Infectious Agents and Cancer

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Evaluation of multidrug resistance in the Gram-negative microbiome of cancer patients and the adverse effects of their metabolites on albino rats and epithelial or fibroblasts cell lines

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Abstract

Background Cancer is a significant global health issue due to its high incidence and mortality rates. In recent years, the relationship between the human microbiota and cancer has garnered attention across various medical fields. This includes research into the microbial communities that influence cancer development, tumor-associated microorganisms, and the interactions between the microbiome and tumor, collectively referred to as the oncobiome.

Methods The negative effects of secondary metabolites extracted from selected multidrug-resistant Gram-negative bacteria within the cancer microbiota were evaluated. These effects included carcinogenicity, mutagenicity, hepatotoxicity, nephrotoxicity, and sperm deformities observed in albino rats after one month of oral ingestion of these microbial extracts.

Results Our findings in the present investigation revealed that among the bacterial community derived from the microbiota, Gram-negative bacteria accounted for 74.87% the total microbiota (146 out of 195) and their spectrum including *Escherichia* sp. (n = 36, 24.66%) followed by *Acinetobacter* sp. (n = 34, 23.29%), *Stenotrophomonas* sp. (n = 29, 19.86%), *Pseudomonas* sp. (n = 26, 17.81%) and *Serratia* sp. (n = 21, 14.38%), as the most prevalent pathogens. All isolates derived from the cancer microbiome exhibited multidrug resistance to a large number of conventional therapies. Out of them *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4 and *Pseudomonas* sp. Esraa 5 strains showed multidrug resistant profile against all antibiotic classes under study including penicillins, cephalosporins, carbapenems, fluoroquinolones, β-lactamase inhibitors combinations, folate synthesis pathway inhibitors, phosphonic, aminoglycosides, polymyxins, tetracyclines, macrolides, and chloramphenicol antibiotics. The adverse effects of oral ingestion of their metabolites were evaluated in albino rats. They induced pronounced carcinogenesis along with severe raise in the inflammatory cytokines, hepatotoxicity, nephrotoxicity, mutagenicity along with sperm deformities in treated animals. Moreover, all metabolites showed marked cytotoxicity against human normal cell lines; human mammary epithelial (MCF10A), human lung fibroblasts (WI38) and human dermal fibroblasts (HDFs).

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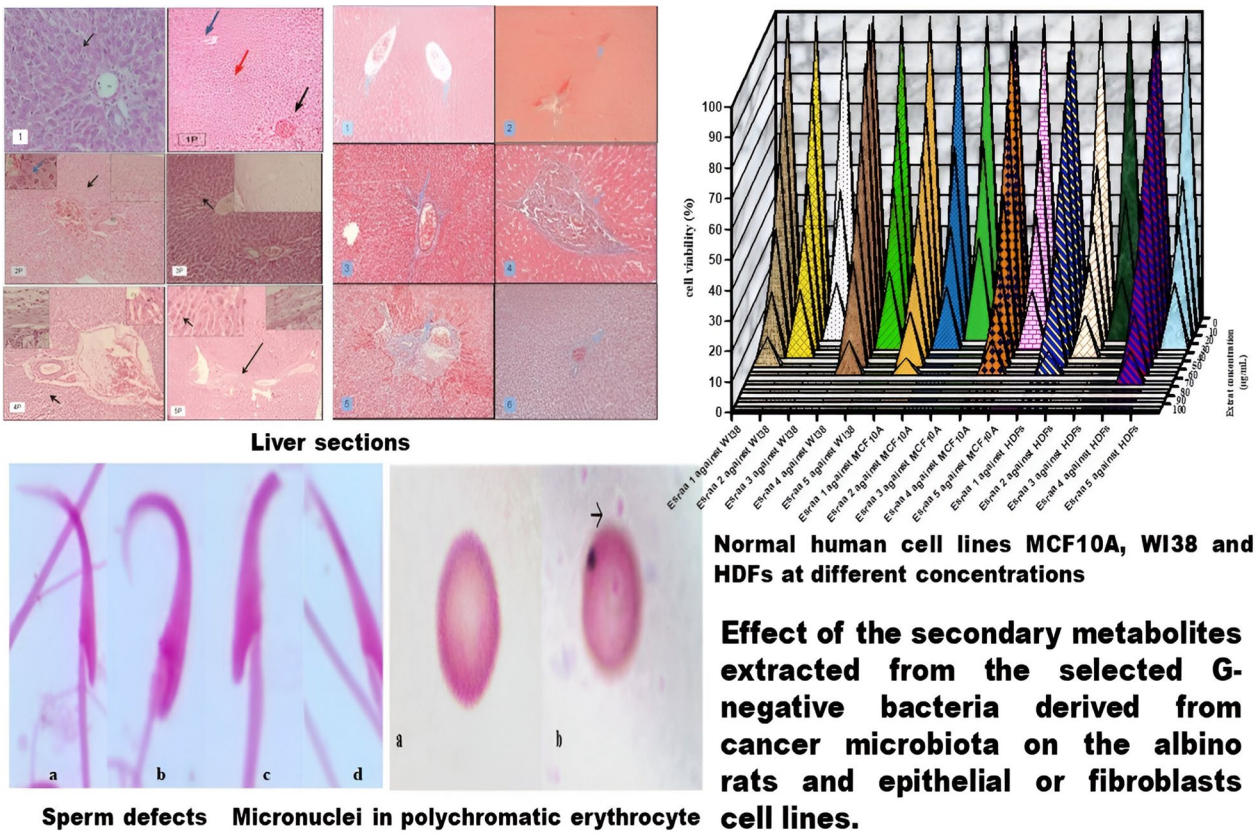
Conclusion These bacterial strains isolated from the cancer microbiome may play significant roles in inducing cancer, inflammation, mutagenesis, hepatotoxicity, nephrotoxicity, and sperm abnormalities, along with histopathological changes in the treated animal groups by orally administrated metabolites in compared to the untreated group.

Keywords Antibiotic resistance, Bacterial extract, Rat, Oral ingestion, Inflammatory cytokines, Carcinogenesis, Non-tumorigenic cell lines

Graphical Abstract



Introduction

Cancer is a major health concern globally because of its high incidence and mortality rates. In recent decades, the microbial association with human body (microbiota) has attracted attention in various medical fields, containing cancer biology and the microbial groups that have any kind of influence on cancer development, tumor-associated microorganisms, and the contact between microbiome and tumor were defined as the oncobiome [1, 2]. The eubiome or oncobiome excretes bioactive metabolites containing short-chain fatty acids, amino acid, hormone-like effects, and other secondary metabolites that trigger cancer initiation or can encourage metastasis creation in tumor like was showed in breast tumor or pancreas adenocarcinoma [3]. Current investigation points out the etiological role of the human microbiome and bacterial infections of the body as risk factors correlated with the development of ovarian, prostate, colorectal and breast cancers by altering the metabolism of steroid hormones and disrupting estrogen metabolism, which play a key role in most cancers [3–5].

Recent research has indicated that the development of pancreatic, esophageal, pancreatic and gynecological cancer is among the most lethal malignancies that are

closely related to the microbiome and influence cancer development through a different mechanism including inflammation and immunomodulation [6–9]. In the past era, the role of host-microbial connections in inducing human tumor plasticity and malignant progress has been documented [10]. Development and alterations in the microbiota negatively impacts the structure and function of different organs as well as the host's behavior [11]. Presently, different countries mentioned that the scale of bacteria in tumor patients has moved from prevalent G-positive to G-negative that seem to take the residence of G-positive, that can be caused by the relatively lower employ of lodging medical devices and lower prescribing of prophylactic antimicrobial usages in tumor patients [12].

Hence in order to effectively inhibit, recognize as well as handle infections, information of the altering epidemiology of infections is critical [13]. It has been displayed that bacterial inflammatory microenvironments mediate the tumor formation due to the contact with certain microorganisms and the immune system resultant in chronic inflammation leading to tumor [14]. Inflammations prompted tumor through various tools containing stimulation of cell proliferation, yield of great levels of metabolically activated free radicals, reactive oxygen species, N-nitrosamines that cause oxidative harm to DNA, regulatory proteins as well as other macromolecules; mutations or even gene deletions and then prompt and impulse different categories of tumor [15]. Amongst the infection of bacterial that prompted tumor, *Salmonella typhi* induce gallbladder cancer through changing the genomic sequence protein cancer p53 and increase of protooncogene c-MYC, *Chlamydia pneumonia* induce lung cancer by variation in apoptosis and cell programming signaling; overexpression of miRNA-328; exciting lung-resident γδ T cells; increase of Myd88-dependent IL-1b and IL-23; increase secretion of cytokines, IL-8, IL-10, and TNF but *Helicobacter pylori* was the cause of distal stomach adenocarcinoma through its capability to cause critical inflammations [16]. Lenický et al., reported that the bacterial *Proteus mirabilis*, *Citrobacter braakii*, *Staphylococcus lentus* and *Escherichia coli* as the most dominant strains had a negative influence on the sperm quality parameters including sperm motility, acrosome integrity and they stated the positive associations was detected concerning the bacterial load and fragmentation of DNA, ROS over-generation, oxidative mutilation to the proteins, and lipids in semen [17].

