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Acclimatization of Bacteria to Bladder Cancer Tissues

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Abstract: Cancer (including bladder cancer) is not a simple disease due to the multiple reasons responsible for its onset in humans; which vary from socioeconomic, environmental, chemical, to genetic reasons. Bladder cancer occupies eighth place amongst the newly diagnosed malignant cases worldwide. Moreover, men diagnosed with bladder cancer are four times higher than women. Early and non-invasive techniques for the diagnosis of bladder cancer are encouraged to avoid medical complications lengthy and expensive tests. Building a bacterial fingerprint of bladder cancer should contribute to the easiness of the diagnosis process and hence speed up the treatment. Standard bacteriological techniques were used to isolate bacteria from 10 samples of cancer tissues collected from the Urology and Nephrology Centre at Mansoura University, Egypt. The bacterial isolation was followed by classical and molecular identification of twenty bacteria. Bacterial protein fingerprinting screening reduced the 20 isolates to five bacteria which were further identified using 16S rRNA gene sequencing, RAPD-PCR DNA fingerprinting, and MALDI mass spectrophotometry analysis. Then all isolated grouped into three bacterial genera namely E. coli (three isolates), P. aeruginosa, and Klebsiella pneumonia. MALDI mass spectrophotometer proved to be the easiest, faster, convenient and cheapest way of bacterial identification.

keywords: Bacteria, bladder, cancer, fingerprinting, and MALDI **.**

1.Introduction

The major reason for making cancer the leading cause of death worldwide is simply due to the lack of effective eradication protocols and scientists are racing to find one. The annual death rate due to cancer exceeds 14.1 million worldwide and newly diagnosed cases reached 8.2 million [11]. The seriousness of this disease arouses of the fact that there are more than 100 types of cancers are being reported and subjected to detailed studies. The National Cancer Institute one of the National Institute of Health in the United States of America lists all kinds of cancer in alphabetical order (A-Z) with different types under each letter. The most common types are bladder, breast, colon and rectal, endometrial, kidney, leukemia, liver, lung, melanoma, Non-Hodgkin, pancreatic, prostate and thyroid cancers. [22]

The involvement of microbes such as viruses, bacteria, helminths and trematodes in initiation and development of cancers are well documented in the literature [4, 24, 21, 17, 9, 28, 33 and 34]

As part of their infection cycle, many bacteria secrete toxins that cause DNA damage in host cells. Genomic instability is a hallmark of cancer development because DNA damage may result in mutations or deletions that inactivate tumour suppressors or activate oncogenes. Intestinal bacteria can express toxins that cause DNA breaks and thus contribute to genome instability, tumour initiation and progression. Currently, four genotoxins are known to induce DNA damage in infected cells, namely colibactin (produced by E. coli), cytolethal distending toxin (CDT; produced by certain Gram-negative bacteria), Shiga toxin (produced by Shigella dysenteriae) and endonucleases (produced by *N. gonorrhoeae*) [13].

Cellular responses to bacterial genotoxins are detailed in this section. Colibactin expressed by *Escherichia coli* pks+ and the CdtB subunit of cytolethal distending toxin (CDT) induce DNA double-strand breaks (DSBs) that are characterized by phosphorylated H2AX (γH2AX) foci; this activates ataxia telangiectasia mutated (ATM) and thus the DNA damage response (DDR). CdtB stimulates either G1–S or G2–M cell cycle arrest, whereas colibactin stimulates G2–M cell cycle arrest. Activated ATM leads to G1–S cell cycle arrest through the phosphorylation of p53 and activation of its downstream effector p21, which inhibits the cyclin-dependent kinase 2 (CDK2)–cyclin E complex. G1–S cell cycle arrest is also triggered by the activation of check point kinase 1 (CHK1), which inactivates cell division cycle 25A (CDC25A), leading to hyperphosphorylation of the CDK2– cyclin E complex. G2–M cell cycle arrest is mediated through the activation of CHK2, which inhibits the phosphatase activity of CDC25C, resulting in the hyperphosphorylation of the CDK1–cyclin B complex. Cells that are exposed to CDT can overcome cell cycle arrest by stimulating the guanine nucleotide exchange factor neuroepithelial cell-transforming gene 1 protein (NET1) to activate the RHOA GTPase, which stimulates the p38 MAPK signalling pathway. Chronic exposure to CDT-producing bacteria, or infection with *E. coli pks+* at high multiplicity of infection (MOI), can induce an irreversible cell cycle arrest (known as senescence) that is characterized by the secretion of pro-inflammatory cytokines and growth factors, which can induce the growth of neighbouring cells. Haematopoietic stem cells are more sensitive to apoptosis after exposure to CDT, which suppresses local immune responses. Finally, in some cases of infection — for example, when cells are subject to *E. coli pks+* at a low MOI — incomplete DNA repair results in mutagenesis and chromosomal instability, which are processes that are also involved in tumour initiation and cancer progression. Red lines represent inhibition, the green arrows represent pathways that are stimulated by toxins and the grey arrows represent the consequences of inducing the respective pathways.

