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The Prevalence of *Pseudomonas aeruginosa* among Baghdad Hospitalised Patients

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Abstract

In order to determine the prevalence of *Pseudomonas aeruginosa* among hospitalised patients, 156 specimens were collected from hospitalized patients referring Baghdad, Iraq hospitals. Bacterial isolates were identified using conventional biochemical tests and then identification was confirmed by the locating of 16SrRNA. *Pseudomonas aeruginosa* constitutes 30.05%. In conclusion, the attention should be paid toward the infections of this opportunistic pathogen.

Keywords: *Pseudomonas aeruginosa, hospital, 16SrRNA, PCR.*

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium that is ubiquitous in different ecosystems and involves in numerous forms of relations with eukaryotic host. It is an opportunistic pathogen extensively spread in humans and animals [(1)], leading to a wide range of infections in community and hospitals [(2, 3)]. Due to the extended spread of *P. aeruginosa* habitat, the control of the organism in a hospital setting is very difficult, and makes it practically impossible to prevent contamination [(4)]. The major threat is the infection of immunocompromised patients or those in burns, neonatal and cancer wards [(5)]. Infection of *P. aeruginosa* is still one of the main causes of death among the critically ill and patients with impaired immune systems in spite of the development of newer and stronger antibiotics [(6)].

Due to the extended spread of *P. aeruginosa* habitat, the control of the organism in a hospital setting is very difficult, and makes it practically impossible to prevent contamination. The major threat is the infection of patients who are immunocompromised or those in burns, neonatal, cancer wards, it is the main cause of morbidity and mortality in cystic fibrosis patients and one of the leading nosocomial pathogens affecting hospitalized patients [(7)].

Due to the innate capacity of resistance to antimicrobial agents, this bacterium is greatly difficult to treat. What's more, such resistance is being progressively a problematic issue because of increasingly development of resistance to agents regarded as powerful therapeutic options [(8)].

In this study we have used a genus specific bacterial 16S PCR to investigate the prevalence and diversity of *P. aeruginosa* among Iraqi hospitalised patients.

Materials and Method

Ethical Statement: All participants agreed to provide the investigator with the specimens. Informed consent according to the Declaration of Helsinki was obtained from all participants.

Pseudomonas aeruginosa isolation and identification: One hundred and fifty-six specimens included mid-stream urine, burn swabs, wound swabs, and blood were collected from hospitalized patients referring Al-Yarmouk teaching Hospital and Baghdad Medical City in Baghdad, Iraq. All specimens were cultured on enrichment media such as blood agar and transferred onto MacConkey agar. Pale colonies on MacConkey agar (lactose non-fermenter) were assayed for the conventional morphological and biochemical characterization comprising Gram stainability was conducted alongside the activities of oxidase and

catalase. Thereafter the primarily identified *P. aeruginosa* isolates were submitted to molecular identification by polymerase chain reaction (PCR) technique.

Detection of 16SrRNA

Extraction of Bacterial DNA: Genomic DNA was extracted using Presto™ Mini gDNA Bacteria (Geneaid, Thailand). Upon the procedure itemized by the manufacturing company, DNA was extracted from overnight cultures of the carefully chosen staphylococcal isolates. Purified DNA concentration was measured using Biodrop (Biodrop, Canada).

PCR: To confirm the identification of *P. aeruginosa* isolates, conventional PCR technique was carried out in accordance to Spilker et al. [9] to amplify a fragment of *16SrRNA* (956 bp). Two microliters of each primer PA-SS-F (5′-GGGGGATCTTCGGACCTCA-3′) and PA-SS-R (5′-TCCTTAGAGTGCCCACCCG-3′), different concentrations of DNA (depending on DNA yield) extracted from each *P. aeruginosa* isolate and deionized D.W. were added to PCR premix tubes (Bioneer, Korea) in order to reach 20 µl as a final volume. The thermocycling conditions (Bio-Rad T100, USA) set at 94°C for 2 min, followed by 25 cycles of 94°C for 20s, 58°C for 20s, and 72°C for 40s. A final extension of 1 min at 72°C was applied. PCR products were visualized using 2% agarose gel stained with diamond nucleic acid dye (Promega, USA).