Antibiotics remain the mainstay of pathogens treatments and the select of empirical antibiotics therapy should be based on the prevalent pathogens and the sensitivity of those microorganisms to antibiotics that vary greatly from region to another then continuous monitoring of the type of drug-resistant local infection is critical [18, 19]. Cancer patients are at risk of multidrug-resistant nosocomial infections because of unsuitable usage of antibiotics [20]. It is expected that there will be more deaths due to antimicrobial resistance than cancer by 2050. The occurrence of drug resistance in cancer patients is a catastrophic problem because it combines the two biggest problems of humanity, multidrug resistance and tumorigenesis [14, 21–23]. Furthermore, Garg et al., reported that in cancer patients among 644 positive bacterial cultures, G-negative isolates were most commonly encountered (84.14%) with the highest level of resistance to various antibiotics [20]. *Acinetobacter baumannii, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter* sp., *Stenotrophomonas maltophilia* and *Escherichia coli* have gained notoriety worldwide as the most prevalent multidrug resistant pathogens that characterized as the most common carcinogens in tumor patients with moveable rate relying on the recording country and continent [24–27].

The present work was designed to measure multidrug-resistant G-negative bacteria (MDRGN) in the microbiome of cancer patients against 37 antibiotics of 12 chemical classes. Moreover, the adverse effects of secondary metabolites extracted from the selected strains that characterized as being XDR strains with the highest MICs values among the cancer microbiota including carcinogenicity, mutagenicity, hepatotoxicity, nephrotoxicity and sperm deformities that occurred in albino rats after oral ingestion of these microbial extracts, individually for one month were assessed. Furthermore, anti-proliferative activities of these extracts against different human normal cell lines including mammary epithelial (MCF10A), lung fibroblasts (WI38) and dermal fibroblasts (HDFs) cell lines were described.

Materials and methods

Antimicrobial susceptibility test

All bacterial isolates susceptible to antimicrobial agents performed according to the CLSI reference broth microdilution technique, EUCAST, or FDA categorical interpretations were used [28–31]. The antibiotics tested were penicillins (ampicillin, ticarcillin, oxacillin, piperacillin and carbenicillin), cephalosporins (cephalothin, cefazolin, cefoxitin, ceftazidime, cefepime, ceftriaxone and cefotaxime), carbapenems (ertapenem, imipenem, meropenem and doripenem), antibiotics with β-lactamase inhibitors (ceftazidime-avibactam, meropenem-vaborbactam as well as imipenem-cilastatin), folate synthesis pathway inhibitors (trimethoprim/sulfamethoxazole), fluoroquinolones (ofloxacin, ciprofloxacin, levofloxacin and moxifloxacin), phosphonic (fosfomycin), aminoglycosides (gentamicin, tobramycin, netilmicin, amikacin,

nitrofurantoin and vancomycin), polymyxins (colistin), tetracyclines (doxycycline, minocycline and tigecycline), macrolides (clarithromycin and azithromycin) and chloramphenicol antibiotics (Oxoid, UK).

Clinical strains and extraction of secondary metabolites

The resistant strains *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4 and *Pseudomonas* sp. Esraa 5 were isolated from cancer patient, cultured and extracted like previously mentioned previously [14]. Briefly, each isolate was inoculated from blood agar plates into tryptic soy broth medium with 5% blood in 500 mL Erlenmeyer flasks and incubated at 37 °C, 150 rpm for two days followed by extraction with ethyl acetate (EtOAc) at pH 4.0. Each ethyl acetate (EtOAc) extractable metabolite was dried, assigned the same code as its producing strain and stored at – 20 °C.

Animals and experimental design

Adult male Sprague–Dawley albino rats (weighting 150–180 g) were obtained from the animal colony (National Research Centre, Egypt), the rats were allowed free access to tap water and food, for a week before starting the trial for acclimatization. The animals were cared for in compliance with the fundamental standards for the handling and use of experimental rats, as approved by the ethical committee of the National Research Center (FWA 00014747), which had previously authorized this investigation. After acclimation, the animals were randomly arranged into 6 groups (7 rats per group) as group 1 healthy rats orally ingested with physiological saline (1.25 mL/kg body weight twice/week) for four weeks (control), while groups 2, 3, 4, 5, and 6 comprised healthy animals those were subjected to oral administration with Esraa 1, Esraa 2, Esraa 3, Esraa 4 and Esraa 5 extracts, respectively at dosage of 1.25 mL/kg twice/week (11.6 g of each extract was dissolved in 8 mL physiological saline) for a similar period.

Blood and tissue sampling

One day after the last administration, the rats were fasted overnight. Then, under diethyl ether anesthesia, blood samples were collected from retro orbital venous plexus, and centrifuged at 3000 rpm under cooling for 15 min; sera were separated, and stored at − 80 °C until the biochemical studies could be performed as soon as possible.

Biochemical measurements

Serum amino transferases (ALAT and ASAT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities as well as serum urea and creatinine, alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), tumor necrosis factor alpha (TNFα) and interleukin-1beta (IL-1β) levels were determined [14].

Histopathological studies

Sections of 5 µm thick from rats' groups treated with extract of Esraa 1, Esraa 2, Esraa 3, Esraa 4 and Esraa 5, individually as well as animals control were cut and stained with hematoxylin and eosin (H & E) and Masson's trichrome for connective tissue, collagen, assessment [32]. The collagen fibers percentage was measured by image analyzer system.

Micronucleus test and sperm morphology deformity

In all animal groups, after the last ingestion of each microbial extract**,** the epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum [33]. Counting the polychromatic erythrocytes and the ratio of micro-nucleated polychromatic erythrocytes (MNPCEs) were established by observing the MN cells numbers from 1000 PCEs per rat [34]. The epididymides were excised and minced in 2.2% isotonic sodium citrate solution. Smears were equipped and sperms were stained with Eosin Y [35, 36]. Thousand sperm/animal were evaluated for morphological deformities.

Preparation of cell lines and The MTT cell proliferation assay and IC_{50} determination

To study the cytotoxic effects of these microbial extracts, their anti-proliferation activities against three different types of human non-tumorigenic cells including mammary epithelial (MCF10A), lung fibroblasts (WI38) and dermal fibroblasts (HDFs) cell lines (American Type Culture Collection; ATCC) were evaluated. MCF10A were preserved in MEBM basal medium added with the MEGM SingleQuot Kit (Lonza Corporation, USA) containing 100 ng/mL cholera toxin (Sigma-Aldrich), WI38 were cultured in Eagle's minimum essential medium ((EMEM, ATCC) complemented with 2 mM glutamine and 1% non-essential amino acids (NEAA) and HDFs were cultured in Dulbecco's modified Eagle's medium (DMEM). All media were accompanied with 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin antibiotics (Gibco, Grand Island, NY, USA), and cultured at 37 °C in a humidified atmosphere with 5% CO_2. At confluence 85%, the cells were trypsinized by trypsin–EDTA

(Sigma-Aldrich), then MCF10A, HDFs and WI38 cells were seeded in 96-well plates (at a density of 2×10^4 cells/mL) and exposed to various concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL) of each bacterial extract individually and trial control with cells only was also involved. After incubation for 48 h, the viability of cells and the half maximal inhibitory concentration (IC_{50}) values were assessed. To measure the rate of cell proliferation, the MTT (3-(4, 5-dimethylthiazolyl-2)−2, 5-diphenyltetrazolium bromide) assay based on the yellow compound MTT-dependent was applied. After the handling time, cells were allowed to react with MTT for 3–4 h in the dark at 37 °C and then dark purple formazan crystals were dissolved and the absorbance was measured spectrophotometry at 595 nm. The cell viability % = [(Optical density {OD} of treated cell − OD of blank) / (OD of vehicle control − OD of blank) × 100] was calculated [37].

Statistical analysis and data reconstruction

The achieved results were statistically analyzed using one-way analysis of variance (ANOVA) followed by post hoc (Duncan) test at $p \leq 0.05$. T-test was used for cytogenetic analysis.

Results

Microbial spectrum of G-negative bacteria isolated from cancer microbiome

In the current study, G-negative bacteria were the most prevalent bacteria in the obtained microbiome with the rate of 74.87% (146/195 isolates, Table 1). Furthermore, data in Table 1 showed that the general ranking of the most common pathogens among G-negative microbiota of oncology patients was *Escherichia* sp. (n = 36, 24.66%) > *Acinetobacter* sp. (n = 34, 23.29%) > *Stenotrophomonas* sp. (n = 29, 19.86%) > *Pseudomonas* sp. (n = 26, 17.81%) > *Serratia* sp. (n = 21, 14.38%).

The prevalence of antibiotics resistance to G-negative bacteria obtained from cancer microbiome

The antibiotic resistance prevalence analysis in Table 1 showed that the obtained G-negative bacteria derived from the cancer microbiome exhibited entirely multidrug resistance to penicillin antibiotics including ampicillin, ticarcillin, oxacillin, piperacillin and carbenicillin (MIC; 128—> 1024, 560—> 1024, 256—> 1024, 512—> 1024 and 440—> 1024 µg/mL), respectively as well as the first-generation cephalosporins including cephalothin, cefazolin and cefoxitin (MIC; 256—> 1024, 360—> 1024 and 480—1024 µg/mL), respectively. Moreover, resistance prevalence to other cephams including ceftazidime, cefepime, ceftriaxone and cefotaxime reached 100% in *Stenotrophomonas* and *Acinetobacter* isolates with MIC ranged from 128 to > 1024 µg/mL (Table 1). Additionally, resistance rate equal to 96.15, 76.92, 65.39 and 57.69% with MIC 400–664, 256–512, 128–512 and 64–512 µg/mL, respectively was recorded in *Pseudomonas* isolates (n = 26) compared to 95.24, 76.19, 66.67 and 61.91% with MIC 512–720, 256–640, 128–560 and 64–512 µg/mL, respectively in *Serratia* sp. isolates (n = 21 isolates) while 88.89, 80.56, 63.89 and 63.89% of *Escherichia* isolates (n = 36 isolates) were resist to ceftazidime, cefepime, ceftriaxone and cefotaxime with MIC 256–512, 128–800, 64–480, and 128–256 µg/mL, respectively (Table 1). Then the cancer microbiota under study can be considered as extended-spectrum β-lactamase producers (ESBL).