Moreover, Microorganisms were used such as *Mycobacterium bovis* (BCG) in the treatment of cancer diseases for more than 10 decades , including successful treatment for bladder cancer [5] Many microbial products have also been used in the treatment of cancer, As in oxidation proteins such as azrin, too,Various mechanisms of action , Some of which activate the immune system , Others lead to apoptosis , Others prevent the formation of new blood vessels. Thus, Tumors are deprived of access to nutrients . Future studies should examine metabolic and ecological interactions between microbes and tumors in order to discover new microbes that can be used to treat cancer.

Because microbes play prominent roles in development of cancers, we decided to study the types of microbes associated with the bladder cancer in Egyptian patients subjected for treatments in the Nephrology and Urology Center at Mansoura University, Egypt.

Materials and Methods

Sample Collection:

Ten cancer bladder tissue samples were collected from inpatients at the Urology and Nephrology Center, Mansoura University, Egypt. Their ages ranged from 39 to 74 years of age, two of them were females and eight were males. All patients were routinely tested for bilharzia, AIDS and HCV; only one female was tested positive for HCV. Examination of their bacterial cultures found that three patients were positive for *E. coli/Serratia,* and *Klebsiella.*

Isolation of Bacteria:

The bladder cancer tissue samples were transferred in a sterile container to the Bacteriology/Molecular Biology laboratory at the Faculty of Science, Mansoura University for isolation and purification of any culturable bacteria associated with samples. Each cancer tissue sample was macerated and suspended in phosphate buffer saline (PBS) and 100ul was spread onto LB agar medium then incubated at 37ºC for 24 to 48 h. The grown bacterial colonies were picked and streaked onto fresh LB plates for purification. The purified bacterial cultures were Gram-stained and examined microscopically to determine the shape and stain affinity [7]. Portion of the purified colonies were preserved in 20%

glycerol and kept at -20ºC for long term storages.

Antibiotic sensitivity

The antibiotic susceptibilities of all purified bacterial isolates were determined according to the standard methods [3]. Seven of the commercially available antibiotics discs tested against each bacterial isolate were Kanamycin (30 µg), Gentamicin (10µg), Vancomycin (30µg), Trimethoprim/ Sulphamethoxazole (25µg), Penicillin (10µg), Erythromycin (15 µg), Ampicillin (10 µ g). The discs were laid onto the bacterial lawn and the bacterial cultures were incubated at 37ºC for 24 h. Then the diameters of the zones of inhibition around each disc were recoded.

Polyacrylamide gel Electrophoresis (SDS-PAGE):

The total cellular protein of each bacterium was analyzed by sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as described by [18]. Where, the SDS detergent coats the polypeptide backbone giving it a negative charge that allows molecules to migrate towards the anode in the electric field. This method separates proteins according to their molecular sizes.

