sample, 16 (64 %) were *E. coli* and only 2 (8 %) was *Klebsiella* spp

Table (1): Distribution of bacterial isolates in colon cancer patient's.

Bacterial isolate	No.(%)
Escherichia coli	13 (50.0 %)
Klebsiella pneumoniae	5 (19.2 %)
Klebsiella oxytoca	1 (3.8 %)
***Proteus* spp.**	3 (11.5 %)
***Enterobacter* spp.**	4 (15.4 %)
Pseudomonas aeruginosa	0 (0.0 %)
Total	26 (100 %)

Bacterial isolated from stool in this study did not necessary correlated with clinical evidence of clinical bacterial infection. The isolated bacteria are an opportunistic commensal microorganism which exists as a part of normal flora of the mucocutanous gastrointestinal area in the healthy and illness human. It was clear from Table (1) that the *E. coli* isolates were the most common species isolated from an colon cancer patients (44.9 %). This results were previously indicated(13) who isolated *E. coli* from stool of colon cancer patien.

The weakness of immune system of cancer patients is one major factor that predisposes danger of infection by bacteria. Most cases of cancer are associated with immune deficiency due to the engagement of body to get rid of tumor cells, which leads to secondary bacterial infection to the patients. The source of secondary infection with bacteria is not determined yet(21). The possibility of getting infection increases in immune compromised patients as patients with cancer, especially when they take anti cancer drugs(22).

The results of present study showed that 19 (86.4 %) of *E. coli* isolates were resistant to both ampicillin and amoxicillin (Table 2). All these isolates were able to grow normally in the final concentrations of 50-10µg/mlofthesetwoantibiotics.

Table (2): β-lactam resistance of *E. coli* and *Klebsiella* spp. Isolated from colon cancer patients.

Isolate	No. of isolates	No. (%) resistant isolates
Escherichia coli	22	19 (86.4 %)
***Klebsiella* spp.**	14	13 (92.8 %)
Total	36	32 (88.8 %)
Cal. X^2 = 0.365 tab. X^2 = 0.004 df = 1 P-value = 0.949		

The reason of β-lactam resistance of *E. coli* isolates is probably due to the production of TEM β-lactamases, which may be genetically localized on the chromosome or on a plasmid. The TEM-1 is the most commonly encountered β-lactamase in Gram-negative bacteria; up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1(23).

The development of antibiotics resistance in these isolates is often related to the overuse and misuse of the antibiotics prescribed. Iraq is one of the developing countries where antibiotics sold over the counter, an attitude that encourages self-medication(24). The present results also showed that 13 (92.8 %) *Klebsiella* spp. isolates were resistant to both ampicillin and amoxicillin. The statistical analysis showed a significance differences ($P \leq 0.01$) among tested isolates. This relatively high ratio is similar to some local studies(25,26) showed that all (100 %) *Klebsiella* isolates were resistant to both ampicillin and amoxicillin, and Al-Muhannak(27) who reported that 93.7% of *Klebsiella* isolates were resistant to both antibiotics, and to Al-Hilli(28) who revealed that 89.4% of *Klebsiella* isolates has the β-lactam resistance. The resistance bacterial isolates to antibiotics is now widespread and possesses serious clinical threats. The organisms develop resistance to antibiotics by any of the following mechanisms: selection, mutation, phage transduction, and transference. Microbial resistance can be either hereditary in the organism or acquired through the environment(29).

In this study, all the 32 β-lactam resistant *E. coli* (n=19) and *Klebsiella* spp. (n=13) isolates were screened for their antibiotic resistance against 18 antimicrobial agents of different classes using Kirby-Bauer disk diffusion method. A strain is considered a multidrug resistant (MDR) if an isolate is resistant to representatives of three or more classes of antibiotics(30).

From table (4), the majority of *E. coli* isolates were highly resistant to amoxicillin-clavulanate (84.2 %) and pipracillin (89.5 %). Resistance is

probably due to indiscriminate antibiotics usage (drug abuse) which could result in plasmid-mediated antibiotic resistance found to be common in *E. coli(31).*

Table (4): Antibiotics susceptibility for *E. coli* (n=19) isolated from colon cancer patients.