The highest resistance rates toward imipenem (73.08% with MIC; 128–512 µg/mL) and doripenem (76.92% with MIC; 264–472 µg/mL) was recorded among *Pseudomonas* species but the highest resistant against meropenem (75.86% with MIC; 256–600 µg/mL) and ertapenem (79.41% with MIC; 400–648 µg/mL) were occurred among *Stenotrophomonas* and *Acinetobacter* species, respectively (Table 1). On the other hand, when ceftazidime, imipenem and meropenem were used together with the β-lactamase inhibitors avibactam, cilastatin and vaborbactam, respectively the rates of resistance in the total number of G-negative bacteria decreased significantly from 95.89, 62.33 and 68.49% to 39.73, 25.34 and 29.45%, respectively (Table 1).

Moreover, data in Table 1 showed that the rates of resistance toward fluoroquinolones antibiotics include ofloxacin, ciprofloxacin levofloxacin and moxifloxacin as well as trimethoprim/sulfamethoxazole (folate synthesis pathway inhibitors) were ≥ 61.91% ≤ 80.95%; ≥ 41.67% ≤ 75.00%; ≥ 69.23% ≤ 80.77%; ≥ 75.86% ≤ 93.10%, and ≥ 73.53% to 100.00% with *Serratia* sp., *Escherichia* sp., *Pseudomonas* sp., *Stenotrophomonas* sp. and *Acinetobacter* sp., respectively. Because aminoglycosides remain, a significant class of agents when microorganisms of interest are resistant to other classes, especially β-lactams and fluoroquinolones as in the current study, resistance to aminoglycosides among G-negative microbiome under study were evaluated (Table 1). High resistance potency was recorded against nitrofurantoin (n = 93, 63.70%) followed by tobramycin (n = 90, 61.64%), netilmicin (n = 85, 58.22%), gentamicin (n = 82, 56.16%), amikacin (n = 69, 47.26%) and vancomycin (n = 57, 39.04%) (Table 1). Therefore, the current spread of resistance to aminoglycosides amongst already MDR microorganisms is an unwelcome event. The data in Table 1 indicated that the cancer microbiome resistance rates under study were estimated to be 63.70, 42.47 and 34.93% with MIC; 240–1000, 128–500, and 32–280 µg/mL against the tetracycline antibiotics (doxycycline, minocycline and tigecycline), respectively but 79.45, 74.66 and 65.07%

Table 1 Multidrug resistance prevalence and MICs of various antibiotics against G-negative bacterial strains derived from the cancer mirobiome

Antimicrobial agent	Isolates and resistance rate (%)																	
	Total G-negative bacteria n = 146/195, 74.87%			*Serratia* sp. n = 21/146, 14.38%			*Esherichia* sp. n = 36/146, 24.66%			*Pseudomonas* sp. n = 26/146, 17.81%			*Stenotrophomonas* sp. n = 29/146, 19.86%			*Acinetobacter* sp. n = 34/146, 23.29%		
	No	%	MIC (μg/mL)	No	%	MIC (μg/mL)	No	%	MIC (μg/mL)	No	%	MIC (μg/mL)	No	%	MIC (μg/mL)	No	%	MIC (μg/mL)
Penicillins antibiotics																		
Ampicillin	146	100	128–>1024	21	100	256–1024	36	100	128–1000	26	100	256–>1024	29	100	256–1024	34	100	>1024
Ticarcillin	146	100	560–>1024	21	100	256–>1024	36	100	512–>1024	26	100	>1024	29	100	>1024	34	100	>1024
Oxacillin	146	100	256–>1024	21	100	256–1000	36	100	256–1000	26	100	512–1000	29	100	512–1000	34	100	640–>1024
Piperacillin	146	100	512–>1024	21	100	512–752	36	100	400–800	26	100	512–1024	29	100	>1024	34	100	>1024
Carbenicillin	146	100	440–>1024	21	100	648–1000	36	100	440–640	26	100	512–1024	29	100	>1024	34	100	>1024
Cephalosporins antibiotics																		
Cephalothin	146	100	256–>1024	21	100/0	256–1000	36	100	256–800	26	100	512–1024	29	100	>1024	34	100	512–>1024
Cefazolin	146	100	360–>1024	21	100	512–1024	36	100	360–640	26	100	512–1024	29	100	>1024	34	100	640–>1024
Cefoxitin	146	100	480 –1024	21	100	480–1000	36	100	512–1000	26	100	640–800	29	100	528–800	34	100	512–1024
Ceftazidime	140	95.89	256–>1024	20	95.24	512–720	32	88.89	256–512	25	96.15	400–664	29	100	>1024	34	100	>1024
Cefepime	128	87.67	128–>1024	16	76.19	256–640	29	80.56	128–800	20	76.92	256–512	29	100	256–>1024	34	100	512–>1024
Ceftriaxone	117	80.14	128–>1024	14	66.67	128–560	23	63.89	64–480	17	65.39	128–512	29	100	256–512	34	100	256–>1024
Cefotaxime	114	78.08	64–1000	13	61.91	64–512	23	63.89	128–256	15	57.69	64–512	29	100	256–1000	34	100	128–840
Carbapenems antibiotics																		
Ertapenem	97	66.44	128–648	12	57.14	128–480	20	55.56	128–256	15	57.69	256–360	23	79.31	256–640	27	79.41	400–648
Imipenem	91	62.33	128–512	9	42.86	264–512	19	52.78	128–320	19	73.08	128–512	20	68.97	264- 512	24	70.59	128–512
Meropenem	100	68.49	128–600	10	47.62	256–400	25	69.44	128–480	18	69.23	256–512	22	75.86	256–600	25	73.53	256–512
Doripenem	87	59.59	128–472	13	61.91	128–400	17	47.22	128–312	20	76.92	264–472	17	58.62	256–564	20	58.82	128–432
Antibiotic + β-lactamase inhibitors																		
Ceftazidime-Avibactam	58	39.73	128–800	8	33.09	256–400	14	38.89	128–512	6	23.08	128–640	13	44.83	240–320	17	50	128–480
Meropenem-Vaborbactam	43	29.45	72–320	8	38.1	128–320	10	27.77	72–280	8	30.77	224–320	8	27.59	512–800	9	26.47	400–800
Imipenem-Cilastatin	37	25.34	64–256	6	28.57	64–120	10	27.77	72–280	8	30.77	224–320	7	24.14	72–256	6	17.65	256–512
Fluoroquinolones antibiotics																		
Ofloxacin	116	79.45	240–800	16	76.19	400–720	27	75	240–600	19	73.08	512–800	25	86.21	360–722	29	85.3	256–800
Ciprofloxacin	113	77.4	128–1000	14	66.67	128–512	22	61.11	128–586	21	80.77	256–640	27	93.1	400–1000	30	88.24	256–752
Levofloxacin	108	73.97	256–960	13	61.91	320–528	20	55.56	256–640	19	73.08	280 512	25	86.2	360–960	28	82.35	320–800
Moxifloxacin	104	71.23	360–1000	15	71.43	480–664	24	66.67	360–720	18	69.23	480–928	22	75.86	640–1000	25	73.53	400–1000
Folate synthesis pathway inhibitors																		
Trimethoprim/sulfamethoxazole	110	75.34	512–1024	17	80.95	512–1024	15	41.67	512–972	20	77.77	560–800	24	82.76	800–928	34	100	600–>1024

Table 1 (continued)

Antimicrobial agent	Isolates and resistance rate (%)																	
	Total G-negative bacteria n = 146/195, 74.87%			*Serratia* sp. n = 21/146, 14.38%			*Esherichia* sp. n = 36/146, 24.66%			*Pseudomonas* sp. n = 26/146, 17.81%			*Stenotrophomonas* sp. n = 29/146, 19.86%			*Acinetobacter* sp. n = 34/146, 23.29%		
	No	%	MIC (µg/mL)	No	%	MIC (µg/mL)	No	%	MIC (µg/mL)	No	%	MIC (µg/mL)	No	%	MIC (µg/mL)	No	%	MIC (µg/mL)
Phosphonic antibiotics																		
Fosfomycin	85	58.22	256–1000	5	23.81	256–512	7	19.44	256–400	24	92.31	360–1000	16	55.17	512–1000	33	97.05	400–960
Aminoglycosides antibiotics																		
Gentamicin	82	56.16	64–320	8	38.1	64–256	14	38.89	128–280	16	61.54	64–296	20	68.97	64–320	24	70.59	256–320
Tobramycin	90	61.64	128–512	12	57.14	128–256	17	47.22	256–400	14	53.85	256–512	25	86.21	256–512	22	64.71	256–512
Netilmicin	85	58.22	128–600	10	47.62	240–400	20	41.67	128–600	19	73.08	256–480	17	58.62	256–600	19	55.88	420–600
Amikacin	69	47.26	256–1000	6	28.57	512–1000	10	27.78	156–600	15	57.69	512–1000	13	44.83	256–1000	25	73.53	512–960
Nitrofurantoin	93	63.7	512–1024	11	52.38	400–784	19	52.78	240–880	20	76.92	256–920	18	62.07	512–1024	25	73.53	512–1024
Vancomycin	57	39.04	128–720	9	42.86	256–512	11	30.56	128–568	11	42.31	256–512	12	41.38	360–648	14	41.17	512–720
Polymyxins antibiotics																		
Colistin	31	21.23	16–160	9	42.86	16–64	2	5.56	32	4	15.39	16–64	10	34.48	64–128	6	17.65	64–160
Tetracyclines antibiotics																		
Doxycycline	93	63.7	240–1000	8	38.1	240–512	17	47.22	320–1000	18	69.23	512–1000	23	79.31	256–1000	27	79.41	320–512
Minocycline	62	42.47	128–500	6	28.57	128–256	11	30.56	128–256	13	50	128–500	14	48.28	256–500	18	52.94	256–500
Tigecycline	51	34.93	32–280	6	28.57	64–240	8	22.22	32–128	12	46.15	100–250	10	34.28	200–280	15	44.12	160–250
Macrolides antibiotics																		
Clarithromycin	116	79.45	256 – > 1024	16	76.19	256–640	22	61.11	128–512	17	65.39	256–> 1024	27	93.1	> 1024	34	100	> 1024
Azithromycin	109	74.66	128 – > 1024	14	66.67	256–512	20	55.56	128–512	19	73.08	512–> 1024	24	82.76	> 1024	32	94.12	> 1024
Chloramphenicol antibiotics																		
Chloramphenicol	95	65.07	128–> 1024	11	52.38	128–256	13	36.11	128–640	21	80.77	512–> 1024	22	75.86	512–> 1024	28	82.35	256–> 1024