Identification of bacteria by mass spectrophotometer:

Five antibiotic–resistant bacterial isolates were grown on LB agar plates and incubated at 37 ºC for 24 h before being identified by MALDI-TOF-MS (VITEK-MS) at the facility of New Surgery Hospital, Zagazig University. Each bacterial isolate was spotted in duplicates directly on the special plate/slide followed by the addition of the matrix (α-Cyano-4-hydroxycinnamic acid) and air dried. The slide was then inserted into the VITEK MS machine. VITEK-MS mass spectrometer was used to generate spectra of the bacterial spots and the Biotype software (version 2.0) was used to analyze the results. Where, the spectra obtained were matched with the spectra stored in the database of VITEK–MS instrument to identify the bacterial isolates. The peaks from these spectra were compared with the characteristic pattern for the species, genus or family of the bacteria, leading to identification of the organism. Scores between 85 and 90 % were considered an acceptable identification. The results were evaluated according to a colored index: green for >90 % identity, yellow for 85-89.9 % identity and white for 90 % considered reliable [16].

Molecular identification using 16S rRNA gene sequening:

The 16SrRNA gene was amplified for each bacterial isolate using specific primers and PCR technology. Total genomics DNA was extracted from all bacterial isolates by the Quick-gDNA mini Prep kit from Zymo Research - USA. Using the purified template DNA from each bacterial isolate, the Mytaq red PCR Master Mix –Bio - 25044 – Bio line was mixed with the forward primer (F) 5′-CAGGCCTAACACATGCAAGTC-3′) and the reverse primer (R) 5′-CGGCGGWGTGTACAAGGC-3′ to amplify the 16SrRNA genes. The annealing temperature was 60°C and the 16S rRNA gene amplicons were obtained after 35 thermal cycler cycles. The amplicons were cleaned and purified using the DNA Clean and Concentrator kit miniprep- D4033 – Zymo Research–USA. The concentration and degree of purity of the purified amplicons were checked using spectrophotometry and agarose gel electrophoresis before being sent to sequenced by the GIS Research Center – 6th of October City, Giza, Egypt. The DNA sequences were further analyzed against the reference 16S rRNA bacterial database deposited in the NBCI GenBank. An nBLAST analysis would determine similarities and degrees of genetic relatedness for each sequence from our local bacterial isolates [1 and 6].

Plasmid analysis:

Plasmid DNAs were extracted by Favor Prep Plasmid Extraction Mini Kit (FAVORGEN Biotech Corp) according to the manufacturer's instructions. The cell pellets from 1-5 ml of bacterial cultures were suspended completely in 250 µl of FAPD 1 Buffer containing RNase A by pipetting. Cell lysis was achieved by add 250 µl of FAPD 2 buffer and gently invert each tube 5-10 times, followed by incubation at room temperature for 2-5 minutes. The cell lysates were neutralized by add350 µl of FAPD 3 buffer and the tubes were inverted 5-10 times immediately followed by centrifuge at 18,000 Xg for 10 min to clarify the lysate. The

supernatants were carefully transferred to the FAPD Column and centrifuge at 11,000 Xg for 30 seconds. Discard the flow-through and place the column back to the Collection Tube. Add 400 µl of W1 buffer to the FAPD Column and centrifuge at 11,000 Xg for 30 seconds, discard the flow-through and place the column back to the Collection Tube. Add 700-µl Wash buffer to the FAPD Column and centrifuge at 11,000 Xg for 30 seconds, Discard the flow-through and place the column back to the Collection Tube. Centrifuge at full speed (18,000 X g) for an additional 3 minutes to dry the FAPD Column. Place the FAPD Column into a new 1.5 ml microcentrifuge tube. Add 50 µl – 100 µl of Elution Buffer or ddH2O to the membrane center of the FAPD Column. Stand the Column for 1 minute. Centrifuge at full speed (18,000 X g) for 1 minute to elute plasmid DNA and store the DNA at -20 C for further analysis by agarose gel electrophoresis.