Results

Isolation and identification: A total of 52 (33.33%) isolates developed a growth on cetrimide agar, pale colonies on MacConkey agar, and succeeded to grow on nutrient agar at 42°C. Moreover, they were oxidase and catalase positive. Hence, these isolates were primarily identified as *P. aeruginosa.*

DNA extraction and preparation: After DNA extraction by Presto™ Mini gDNA Bacteria Kit, DNA concentration was between 24 and 78 ng/ml; whereas, purity was about 1.82- 1.99. A ratio of 1.8 -2.0 is generally accepted as "pure" for DNA [10]. Gel electrophoresis was done to confirm the integrity of extracted DNA.

Molecular Detection: The current results revealed that *16SrRNA* was located in 50 (96.1%) out of 52 biochemically *P. aeruginosa* isolates. Correspondingly, two isolates were identified using traditional method as *P. aeruginosa*, they did not have this gene.

Discussion

Pseudomonas aeruginosa is an opportunistic pathogen capable of causing a wide array of life threatening acute and chronic infections particularly in patients with compromised immune defense [11]. Earlier reports have shown that the antibiotic resistance of bacteria due to biofilm formation contributes to the persistence of bacterial cells and causes problems in the complete eradication of infection [12, 13].

The present work is in agreement with [14] as they found that the results of *16S rRNA* showed that all *P. aeruginosa* isolates were resistance to gentamicin harbored this gene. Al-Derzi [15] stated that out of 8038 and 1878 clinical specimens submitted for culture in Mosul and Duhok, respectively, 180 and 21 clinically significant isolates of P. aeruginosa were isolated, resulting in a prevalence of 5.2% and 1.6%, respectively. Moreover, cultural and biochemical identification revealed that 63 *P. aeruginosa* isolates were recovered from 158 samples in Karbala, Iraq [16]. Also Hasan et al. [17] reported that 21.6% *P. aeruginosa* isolates were collected form 185 swab samples in Kirkuk City, Iraq.

The variability in *P. aeruginosa* isolation percentage may be attributed to geographic, climatic, and hygienic factors among different areas. As well as, the high prevalence of *P. aeruginosa* in our community may be related to the rise of burn and wound patients than other samples in our population; which may be the result of different increased kitchen accidents, terrorist incidents, and electrical fire [18].

Conclusion

Pseudomonas aeruginosa was isolated in relatively considerable number; consequently, much work is needed to overcome and control this opportunistic pathogen.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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In vitro and *invivo* Study of Banana Peel Extract Anti Toxicity

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Abstract

In this study, extraction of the banana peels were done by two solvent kinds and water. Cytotoxic activity of the extracts were tested by using assay of MTT. Inhibition of cells was 68.2% when banana peel was extracted by hexane, and the effect of its cytotoxicity was the highest at 100 µg/ml. On the other hand, growth inhibition of MCF-7 cells was recorded at this concentration. While, ethanol extract was at the second stage according to its cytotoxicity which reached 54.1% followed by watery extract which showed 46.9%.Antimicrobial activity of banana peel extractswas tested against some pathogenic bacteria and showed the capacity to have abroad range of inhibition activities against isolates *E. coli, klebsiellaspp, S. aureus, P. aeruginosa*, extracts demonstrated inhibition zones which were greater than 20,10,15 mm against *E. coli*,*klebsiella* and*staph. spp.* respectively, but did not affect *P. aeroginosa* Also, experimental animals were exposured to these extracts. It was revealed that NTEC (CNF2) toxin made few chronic inflammatory cells proliferation, hyperplasia of lymphoid tissue and some cases of atrophy in the villi. Current results showed low impact on tissue cells when banana peel extracts were used.

Keywords: *Banana peel, anti toxicity, invivo, In vitro.*

Introduction

Banana's peel is known to contain many material that had biological activity and medicinal properties[1,2]. For example, tannins, alkaloids, flavonoids, phlobatannins, terpenoids and glycosides are known to be the bioactive compounds that are usually available in peel of banana. It have anticancer, antioxidant activity, in addition to pharmacological and antibiotic effect[3,4,5] . The present study aimed to prepare extracts of banana peel and assessment the inhibition activity against the cancer cell line Hela cell, MCF-7 and normal cell lines (REF) and estimate their ability to inhibit toxin *in vivo*.