of G-negative isolates were resistant to clarithromycin, zithromycin (macrolides) and chloramphenicol, respectively with MIC ranged between 128 and > 1024 µg/mL. Of the results presented in Table 1, the highest sensitivity profile was recorded against colistin followed by imipenem-cilastatin, meropenem-vaborbactam, tigecycline, vancomycin and ceftazidime-avibactam (78.77, 74.66, 70.55, 65.07**,** 60.96 and 60.27%), respectively (Table 1). The isolates *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4 and *Pseudomonas* sp. Esraa 5, which showed resistance against all the antibiotics under study and then characterized extensive drug-resistant (XDR) strains with the highest MIC values, were selected for the further studies.

Carcinogenic activities of bacterial extracts

After oral ingestion of microbial extracts, under study by the five animals' groups, the values of serum cancer markers (AFP, CEA, and LDH) increased significantly in all treated animals' groups comprised to the healthy animals' group (Table 2). Serum AFP levels was significantly increased from 0.52 ± 0.019 ng/mL (healthy group) to 2.08 ± 0.07 ng/mL (300%) after treating with *Acinetobacter* sp. Esraa 3 followed by *Pseudomonas* sp. Esraa 5, *Stenotrophomonas* sp. Esraa 2, *Escherichia* sp. Esraa 4, and *Serratia* sp. Esraa 1 extracts (1.92 ± 0.41; 269.2%, 0.85 ± 0.04; 63.5%, 0.73 ± 0.04; 40.4%, and 0.57 ± 0.02 ng/mL; 9.6%), respectively (Table 2). Furthermore, serum CEA level increased in descending order in animals' groups handled by extracts of *Pseudomonas* sp. Esraa 5, *Stenotrophomonas* sp. Esraa 2, *Escherichia* sp. Esraa 4, *Acinetobacter* sp. Esraa 3, and *Serratia* sp. Esraa 1 (07.69 ± 0.71; 311.2%, 6.08 ± 0.13; 225.1%, 4.41 ± 0.79; 135.8%, 3.53 ± 0.07; 88.8%, and 1.99 ± 0.14 ng/mL; 6.4%), respectively compared with the control group (1.87 ± 0.04 ng/mL), indicating the carcinogenic activity of the studied G-negative bacteria (Table 2). Moreover, activity of serum lactate dehydrogenase (LDH) significantly increased to 2248 ± 81.1, 1851 ± 73.4, 789 ± 7.49, 623 ± 18.5, and 402 ± 11.6 IU/L (464.8, 365.1, 98.2, 56.5, and 1.1%) in rats after oral ingestion of the extracts of strains Esraa 3, Esraa 5, Esraa 2, Esraa 4, and Esraa 1, respectively comprised with 398 ± 3.74 IU/L in healthy animals' group (Table 2). For instant, the higher carcinogenic activity with *Escherichia* strain was achieved by subcutaneous-injection while *Serratia* sp. Esraa 1 or *Pseudomonas* sp. Esraa 5 extracts markedly increased serum level of CEA in the case of oral ingestion. However, there is no significant difference in the effect of *Stenotrophomonas* sp. Esraa 2 extract whether administered orally or by injection while *Acinetobacter* sp. Esraa 3 extract increased serum LDH activity by 171.5 and 98.2% post subcutaneous-injection and oral administration, respectively.

Hepatotoxic effect of bacterial extracts

Oral ingestion of the microbial extracts in treated animals stimulated liver dysfunction that was monitored through increased activities of liver enzymes. ALAT activity markedly increased from 23.1 ± 1.8 U/L (control group) to 27.1 ± 1.98, 85.3 ± 6.56, 27.7 ± 2.06, and 120.6 ± 3.2 U/L (17.3, 269.3, 19.9, and 422.1%) in the animal groups ingested with the extracts of strains Esraa 2, Esraa 3, Esraa 4, and Esraa 5, respectively; while it decreased by 7.8% post-handling with the extract of strain Esraa 1 (Table 3). Similarly, serum ASAT activity increased from 22.3 ± 0.3.6 U/L (control group) to 25.2 ± 4.1, 29.0 ± 3.8, 78.6 ± 9.6, 25.4 ± 4.12, and 101.7 ± 3.13 U/L (13.0, 30.0, 252.5, 13.9, and 356.1%); GGT increased from 45.6 ± 2.83 U/L (control group) to 52.1 ± 1.44, 64.8 ± 2.84, 142.1 ± 3.8, 48.7 ± 1.38, and 102.7 ± 13.19 U/L (14.3, 42.1, 211.6, 6.8, and 125.2%); as well as serum ALAP activity increased from 80.7 ± 4.38 (control group) to 93.9 ± 9.3, 138.3 ± 6.9**,** 375.4 ± 10.5**,** 89.7 ± 1.83, and 256.4 ± 7.43 U/L (16.4, 71.4, 365.2, 11.2, and 217.7%) after the oral ingestion of the extracts of

Table 2 Effect of oral ingestion of selected bacterial extracts on some tumor markers in treated albino rats' groups compared to the normal group

Animal group	Treatment	CEA		AFP		LDH	
		ng/mL	%	ng/mL	%	IU/L	%
1	Control	1.87 ± 0.04	100.0	0.52 ± 0.01	100.0	398 ± 3.74	100.0
2	Esraa 1 extract	1.99 ± 0.14	106.4	0.57 ± 0.02	109.6	402 ± 11.6	101.1
3	Esraa 2 extract	6.08 ± 0.13*	325.1	0.85 ± 0.04*	163.5	789 ± 7.49*	198.2
4	Esraa 3 extract	3.53 ± 0.07*	188.8	2.08 ± 0.07*	400.0	2248 ± 81.1*	564.8
5	Esraa 4 extract	4.41 ± 0.79*	235.8	0.73 ± 0.04*	140.4	623 ± 18.5*	156.5
6	Esraa 5 extract	7.69 ± 0.71*	411.2	1.92 ± 0.41*	369.2	1851 ± 73.4*	465.1

All results are given as mean ± SEM. Mean with superscript symbol * is significantly different from that of normal control

Table 3 Effect of oral ingestion of selected bacterial extracts on liver function enzymes in treated albino rats' groups compared to normal animals

Animal group	Treatment	ALAT		ASAT		GGT		ALAP	
		U/L	%	U/L	%	U/L	%	U/L	%
1	Control	23.1 ± 1.8	100	22.3 ± 3.6	100	45.6 ± 2.83	100	80.7 ± 4.38	100
2	Esraa 1 extract	21.3 ± 2.17	− 7.8	25.2 ± 4.1	113	52.1 ± 1.44	114.3	93.9 ± 9.3	116.4
3	Esraa 2 extract	27.1 ± 1.98	117.3	29.0 ± 3.8*	130	64.8 ± 2.84*	142.1	138.3 ± 6.9*	171.4
4	Esraa 3 extract	85.3 ± 6.56*	369.3	78.6 ± 9.6*	352.5	142.1 ± 3.8*	311.6	375.4 ± 10.5*	465.2
5	Esraa 4 extract	27.7 ± 2.06	119.9	25.4 ± 4.12	113.9	48.7 ± 1.38	106.8	89.7 ± 1.83	111.2
6	Esraa 5 extract	120.6 ± 3.2*	522.1	101.7 ± 3.13*	456.1	102.7 ± 13.19*	225.2	256.4 ± 7.43*	317.7

All results are given as mean ± SEM. Mean with superscript symbol * is significantly different from that of normal control

Esraa 1, Esraa 2, Esraa 3, Esraa 4, and Esraa 5, respectively (Table 3). These results referring to sever hepatotoxic potential of these extracts; the highest hepatotoxic effect was exhibited by Esraa 3, and Esraa 5 (Table 3).

Nephrotoxic effect of bacterial extracts

The extracts of cancer microbiota exhibited nephrotoxic effects on the kidneys, which were achieved through a respectable increase in the concentricity of urea and creatinine in the blood compared to normal rats. Esraa 3 extract performed the most nephrotoxic properties as it increased the serum levels of urea and creatinine to 110.4 ± 3.95 (253.9%), and 1.78 ± 0.42 mg/dl (114.5%), respectively; while the extracts of Esraa 1, and Esraa 2 exhibited the smallest effect as they performed the minimal increase in serum urea (34.7 ± 3.25 mg/dl, 11.2%) and creatinine (0.84 ± 0.07 mg/dl, 1.2%), respectively (Table 4). This study revealed that these tumors-associated microbial communities have some sort of influence on tumor development. Therefore, this correlation can be considered as demonstrated that it can be defined as an oncobiome.