DNA fingerprinting

Genomic DNAs (gDNA) were isolated from the bacterial pellets according to the instructions of the GeneJET gDNA purification kit (Thermo Scientific, Germany). The purified gDNA were fingerprinted using four polymorphic DNA primers randomly. The four primers manufactured by OPERON were OPU-16 -5′-CTGCGCTGGA- 3′,(OPT-16-5′-GGTGAACGCT3′, OPK-02-5′-GTCTCCGCAA-3′ and OPI- 17 5′-GGTGGTGATG-3′. Each of the reaction mixtures was adjusted to a total volume of 20ul: 1ul DNA template, 4ul 5X buffer, 2ul primer, 0.5ul Taq DNA polymerase, and 12.5ul distilled water. The thermal cycler was programmed to denature the template DNA at 94°C for 3 minutes, followed by 40 cycles; each consisted of denaturation at 94°C for 1 minute, annealing at 30°C for 30 s, and extension at 72°C for 1 minute; this followed by one extra extension at72°C for 5 minutes. The products of PCR were electrophoresed on 1.2 % agarose gel containing ethidium bromide and documented by photography.

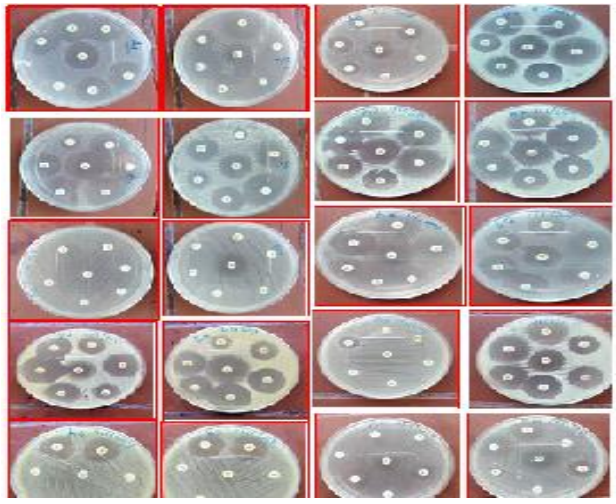

Fig. 1. The antibiotic sensitivity assays for all 20 isolates from the cancerous tissues.

The antibiotic sensitivity of all 20 bacterial isolates from the cancerous tissues showed different responses to the antibiotics tested (Fig.1). It is important to note that any two bacterial isolates from the same cancer tissue sample showed almost the same degree of either sensitivity or resistance to any of the antibiotics tested. Isolates A3 and B3 were resistant to all tested antibiotics and B3 differed from A3 in its sensitive to gentamycin only. Isolates A5 and B5 were resistant to the same five antibiotics (ampicillin, erythromycin, penicillin, trimethprim/sulfamethoxazole, and vancomycine) bothe isolates were sensitive to gentamycin and differ in the level of sensitivity to kanamycine; A5 showed intermediate sensitivity (21mm inhibition zone diameter) while B5 showed higher semnsitivity with 23 mm zone of inhibition diameter. While, isolate A9 was resistant to all seven antibiotics tested, isolate B9 showed sensitivity to four antibiotics (gentamycine, kanamycine, penicillin, and trimethprim/sulfamethoxazole) intermediate sensitivity to ampicillin, erythromycin and

Results

Isolation and Purification of Bacteria:

Twenty bacterial isolates were purified from the collected cancerous tissues using the streaking method on LB agar plates as detailed in the materials and methods section. The growth was recorded after 48 h of incubation at 37 ºC. The purified bacterial isolates were identified and stored as glycerol stock at –20ºC freezer.

Antimicrobial Susceptibility test:

The antibiogram of the purified isolates with different antibodies is recorded in Fig (1). An allergy test for microbes was performed for certain types of antibiotics using a standard disc diffusion method.

vancomycine. The isolates showing the highest level of resistance (A3, A5, A9, B3, and B5) were selected for further studies and characterization. Isolates A3 and A9 were resistant to all antibiotics, B3 was only sensitive to gentamycin, A5 and B5 were sensitive to gentamycin and kanamycin, however, A5 was the only showed isolate intermediate sensitivity to kanamycin.