Material and Method

Extract Preparation: 50g of banana peels were dried in order to obtain powder which was extracted by dissolving with 250 ml n-hexane, ethanol and water, separately . These extracts were dried by using hot plate at 40°C, then were filtrated and dried by a rotary evaporator[6].

Antimicrobial Assay: The diffusion method was used to determine inhibition effects for banana peels extraction toward many pathogenic bacteria such as *Escherichia coli, klebsiellaspp, Staphylococcus aureus*, *Pseudomonas aeruginosa* isolates which were obtained from lab of graduate studies in Biology Department/Sciences Collage/Baghdad University/ Iraq. Culture media were used to inoculate pathogenic bacteria,Wellswere made in the media toinoculate 100 µL of banana peels extracts and incubatedat 37°C for 18 hrs. After that measurement of inhibition zones was performed [7].

Cell lines and growth conditions: MCF-7 (breast cancer), HeLa (cervical cancer) cells and normal cell line (REF) were used to determine the effect of banana peel extracts. These cells were cultured on RPMI and MEM media which were enhanced with penicillin-streptomycin mixture (1%) and FBS (10%). The experimental conditions was 37 °C and incubation at 5% CO_2[8].

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Assay of MTT on Cytotoxicity: Investigation of the extracts cytotoxicity on the adherent cells proliferation in 96-well microtiter plate, procedure was performed according to [9].

***In vivo* study**

Animals: 18 Male BALB/C mice were used in this study. They were 3-4 weeks old and weighed 20-24 g. Mice were challenged with CNF2(cytotoxic necrosis factor2). All animals were fed on sterile food and water.

Inoculation procedure: Two method of animal inoculation were used in this study which were orally and peritoneally injection. Division of the tested animals were done for three groups, each group composed of three mice for each route of inoculation. No mortality of mice were occurred during or after inoculation.

1. First group was exposured to 100 µl of (100 µg/ml) with toxin only.
2. Second group was exposured to 100 µl of (100 µg/ml) toxin +100 µl banana peel extracts (n-hexan).
3. Third group was exposured to 100 µl of normal saline (control group).

10 days post inoculation, mice were killed.

Determination of Banana peel extracts effect on mice intestine: histopathological studies were done according to[10].

Statistical analysis: SPSS program was used for Statistical analysis[11].

Results and Discussion

Banana Extracts: Results of the current study demonstrated that the high amount of extract yield was obtained by using water followed by hexane and ethanol respectively (Table 1).

Currently, alcohol and hexane organic solvent were used to prepare extracts. Obtained results revealed that hexan was the best extract in its cytotoxicity on cancer cells which showed 68.2% for McF -7 and 62.3 for Hela cells than the others, followed by ethanolic and watery extracts . These results can be referred to bioactive compounds that are found in the organic extracts such as flavonoids, tannins and alkaloids that are responsible for their activity. While, the water extract contains only glycosides and alkaloids[12,13]. On the other hand, these result can be explained by that the solvents that are used have the ability to dissolve compounds that have biological activity more than that gained when water is used[14].

Antimicrobial activity: Antimicrobial activity of banana peel extractswas tested against some pathogenic microorganisms, and showed the capacity to have abroad range of inhibition activities against isolates *E. coli, klebsiellaspp, S. aureus, P. aeruginosa*, (table 2) extracts demonstrated inhibition zones which were greater than 20,10,15 mm against *E. coli*,*klebsiella* and*staph. spp.* respectively, but did not affect *P. aeroginosa*. Banana peel extracts can inhibit pathogen colonization and consequently prevent contamination. Present investigation exhibited that the chosen extracts of banana peel are great probiotic materials, which concurred with previous study[15].