Effect of the bacterial extracts on the inflammatory cytokines

Inflammatory cytokines (IL-1β and TNF-α) levels were markedly increased post-oral ingestion of each bacterial extracts under study. *Pseudomonas* sp. Esraa 5 extract induced the highest production of IL-1β followed by Esraa 2, Esraa 3, Esraa 4, and Esraa 1 extracts (30.2 ± 2.41; 297.4%, 24.5 ± 2.18; 222.4%, 18.6 ± 1.89; 144.7%, 15.3 ± 1.08; 101.3%, and 7.8 ± 1.44 ng/L; 2.6%), respectively compared to 7.6 ± 1.65 ng/L of control group (Table 4). Moreover, TNF-α level was increased by (11.08 ± 0.94; 6.5%, 29.8 ± 1.71; 186.5%, 27.5 ± 0.65; 164.4%, 19.1 ± 2.44; 83.7%, and 41.9 ± 5.32 ng/mL; 302.9%) after oral administration of Esraa 1, Esraa 2, Esraa 3, Esraa 4, and Esraa 5 extracts, respectively compared to 10.4 ± 0.24 ng/mL of control group (Table 4).

Histopathological studies

The histopathological studies of the liver sections of untreated animals (group 1), and oral ingestion treated groups with Esraa 1 (group 2), Esraa 2 (group 3), Esraa 3 (group 4), Esraa 4 (group 5) and Esraa 5 (group 6) extracts, stained by Masson trichrom to examine the

Table 4 Effect of oral ingestion of selected bacterial extracts on kidney function tests and inflammatory cytokines in the treated albino rats' groups compared to the normal animals

Animal group	Treatment	Urea		Creatinine		IL-1β		TNFα	
		mg/dl	%	mg/dl	%	ng/L	%	ng/L	%
1	Control	31.2 ± 2.97	100.0	0.83 ± 0.07	100.0	7.6 ± 1.65	100.0	10.4 ± 0.24	100.0
2	Esraa 1 extract	34.7 ± 3.25	111.2	0.91 ± 0.07	109.6	7.8 ± 1.44	102.6	11.08 ± 0.94	106.5
3	Esraa 2 extract	48.1 ± 4.52*	154.2	0.84 ± 0.07	101.2	24.5 ± 2.18*	322.4	29.8 ± 1.71*	286.5
4	Esraa 3 extract	110.4 ± 3.95*	353.9	1.78 ± 0.42*	214.5	18.6 ± 1.89*	244.7	27.5 ± 0.65*	264.4
5	Esraa 4 extract	42.1 ± 4.9*	134.9	0.99 ± 0.08*	119.3	15.3 ± 1.08*	201.3	19.1 ± 2.44*	183.7
6	Esraa 5 extract	62.8 ± 10.1*	201.3	1.74 ± 0.31*	209.6	30.2 ± 2.41*	397.4	41.9 ± 5.32*	402.9

All results are given as mean ± SEM. Mean with superscript symbol * is significantly different from that of normal control

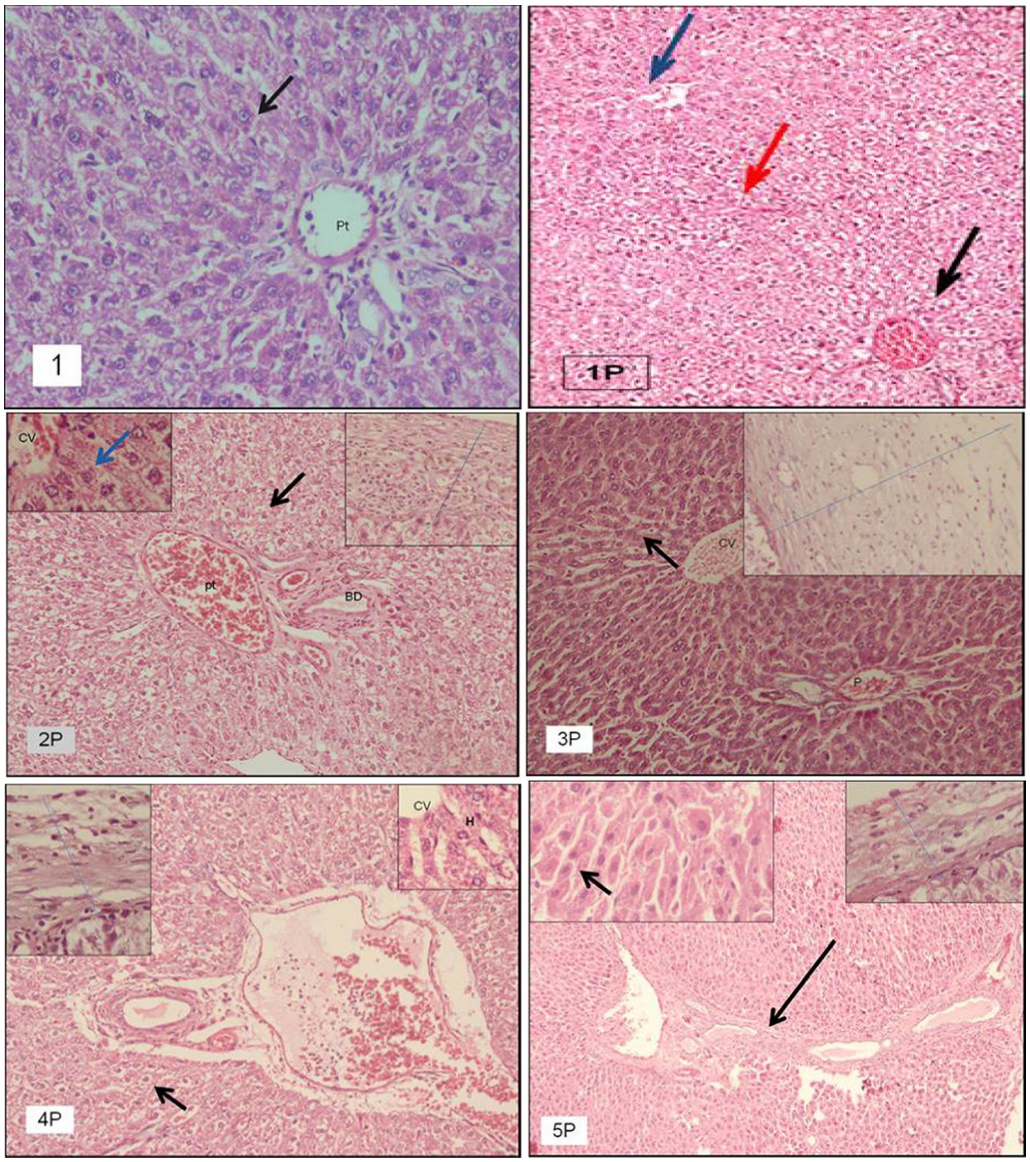


Fig. 1 A section in liver of control and oral treated animals; (section 1; control) group 1 (Hx. & E. × 400), (section 1P) group 2 treated with *Serratia* sp. Esraa 1 extract (Hx. & E. × 400), (section 2P) group 3 oral treated with *Stenotrophomonas* sp. Esraa 2 extract (Inset × 400) (Hx. & E. × 400), (section 3P) group 4 treated with *Acinetobacter* sp. Esraa 3 extract (Hx. & E. × 300), (section 4P) group 5 treated with extract of *Escherichia* sp. Esraa 4 (Hx. & E. × 300), and (section 5P) group 6 oral treated with extract of *Pseudomonas* sp. Esraa 5 (Hx. & E. × 200)

alterations in collagen and fibrous tissues are illustrated in Figs. 1 and 2. Liver section of the control group in Figs. 1 and 2 (section 1) showed branching cords of hepatocytes with vesiculated nuclei and intervening blood sinusoids lined with endothelial cells, hepatocytes as polygonal cells with acidophilic granular cytoplasm around the portal tract. The control group has the minimum amount of collagen or fibrous tissues compared to the collagen and fibrous tissues in all other orally treated groups. The hepatic sections of animals group 2 treated with the extract of Esraa 1 exhibited disrupted arrangement of hepatocytes around the portal tract (red arrow) together with severe cytoplasmic vacuolation and pyknotic nuclei (black arrow) but hepatocytes around the central vein are organized and nearly normal (blue arrow, Fig. 1; section 1P and 2; section 2). Treated animals by extract of Esraa 2 (group 3) exhibited disrupted arrangement of hepatocytes around the portal tract (pt) with vacuolization and pyknotic nuclei (arrow) but hepatocytes around the central vein are organized and nearly normal (inset) along with marked increase in connective tissue in liver capsule (right inset) were observed (Fig. 1; section 2P and