Microscopic examination of Bacterial isolates:

From the original 20 bacterial isolates five multidrug resistant (MDR) (A3, A5, A9 and B3, and B5) were selected for further characterization. All isolates were grown in/on LB/LB agar for overnight and smears were prepared for Gram staining. All of the five MDR isolates were rod in shape and positive to Gram's stain (A5, A9, B3, and B5), except A3 which was Gram-negative. However, A5 and A9 looked as coccobacilli.

Molecular identification:

1- Protein Profile:

Depending on the antibiotic susceptibility tests five bacterial isolates showing resistance to numerous antibiotics (multidrug resistance, MDR) were selected for protein analysis by polyacrylamide gel electrophoresis (SDS–PAGE) under denaturing conditions. The protein banding patterns of the five MDR isolates further grouped them into: group one contained A3 isolate alone, group two contained isolates A5, B3 and B5, and group three contained isolate A9 only. The protein banding patterns of the each representative of the three groups or isolates A3, A5 and A9 showed distinctive differences that rationalized their different identities. The three isolates were further characterized by the 16S rRNA gene sequencing and fingerprinted by the RAPD-PCR technology.

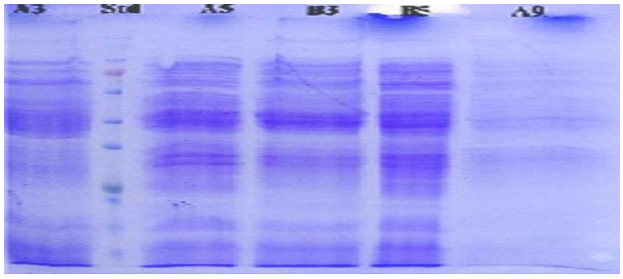

Fig. 3: The protein banding patterns of the five MDR bacterial isolates: A3, A5, A9, B3, and B5. The Std lane is the protein standard marker (245,180, 135, 100, 75, 63, 48, 35, 25, 20, 17, 11, kDa).

2. Plasmid Profile:

The plasmid profile of the 5 MDR bacterial isolates showed distinctive different plasmid contents for all of them (Fig.4). The group 2 isolates consisted of A5, B3, and B5 (from SDS-PAGE analysis) did not produce the exact plasmid patterns; i.e. variability does exist amongst the member of this group. They shared the highest plasmid band and differed in the smaller plasmid one. B3 showed a single higher DNA band, A5 showed the higher DNA and a lower band, and B5 showed also two DNA bands but the electrophoretic mobility of the apparently lower band is smaller than that existed in the profile of the A5 one.

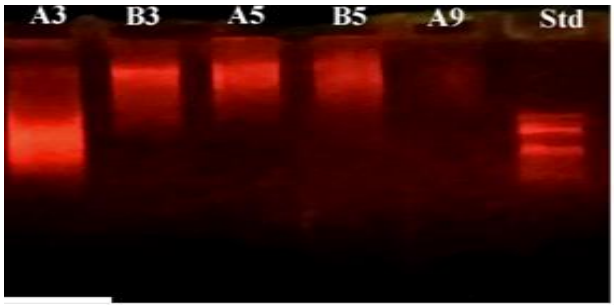

Fig. 4. The plasmid profile of the five MDR bacterial isolates. Lan Std showing the sizes of the standard DNA molecules.

3. Identification of the MDR isolates using MALDI:

All five isolates (A3, A5, A9, B3, and B5) analyzed by the MALD-TOF MS were negative to Gram stain and rod-shaped. MALDI spectra acquired as the intensity (relative abundance of specific ion, an arbitrary unit) of total proteins of the five laboratory isolates by the VITEK-MS instrument (Fig. 5 A, B and C). The height of each band is a function of mass (m) divided by the charge number of ions (z) or electrons removed after laser burst (m/z value). Analysis of the spectra showed that three of the isolates (A5, B3, and B5) were identified by the built-in library of the MALDI instrument as member of the bacterium E. coli with different matching values; isolate A5 has the highest matching value of 99.9 % with the values in the built-in library, while the other two isolates B3 and B5 produced matching values of 56.1% and 49.1 %, respectively. The acquired MS spectra of