Type of antibiotic	No. (%) of Resistant Isolates	No. (%) of Intermediate Isolates	No. (%) of Sensitive Isolates
Amikacin	1 (5.3 %)	0 (0.0 %)	18 (94.7 %)
Amoxicillin-Clavulanate	16 (84.21 %)	3 (15.79 %)	0 (0.0 %)
Azteronam	13 (68.4 %)	3 (15.79 %)	3 (15.8 %)
Cefotaxime	14 (73.7 %)	1 (5.26 %)	4 (21.0 %)
Cefoxitin	8 (42 %)	1 (5.26 %)	10 (52.6 %)
Ceftazidime	5 (31.6 %)	2 (10.53 %)	12 (63.2 %)
Ceftriaxone	14 (73.7 %)	1 (5.26 %)	4 (21.0 %)
Chloramphenicol	1 (5.26 %)	2 (5.26%)	16 (84.2 %)
Cifixime	10 (52.6 %)	2 (10.53 %)	7 (36.8 %)
Cefepime	15(78.9%)	1 (5.26 %)	3 (15.8 %)
Ciprofloxacin	11 (57.9 %)	2 (10.53 %)	6 (31.6 %)
Co-trimoxazole	9 (47.4 %)	0 (0.0 %)	10 (52.6 %)
Gentamycin	9 (47.4 %)	1 (5.26 %)	9 (47.4 %)
Nalidixic acid	8 (42.1 %)	2 (10.53 %)	9 (47.4 %)
Pipracillin	17(89.5 %)	1 (5.26 %)	2 (10.5 %)
Rifampine	7 (36.8 %)	0 (0.0 %)	12 (63.2 %)
Tetracycline	10 (52.6%)	1 (5.26 %)	8 (42.1 %)
Trimethoprim	8 (42.1%)	2 (10.35 %)	9 (47.4 %)

The high-level resistance against many antibiotics in the present study may be as a result of both intrinsic and acquired mechanisms. This resistance is widespread and constitutes serious clinical threats(32).

Table (5) shows that the majority of tested *Klebsiella* isolates were resistant to amoxicillin-clavulanate .It was also clear from this table that all *Klebsiella* isolates were sensitive to

ciprofloxacin. This result is close to Al-Husseiny(13) who found that all tested *Klebsiella pneumonia* isolates that isolated from cancer patient were sensitive to ciprofloxacin.

Table (5): Antibiotics susceptibility for *Klebsiella* spp. (n=13) isolated from colon cancer patients.

Type of antibiotic	No. (%) of Resistant Isolates	No. (%) of Intermediate Isolates	No. (%) of Sensitive Isolates
Amikacin	1 (7.69 %)	0 (0.0 %)	12 (92.31 %)
Amoxicillin-Clavulanate	12 (92.3 %)	1 (7.69 %)	0 (0.0 %)
Azteronam	8 (61.5 %)	0 (0.0 %)	5 (38.5 %)
Cefotaxime	7 (53.8 %)	0 (0.0 %)	6 (46.15 %)
Cefoxitin	5 (38.5 %)	0 (0.0 %)	8 (61.5 %)
Ceftazidime	6 (46.1 %)	1 (7.69 %)	6 (46.15 %)
Ceftriaxone	8 (61.5 %)	0 (0.0 %)	5 (38.46 %)
Chloramphenicol	1 (7.69 %)	0 (0.0 %)	12 (92.31 %)
Cifixime	9 (69.2 %)	0 (0.0 %)	4 (30. 8 %)
Cefepime	9 (47.4 %)	1 (7.69 %)	3(23.1%)
Ciprofloxacin	0 (0.0 %)	0 (0.0 %)	13 (100 %)
Co-trimoxazole	8 (61.5 %)	0 (0.0 %)	5 (38.46 %)
Gentamycin	10 (76.9 %)	0 (0.0 %)	3(23.1%)
Nalidixic acid	3 (23.1 %)	4 (30.78 %)	6 (46.15 %)
Pipracillin	11 (84.6 %)	2 (15.38 %)	0 (0.0 %)
Rifampine	10 (76.9 %)	0 (0.0 %)	3(23.1%)
Tetracycline	9 (69.2%)	0 (0.0 %)	4 (30.78 %)
Trimethoprim	8 (61.5 %)	0 (0.0 %)	5 (38.46 %)

The results of present study indicated that there was a moderate level of resistance to third generation cephalosporins among *Klebsiella* isolates; 46.1% of these isolates were resistant to ceftazidime, while 61.5% and 53.8% isolates were resistant to ceftriaxone and cefotaxime respectively. Table (6) shows that out of 19 *E. coli* isolates only 11 (57.9 %) were β-lactamase producers.

Table (6): β-lactamase-producing *E. coli* and *Klebsiella* spp. by rapid iodometric method.

Type of isolate	No. of isolate	No. of β-lactamase producers
E. coli	19	11 (57.9 %)
Klebsiella spp.	13	8 (61.3 %)
Total	**32**	**19 (59.4 %)**
Cal. X^2 = 0.042	**tab X^2 = 0.026**	**df = 1** **P-value = 0.83761**

This present study also showed that out of 13 *Klebsiella* spp. isolates, only 8 (61.3 %) were positive with rapid iodometric method. The statistical analysis showed a significance differences ($P \leq 0.01$) among tested isolates.

In conclusion, the E.coli and Klebsiellae isolates are the predominant species recovered from stool samples of cancer patients and all of the tested *E. coli* and *Klebsiellae* isolates are multidrug resistant; therefore, such organisms represent a serious therapeutic challenge in cancer patients.

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