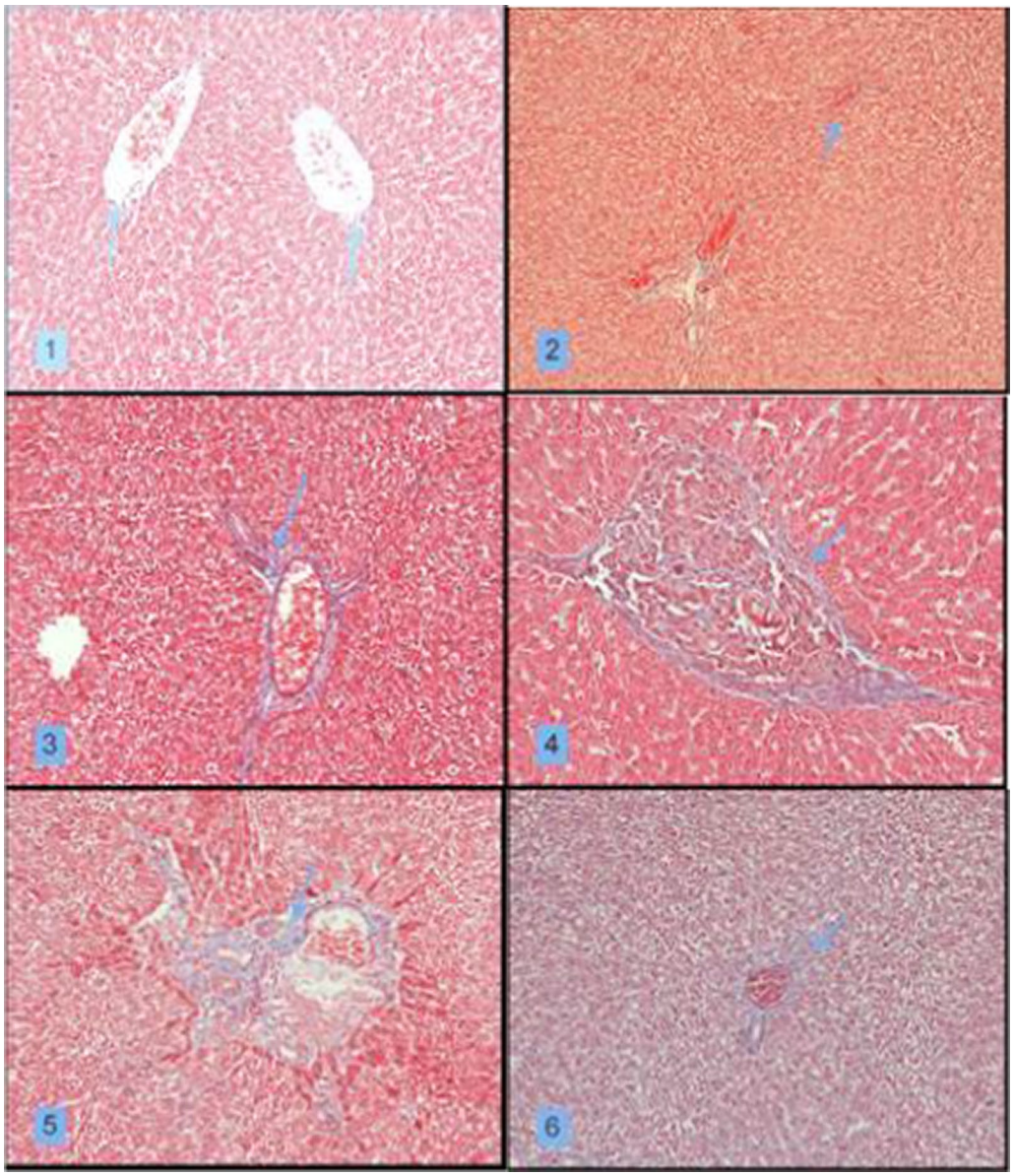


Fig. 2 Photomicrographs of liver sections of 1; untreated and 2 –6; oral treated animal groups with extracts of *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4 and *Pseudomonas* sp. Esraa 5 stained with Masson trichrom (Masson's trichrom reaction × 200), respectively

2; section 3). Radiating hepatocytes from the central vein, arranged, nearly normal and detached by blood sinusoid in addition to some inflammatory cells around the portal tract along with marked increase in connective tissue in liver capsule were observed in the liver sections of animals group 4 orally treated with Esraa 3 extract, (inset, Fig. 1; section 3P and 2; section 4). Data in Fig. 1 (section 4P) and 2 (section 5) illustrated the liver of animals group 5 with vacuolar and pyknosis in hepatocytes around the dilated and congested portal tract area with marked increase in normal arrangement of hepatic cells around the central vein (right inset) in connective tissue of liver capsule (left inset) after oral ingestion of *Escherichia* sp. Esraa 4 extract. Furthermore, the liver sections of the treated group with Esraa 5 extract (group 6) exhibited many hepatocytes with eosinophilic cytoplasm, pyknotic nuclei, massive increase in inflammatory cells around the dilated portal tract and central vein (left inset) and

Table 5 Ratio of micronuclei in polychromatic erythrocyte and number of sperm defects stimulated in the treated male rats by selected bacterial extracts as compared to the normal rats group

Animal group	Treatment	No. of MN	MN Mean ± SE	Abnormal sperm		No. of different types of sperms				Coiled tail
				No	Mean (%) ± SE	Straight	Banana	Amorphous	Without hook	
1	Control	38	0.76 ± 0.48	85	1.70 ± 0.56	27	37	3	18	–
2	Esraa 1 extract	296	5.92 ± 0.58**	301	6.02 ± 0.54**	34	153	41	53	20
3	Esraa 2 extract	321	6.42 ± 0.50**	314	6.28 ± 0.60**	47	161	45	39	22
4	Esraa 3 extract	227	4.54 ± 0.63**	291	5.82 ± 0.58**	40	148	37	48	18
5	Esraa 4 extract	392	7.84 ± 0.68**	368	7.36 ± 0.65**	52	157	63	61	35
6	Esraa 5 extract	297	5.94 ± 0.69**	351	7.02 ± 0.68**	57	191	47	31	25

The total number of scored cells and sperms are 5000 ((1000/rat, 5/7 rats/group)

** Highly significant compared to – ve control ($p < 0.01$) (t-test)

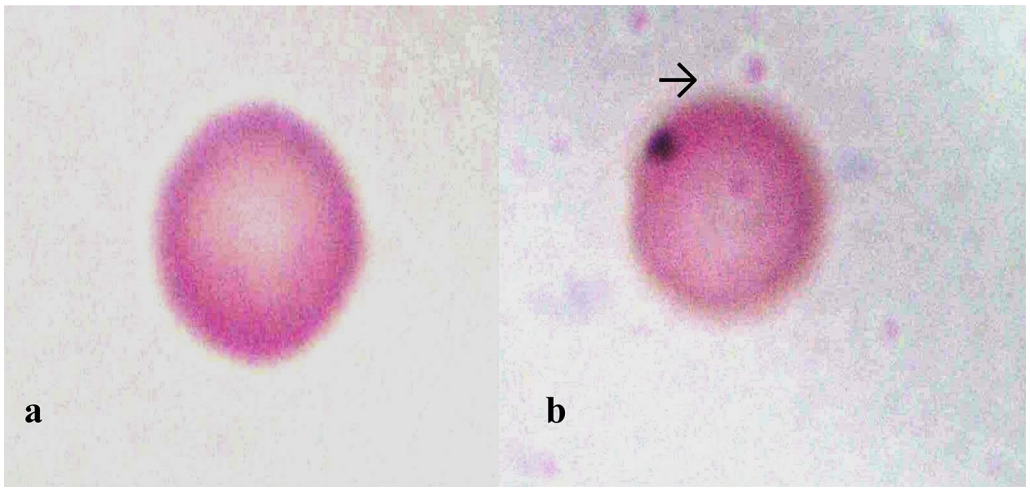


Fig. 3 Micronuclei in polychromatic erythrocyte prompted in rats' bone marrow cells handled with the oral G-negative bacteria extracts; normal cell (**a**) and micronuclei cell (**b**)

marked increase in connective tissue in liver capsule (right inset, Figs. 1; section 5P and 2; section 6).

Frequency of micronuclei (MN) induced by the selected bacterial extracts in male rats

Results in Table 5 and Fig. 3a and b indicated that the ratio of micronuclei in poly chromatic erythrocytes from rat bone-marrow cells was statistically significant ($p < 0.01$) in all treated animals' groups by oral ingestion compared to negative control. The numbers of MN cells in animals group that took orally the extract of *Escherihia* sp. Esraa 4 were markedly increased 10.32-fold (n = 392) followed by the animals' groups that were administered orally extracts of Esraa 2, Esraa 5, Esraa 1, and Esraa 3 (8.45-fold; n = 321, 7.82-fold; n = 297, 7.79-fold; n = 296, and 5.97-fold; n = 227), respectively when compared to n = 38 in the untreated group (Table 5). Moreover, compared to untreated group (0.76 ± 0.48) the ratio of micro-nucleated polychromatic erythrocytes (MNPCEs) that specified by analyzing the number of MN cells from 1000 PCEs per animal was described to be 5.92 ± 0.58, 6.42 ± 0.50, 4.54 ± 0.63, 7.84 ± 0.68, and 5.94 ± 0.69 after oral ingestion by Esraa 1, Esraa 2, Esraa 3, Esraa 4, and Esraa 5 extracts in the current study (Table 5).