A3 and A9 isolates coincided with the databases (99.9%) of *Pseudomonas aeuroginosa* and *Klebsiella pneumoniae*, respectively. It is also noticeable that four isolates (A5, A9, B3, and B5) are members of the family Enterobacteriaceae, while isolate A3 belongs to the Pseudomonadaceae. Two

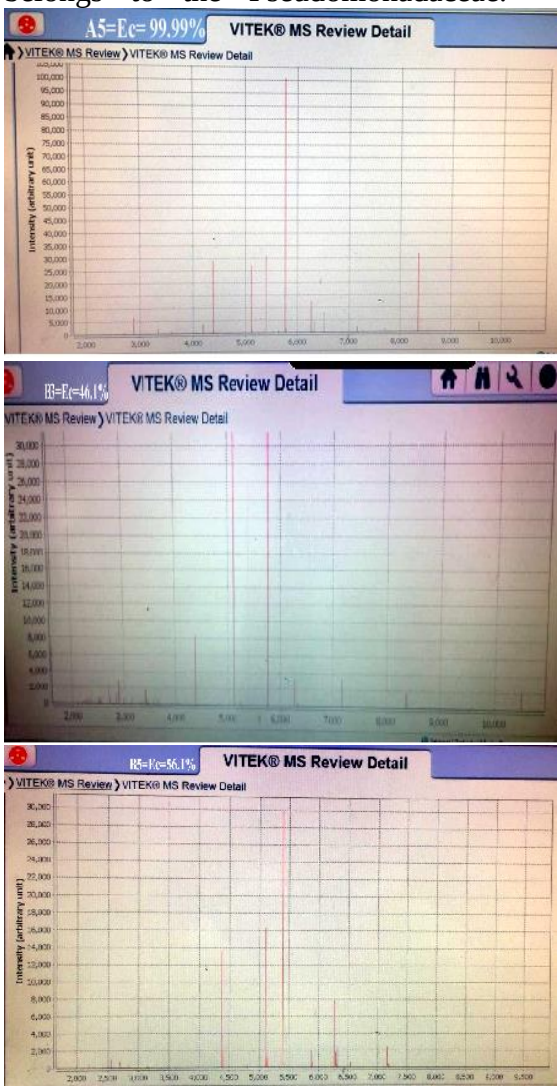

Fig. 5. The MALDI-TOF-MS acquired spectra for the three bacterial isolates A5, B3, and B5 from bladder cancer tissues.

4. 16S rRNA gene Sequencing:

The nucleotide sequences of the five local isolates from collected cancer tissues (A3, A5, A9, B3, and B5) were analyzed (Parsed nBLAST) against 16S rRNA bacterial database (RDP-II). This was helpful to assign the most similar type strains and then draw the dendrogram trees using the MEGA 7 program (Fig. 6). Most isolates showed 98–100% similarities in their sequences to their closest relatives in the GenBank database. The analyses of the nucleotide sequences of the

important observations on these spectra are evident. The number of peaks was not the same in each of the three *E. coli* strains, but two common peaks are evident in all of them. These appeared at m/z value of 5100 and 5, 800.00 m/z value. (Fig. 5 panels A5, B3, and B5).

cultivable isolates B3, B5 and A5 on LB medium identified them as strains of *E. coli* bacterium

Phylogenetic analysis of the isolated strains

Figure (6) illustrates the 16SrRNA gene phylogeny of the A5 bacterial isolate and its closest type strain. The results indicated that both BLAST results and phylogenetic evaluation corroborate the identity of the isolated strains to be *E. coli*. The data for the two bacterial isolates (B3 and B5) are not shown because of redundancy and the three are being identified as *E. coli*.