Cell viability assay: cytotoxicity was performed by utilizing MTT technique. As appeared in table (3), increasing of concentrations and incubation period the extracts resulted in decreasing of HeLa and MCF-7 cells viability. Results showed a significant inhibition effect against HeLa ($P<0.05$) in most incubation periods and concentrations that were used. Highest concentration (100µg/mL) of n-hexan extract caused maximum inhibition effect against MCF-7 in maximum time.

High cytotoxic effect against MCF-7 and Hela was observed when treated with hexane extract which were 68.2% and 62.3% respectively. While, the watery extract and ethanolic one resulted in little inhibition effect against MCF-7 and Hela. Whereas, they had week activity against the normal cell line. Well growing of normal cells was observed in about 94% . An explanation of these results can be due to that the death of cancer cells was occurred by apoptosis which is known to be a controlled event. Production of cytokines that are known to be anti-inflammatory molecules in addition to phagocytosis can lead to this type of cell damage[16].As a result, it was thought that the banana peel had biological activity which may inhibit cancer cell proliferation[17] .

Histopathological studies: In the histological examination of intestinal and peritoneal sections, it was appeared that CNF2 made few chronic inflammatory cells proliferation, hyperplasia of lymphoid tissue and some cases of atrophy in the villi. While results elicited low effect on tissue cells when banana peel extracts were utilized. These results are accompanied with a previous study which demonstrated that the injection of the toxin

in the peritoneal area led to agglutination of blood veins and decrease in the platelet and finally death of the cells and caused death of the mouse[18] Additionally, the high doses of CNF2 caused the death byhemorrhagic shock and necrosis in the tissues[19]. Most the toxins from gram negative bacteria stimulate the inflammatory cells to release large amount of TNF and IL-1 which cause tissue necrosis and death. The pathogenic changes which were happened in the cells were removed after removing the causative agent and the cells were returned to normal state[20].

As appeared in figure (1), section of peritoneal tissue was exposed to toxin caused hyperplasia of lymphoid tissue, while, figure (2) showed severe atrophy of intestinal villi when exposed to toxin. These changes were removed when using toxin in addition to the tested extracts as can be seen in figure (3).

Table 1: Yield of banana peelextracts

Banana Part	Solvents	Yield %
Peel	Water	77.3
	n-Hexane	58.6
	Ethanol	55.1

Table 2: The inhibitory effect of banana peel extract against pathogenic bacteria

Pathogenic bacteria	Susceptibility
Escherichia coli	ES
S. aureus	S
Klebsiellaspp	I
P. aeruginosa	R

R = Resistant, S = Sensitive (12-15), Intermediate (7-11), ER = Extra sensitive >16

Table 3: Cytotoxic effects IC_{50} of banana peelextracts against cell lines

Cell line			Solvent
REF	McF -7	Hela	
5.5	68.2	62.3	n-Hexane
6.2	54.1	48.6	Ethanol
4.8	46.9	44.1	Water

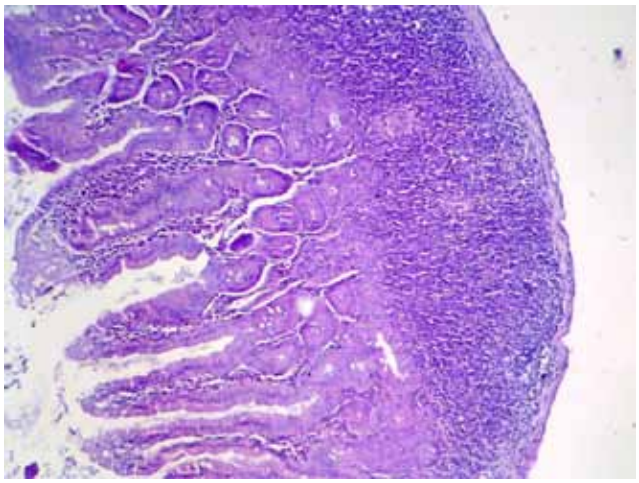

Figure (1) Section of small intestinal tissue showing hyperplasia of lymphoid tissue peyer,s patch, lymphocyte extension inside the villi when exposed to toxin, Hematoxilin –Eosin stained ×200