Sperm-shape abnormalities

Various forms of head abnormalities as straight (n =, 34, 47, 40, 52, and 57), banana (n = 153, 161, 148, 157, and 191), amorphous (n = 41, 45, 37, 63, and 47) and without hook (n = 53, 39, 48, 61, and 31) in addition to the coiled tail (n = 20, 22, 18, 35, and 25) were discovered in groups treated by Esraa 1, Esraa 2, Esraa 3, Esraa 4, and Esraa 5 extracts, respectively compared to 27, 37, 3, 18, and 0, respectively in the untreated animals (Table 5 and Fig. 4a - d). The highest sperm abnormalities number in

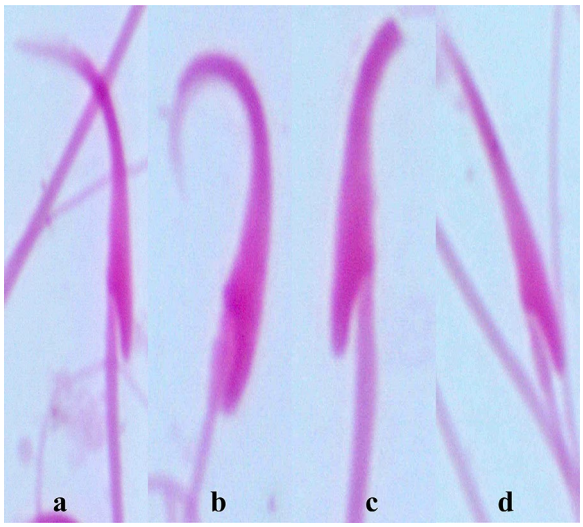


Fig. 4 Sperm defects stimulated in male rats treated by the oral G-negative bacteria extracts; normal sperm (**a**), amorphous (**b**), banana and without hook (**c**) and straight (**d**)

all groups was recorded for banana shape but the lowest was detected for coiled tail, that accompanied by male sterility (Table 5).

Assessment of anti-proliferative activities of the selected bacterial extracts against human normal cell lines

Anti-proliferative activities of Esraa 1, Esraa 2, Esraa 3, Esraa 4, and Esraa 5 extracts individually against three different types of human normal cell lines including mammary epithelial (MCF10A), lung fibroblasts (WI38), and dermal fibroblasts (HDFs) are presented in Fig. 5. Total inhibition in the proliferation of WI38, MCF10A, and HDFs, when they were treated by the *Serratia* sp. Esraa 1 extract at concentrations of 60, 70, and 70 µg/mL and IC_{50} equal to 22.50, 26.80, and 32.28 µg/mL, respectively (Fig. 5). Stimulation of 100% death in WI38, MCF10A, and HDFs cells was noticed when they treated with Esraa 2 extract at doses of 50, 40, and 50 µg/mL with IC_{50} equal to 20.1, 12.80, and 11.20 µg/mL**.** Moreover, *Escherichia* sp. Esraa 4 extract at concentrations ranged between 70, and 80 µg/mL entirely inhibited the proliferation of WI38, MCF10A, and HDFs with IC_{50} ranged from 30 to 40 µg/mL, while their growth

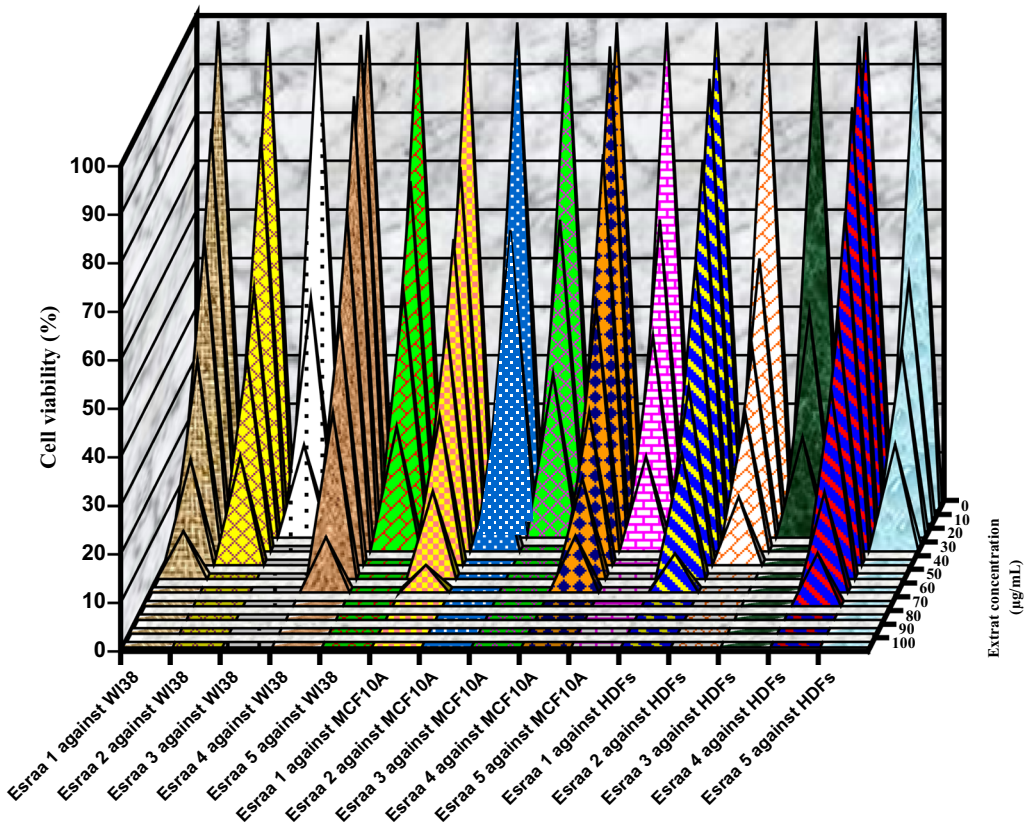


Fig. 5 Effect of the secondary metabolites extracted from the selected G-negative bacteria derived from cancer microbiota on the normal human cell lines MCF10A, WI38 and HDFs at different concentrations

was totally inhibited at 40 µg/mL of *Pseudomonas* sp. Esraa 5 extract with 50% inhibition in their proliferation (IC_{50}) achieved at doses of 20.0, 15.38, and 10.0 µg/mL, respectively. Interestingly, the extracted metabolites of *Acinetobacter* sp. Esraa 3 showed the highest cytotoxic effects against all human normal cells under study. Esraa 3 extract at a concentration of 30 µg/mL caused total inhibition in the growth of WI38, MCF10A, and HDFs cell lines with IC_{50} estimated to be 9.5, 14.12, 8.90 µg/mL, respectively (Fig. 5).

Discussion

Continuous monitoring of multidrug-resistant microorganisms as well as drug resistance patterns is needed to avoid treatment failure and decrease selective pressure [38]. Gram negative bacteria dominated the microbiome in this study, representing 74.87% of isolates. The most common pathogens among oncology patients were *Escherichia* sp. (24.66%) > *Acinetobacter* sp. (23.29%) > *Stenotrophomonas* sp. (19.86%) > *Pseudomonas* sp. (17.81%) > *Serratia* sp. (14.38%). Previous investigations in developed countries indicated a shift in the causative microorganisms to G-positive bacteria, but G-negative bacteria remain the dominant causative pathogen amongst developing countries [39]. Montazeri et al., described that G-negative bacteria were the most prevalent bacteria isolated from cancer patients (74%) and all of them were multidrug resistant strains [27] as well as Bhat et al., reported that G-negative bacteria constituted 66.96% of the strains, which is related to our findings of 74.87%, then the current study focused on the G-negative bacteria [13]. In the study of Siddaiahgari et al., in Mumbai the most common pathogens among microbiota of oncology patients were *Pseudomonas* sp. (26.2%) > *Enterococcus* sp. (11.66%) > *E. coli* (11.34%) > *Klebsiella* sp. (10.59%) > *Acinetobacter* sp. (9.95%) > *Enterobacter* sp. (3.1%) > *Burkholderia* sp. (2.35%) [40]. The rank order in the study of Bhat et al., was *Klebsiella* sp. (18.30%) > *Pseudomonas* sp. (17.65%) > *Escherichia coli* (14.71%) > *Acinetobacter* sp. (6.21%) > *Enterococcus* sp. (3.92%) > *Proteus* sp. (2.61%) > *Haemophilus* sp. (1.96%) [13]. Furthermore, Jungrungrueng et al., described the pattern of causative microorganisms in oncology patients and they found that of 192 isolates; the most common bacteria were *E. coli* (38/154, 24.7%), *K. pneumoniae* (19/154, 12.3%), and *A. baumannii* (14/154, 9.1%) [18] as well as Kabanangi et al., reported that the most obtained G-negative bacterium was *P. aeruginosa* (39.0%), followed by *Acinetobacter* sp. (28.7%) and *Klebsiella* sp. (16.2%) as well as among them 80.1% were MDRGN strains [41]. These data indicated that the structure of cancer microbiome varies greatly from region to region, and then continuous monitoring of bacterial pattern in the cancer microbiome is critical.

It is complicated to treat the G-negative microbiome in cancer patients due to the uncontrolled and continuous use of a large number of antibiotic therapies for a prolonged period which has resulted in selective pressure on bacteria, thus increasing the prevalence of antibiotics resistance [14]. In line with our data, Kabanangi et al., reported that 78.6% of *Enterobacteriaceae* are extended spectrum producers of β-lactamase and amongst them 100% of *Klebsiella* sp. and *E. coli* were MDR [41]. Interestingly, resistance against carbapenems including ertapenem, imipenem, meropenem and doripenem was detected in 66.44, 62.33, 68.49, and 59.59% of G-negative bacteria, respectively. These findings raise the alarm because when G-negative microbiome isolated from cancer patients are resistant to third-generation cephalosporines or/and carbapenems treatment options become limited or even result in treatment failure [42]. Smith et al., mentioned that combine β-lactamase inhibitors with antibiotics as ceftazidime-avibactam, imipenem-cilastatin and meropenem-vaborbactam have high potency against isolates carried carbapenem, multidrug, colistin and tigecycline; resistance mechanisms and inhibited activity of *Enterobacterales*, MDR and XDR isolates by 99.1, 96.5, and 82% of respectively, which are much higher than the current study [43]. Doi et al., reported that aminoglycoside resistance may occur via enzymatic modification mediated by aminoglycoside acetyltransferases, nucleotidyltransferases or phosphotransferases and appears to converge with the carbapenemase epidemic, facilitating the emergence of XDR and in some cases, pan-drug resistant microorganisms [44]. Colistin has regained worldwide interest due to the high prevalence of multidrug resistance [38]. Nevertheless, the incidence of G-negative MDR-resistant microorganisms as *P. aeruginosa*, *A. baumannii*, *Serratia* sp., *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp. that considered a serious problem due to the lack of alternative antibiotics in cancer patient is mutable depending on the stating country and continent coupled with inadequate empirical/therapeutic antibiotics therapy exposes these patients to increased risk of adverse outcome [12, 45, 46] as well as necessitates more research and treatment development as previously were reported [14, 21, 45–50].