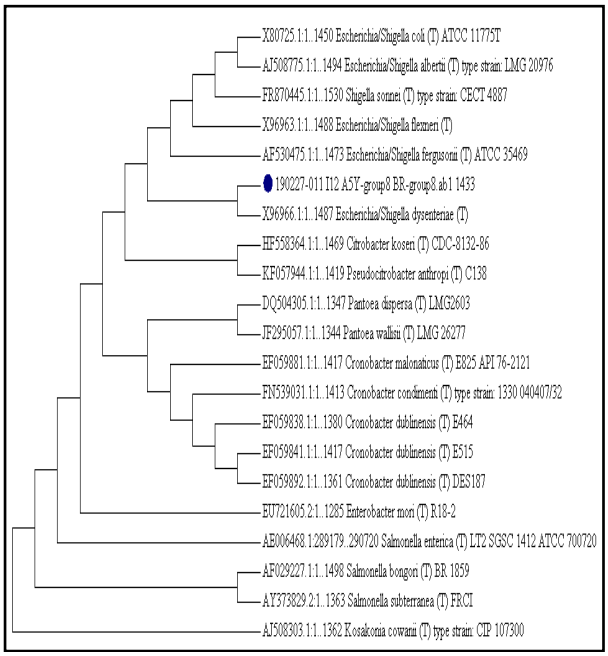

Fig. 6. The dendrogram of the phylogenetic tree based on the 16S rRNA nucleotide sequences of isolate A5 from bladder cancer tissue sample five. Values shown in each node of the tree are bootstrap values; 2,000 bootstrap replicates were performed.

5. The DNA fingerprinting

The three final isolates A3, A9 and B5 did not react with oligonucleotide primers used in random PCR fingerprinting. The A3 isolate lacked complementary DNA sequences for primers OP-T16, OP-K02, and OP-I17 and produced two DNA fragments with OP-U16

primer at a molecular size less than500bp. Primer OP-U16 produced three DNA bands with approximate molecular weights of 550, 650, and 1100bps and two DNA bands with primer OP-T16 with molecular sizes of about 550 and 400bps. The B5 interacted only with primers OP-T162 and OP-I17, the OP-I17 primer produced extra DNA band with electrophoretic mobility close 100bp (Fig. 7).

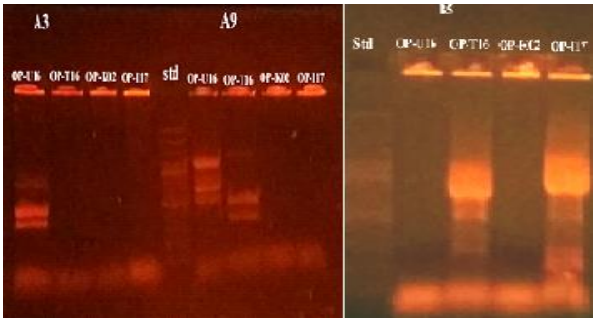

Fig.7. DNA fingerprinting of three MDR isolates A3, A9 and B5 using five random primers from the OPERON kits.

DISCUSSION

During this study, three different bacteria (*E. coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa)* belonging to two different families were isolated from bladder cancer tissue samples collected the Urology and Nephrology Center at Mansoura University Egypt. These internal tissues supposed to be free of microbes and comparing to the existence of human microbiome members with all body parts these three bacteria must have played significant roles in the development of the bladder cancer in their patients. However, the objective of this research was the search for a correlation between the type of bladder cancer and bacteria associated with it and a trial to build a case for a microbial fingerprint to bladder cancer among Egyptian patients. This is no surprise since our gut bacterial population, the unseen microbes live in our digestive system, is now known to affect not only all of our body parts but their functions as well. Increasing scientific attention for the role of these microbes on the overall human health is mounting. Due to these recently known facts being circulated in the literature about the interaction between the gut microbiome and our body parts may lead to innovation of new trend in medical treatments. Scientists working with gut microbiota concluded its complexity and personal status that qualified it to be taken as a fingerprint. The extensive work with the human gut microbiome has discovered that huge numbers of diverse bacterial inhabit our digestive system and their numbers are ten times the number of cells in the human body. Some estimated that the genetic pool of these bacterial species is 150 times the human genome, which adds more functions than we imagined and have bigger effects on our health and functions.