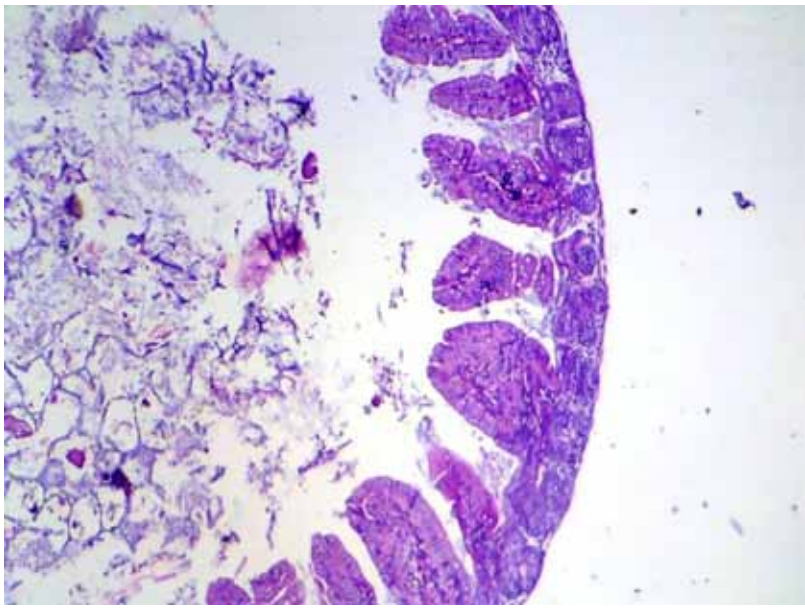

Figure (2) Section of small intestinal tissue showing severe atrophy of intestinal villi with its broadening and the crypts atrophied exposed to toxin, Hematoxilin –Eosin stained ×200

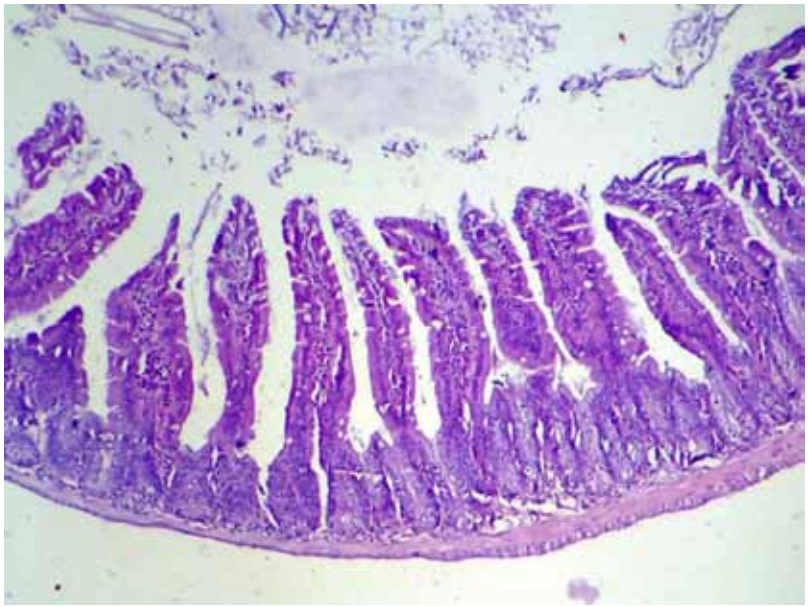

Figure (3) Section of small intestinal tissue showing elongation of intestinal villi and loo k-like normal also the crypt, NFs normal when exposed to toxinafter added peel banana, Hematoxilin –Eosin stained ×100

Conclusion

The banana peels have Inhibition of cells was 68.2% when extracted its by hexane, and the effect of its cytotoxic was the highest at 100 µg/ml. growth inhibition of MCF-7 cells .and Antimicrobial activity of extracts was tested against some pathogenic bacteria and showed the have of highest inhibition against *E. coli*, inhibition zones which were greater than 20mm Also, in vivo tested results showed low impact on tissue cells when extracts were compared toxin used.

Conflict of Interest: No conflict of interest

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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