Numerous trials have been prepared on the etiology of tumor and most of them have been assessed genetically or environmentally; however, the prospective role of bacterial strains in expanding of adenocarcinoma has not been discussed [51]. These findings are consistent with Al-Hilu and Al-Shujairi, who reported that certain bacteria are associated with stimulation of a specific kind of tumor through various molecular and biochemical mechanisms;

among them *H. pylori* caused gastric cancer through its capability to cause intense inflammations, *S. typhi* associated with gallbladder carcinoma, *C. pneumoniae* is one of the ethological reasons of lung cancer but *Klebsiella* sp., as well as *Proteus mirabilis* caused bladder cancer [16]. New studies show that the spread of tumor may be exceedingly linked to the microbiome [52]. In harmony with current findings, EL-Gendy et al., indicated that the subcutaneous-injection of *Pseudomonas* sp. extract significantly increased CEA and AFP in hepatic tissue and LDH in rats' serum; however, MRSA strains significantly increased various tumor markers including CEA, AFP and LDH [21, 22]. Our previous results dealing with the risks of subcutaneous injection of bacterial extracts into animals [14] and the current work results representative the harmful effects of their oral administration suggested that the risk of each bacterial extract on animals' groups is affected by the route of exposure.

Some microbial metabolites are carcinogens that undergo metabolic activation to form metabolites that react with cellular macromolecules and initiate carcinogenesis via DNA damage [8, 9]. Our data supported the previous results of El-Gendy et al., they described that the extracts of different *Pseudomonas* and *S. aureus* strains prompted a considerable increase in the serum activities of ASAT, ALAT and ALAP because of hepatic harm and cell necrosis of several tissues [21, 22]. EL-Gendy et al., reported that the levels of urea and creatinine were increased considerably post repeated administration of *Pseudomonas* sp. extract; this effect was attributed to the deterioration of renal function [22]. Thus, with a best comprehension of how bacterial microbiota contribute to carcinogenesis, new strategies for tumor prevention and treatment by targeting the cancer microbiome can be developed [6, 7]. There is a potential association between the serious clinical syndromes including progression of specific cancers and antibiotic resistant G-negative bacteria in patients with malignancy [14, 21, 53]. Furthermore, current investigations have displayed a causal relationship between bacterial infection and emergence of tumor in organs as kidney, cervix, liver, lungs and colon, which are constantly exposed to bacteria [10]. Microbiota colonization has been associated to G-negative bacteria with high inflammatory prospective and then local inflammation possibly participates in the initiation and continuation of carcinogenesis as seen in ovarian cancer that characterized by oncobiosis in neoplastic diseases [3]. Furthermore, Lenický et al., revealed that the bacterial strains *E. coli*, *P. mirabilis*, *S. lentus*, and *Citrobacter braakii* in turkey induced inflammatory developments, oxidative stress, structural deterioration increased pro-inflammatory markers levels, such as IL-1, IL-6 and C-reactive protein [17].

El-Gendy et al., stated that Masson's trichrome stained tissues of non-treated group exhibited few collagen or fibers around the portal tract while the collagen fiber content in portal zone is apparently increased while subcutaneous injected animals with *Acinetobacter*, *Serratia*, *Escherichia*, *Stenotrophomonas* and *Pseudomonas* species extracts exhibited disorganization of hepatocytes with vacuolization, ballooning and apoptotic nuclei, inset the pyknotic nuclei appear near from the central vein (abnormal hepatocytes) and fibrous tissues are amplified around the portal abroad tract zone [14]. In a previous study animals treated by *pseudomonas* extract exhibited intense histopathological changes as derangement of hepatic cords with granular alterations in the cytoplasm, multifocal swelling of hepatocytes with congestion of central and portal blood vessels, focal degenerative, necrotic alterations along with mononuclear cell infiltration, moderate to intense nephropathic alterations, distinct cellular changes, marked degenerative, necrobiotic alterations with inflammatory reaction and tubular changes as diffuse tubular swelling and loss of tubular epithelium [22]. Petridou et al., stated that microscopic examination of buffalo liver sections infected by *S. maltophilia* showed edema, loss of hepatocytes, fibrosis, capillary thrombosis, thrombi comprised of neutrophils, plasma cells and fibrinous material as well as hemorrhages and foci of coagulative necrosis with infiltrates of neutrophils, macrophages and plasma cells present around the centrilobular vein [54].

These data clearly indicated that oral administration of Esraa 1, Esraa 2, Esraa 3, and Esraa 4 extracts caused higher propagation in the number of micronuclei than their subcutaneous injection which previously reported to be 233, 308, 214 and 341 respectively, while subcutaneous injection of Esraa 5 exhibited higher mutagenicity (MN = 375) in albino rats [14] than its oral ingestion (MN = 297) in the current study. These data supported the severe mutagenicity and genotoxicity of these microbial extracts might be through prompt the construction of DNA cross-links, single- or double-strand breaks that can lead genomic instability and mutations through oncogenes and tumor suppressor genes [6, 7]. It has been found that carcinogenic bacteria gave abroad variation of metabolites, which vary in their chemical configuration but possess a common capability to form high levels of free radicals, inflammations, and chemical bonds with DNA, resulting in the construction of DNA adducts which is known as the first stage in carcinogenesis [16].

Bacterial infection of semen is present recognized global as an important cause contributing to sperm sterility as well as prompted sperm deformities suggesting point mutations in germ cells, presumably causing structural variations in cell organelles, which leads to the

occurrence of sperm abnormalities [55]. In parallel with our data, Eini et al., suggested that genital inflammation caused by bacteria can affect the male reproductive system in different ways and bacteria remarkably connected with leukocytospermia could weaken male fertility prospective through lowering sperm concentration, motility, morphology and DNA integrity [56]. Hamazah and Al-Dahmoshi, found that G-negative bacterial strains combine 40/70 (57.1%) were *E. coli* 30/70 (42.9%) Followed by *E. aerogenes* 8/70 (11.4%) and *Proteus* sp. 2/70 (2.9%) as well as bacteria mirobiome among bacteriospermic-pyospermic showed high resistant rates [57]. Alcántar-Curiel et al., reported that up to 58% of carbapenem resistant bacteria including *A. baumannii* outbreak in HRGIZ strains were capable to adhere to A549 epithelial cells and 14.5% of them caused cytotoxicity of over 50% [58]. Chen et al., revealed the function of gastrointestinal microbiome in occurrence of breast tumor as well as how the intestinal bacterial microbiome, in particular, enteric bacterial genes able of metabolizing estrogens (estroboloma) could influence the risk appearance of "estrogen receptor positive" breast cancer after menopause [59]. Furthermore, Banerjee et al., described that dysbiosis of the microbiota have been linked with pathology containing tumor and suggest a robust and specific microbiota associated with ovarian cancer [60].

Conclusion

In conclusion, regular monitoring of susceptibility pattern of G-negative of cancer microbiome in cancer patients to various antibiotics commonly used in routine chemotherapies is critical for antibiotic policy development to manage multidrug-resistant (MDR) pathogens in cancer microbiota and reduce morbidity and mortality. Therefore, in the present work, we highlight and describe the monitoring of antimicrobial resistance among clinical G-negative bacteria obtained from tumor patients. There was high ratio of drug-resistant G-negative bacterial in the oncology microbiome under study. Among them the selected isolates *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4, and *Pseudomonas* sp. Esraa 5 showed resistant against all antibiotics classes under study and then they were characterized as extensively drug resistance strains (XDR). The oncobiome and the most likely bacterial metabolites play vital roles in mediating the initiation and propagation of cancer as well as the effectiveness of chemotherapy. To advance our understanding of the potential primary or co-factorial possible roles of this bacterial microbiome derived from cancer patients; the trials based on oral ingestion of extracts derived from the G-negative bacteria *Serratia* sp. Esraa 1, *Stenotrophomonas* sp. Esraa 2, *Acinetobacter* sp. Esraa 3, *Escherichia* sp. Esraa 4, and *Pseudomonas* sp. Esraa 5 were performed by albino rats individually. The current study proved that these bacterial strains isolated from the cancer microbiome have potential roles in the induction of cancer, inflammation, mutagenesis, hepatotoxicity, nephrotoxicity and sperm abnormalities along with histopathological changes in the treated animal groups by orally administrated extracts in compared to the untreated group. Moreover, we assessed the cytotoxicity of their extracts on three different human cell lines WI38, MCF10A, and HDFs and they exhibited marked anti-proliferative activities against these human cell lines.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethical Committee of National Research Centre (FWA 00014747), Egypt.

Competing interests
The authors declare no competing interests.

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