This study has isolated 20 different bacterial colonies from the collected bladder cancer tissue samples. Molecular identification and MALDI analysis named them *E. coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* In addition, two identical bacterial colonies were isolated from each tissue sample and the number was reduced from 20 to 10 isolates. Further analysis reduced them five isolates and final MALDI and 16s rRNA sequencing reduced them to three genera. It has been observed that 16S rRNA analysis, phylogenetic analysis, and MALDI analysis produced the same identical results. In DNA sequencing of the 16S rRNA gene, the newly determined bacterial sequences were compared with a known existing database for the identification and determination of the identity of bacteria. Additionally, identification genetic markers can be developed depending on unique sequences of each bacterium and these can be used for rapid fingerprinting. Our data suggested that molecular techniques are far more accurate than phenotypic identification of bacteria. Besides the many advantages of the 16S rRNA gene sequencing phylogenetic analysis, and MALDI have over classical identification methods, these methods can expand our understanding of microbial diversity in different ecosystems. However, it is costly, time-consuming and requires specialized equipment.

Until the beginning of this new millennium, it was perceived that the association of the bacteria with different parts of the human body has no consequences on our bodies. Nowadays, this belief had changed and many functions have been attributed to them and no longer considered hitchhikers or meaningless. This was supported by the scientific evidence of altered microflora associated with oral squamous cell carcinoma, cervical cancer,

cirrhosis, and liver cancer that has been published and augmented this new argument.

In recent years a number of published reports about urinary microbiome have been coined and concluded the existence of a definitive link between bladder cancer and specific bacterial fingerprints [www.cancer.gov/types/bladder]. Michael H. Hsieh, M.D., Ph.D. has published an online article about the association of specific bacteria with bladder cancer in 2018. He found that the population of bacteria at the beginning of his experimental model was different from bacteria isolated and identified after four months from the onset of bladder cancer. He found that the most prominent urinary bacteria associated with invasive carcinoma were *Escherichia* and *Kaistobacter* and *Rubellimicrobiu*m; a rarely bacterial species that was not known to be associated with the disease. At the end of month four, he found that *Gardnerella* and *Bifidobacterium* were the major bacterial species associated with the development of bladder cancer. A large number of *Bifidobacterium* present was not justified since this bacterium is known to support the immune system against cancer progression. His findings contradicted the presence of *Escherichia, Prevotella, Veillonella, Streptococcus, Staphylococcus,* and *Neisseria* with bladder cancer published by other researchers [27].

The harmful roles of microbes in the development of several kinds of cancers are well documented and microbes are the causative agents of about 20% of human cancers. Good examples of the microbial *involvement in human cancers are of* human papillomavirus in cervical cancer and *Helicobacter pylori* in gastric cancer [14 and 10]. A number of prominent studies have indicated the existence of a unique microbial community in the urinary tract [32]. Moreover, these commensal microbial communities differ from males to females and also with age [2 and 19]. The health and the disease status of the examined individuals also affected the outcome of urinary tract microbiome and no consensus yet on such microbiome members; e.g the urinary microbiome of overactive bladder patients [8 and 29], urinary incontinence [30 and 31, and 26], neuropathic bladder [15 and 12] type 2 diabetes mellitus patients [20], interstitial cystitis [29], sexually transmitted infections 20 or chronic prostatitis/chronic pelvic pain syndrome [23 and 25].

Building DNA fingerprinting for cancer bladder is not only cost effective, but not invasive as well if it can be done using urine samples instead of tissue samples. Examination of urine samples instead of tissue sample is based on the exfoliation bladder epithelium with their microbial content; considering the patients do not suffer from infection.

In conclusion, the total cellular protein fingerprinting, the MALDI-TOF-MS, RAPD-PCR fingerprinting, and the 16S rDNA gene sequencing-based approaches are very helpful in characterizing any bacterial population associated with the urinary bladder cancer and may help make a close correlation between the type and relative abundance of specific bacteria in bladder cancer patients. This is the hope of developing novel diagnostic and prognostic options that may be helpful in personalizing treatments in the near future.

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