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Prevalence of Ochrobactrum Intermedium in Gastric Biopsy Specimens of Patients with Gastritis and Dyspepsia and the Impact of Amniotic Membrane as Antibacterial

WAFAA ELSHABSHIRY, M.Sc.[1]; RATEB ABBAS, Ph.D.[2]; ELHAM AWAD, Ph.D.[3]; MEDHAT HASHEM, Ph.D.[4]; ASHRAF EID, Ph.D.[5]; AHMED SEIF, Ph.D.[5] and OMAIMA KHAMISS, Ph.D.[6]

1-Animal Cell and Tissue Culture, Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute University of Sadat City,
2-Professor of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute University of Sadat City, Egypt.
3-Assistant Professor of Medical Microbiology and Immunology, Shebin El-Kom Teaching Hospital, Shebin El-Kom, Egypt
4-Professor of Genetics, Genetic Engineering and Biotechnology Research Institute University of Sadat City, Egypt.
5-Professor of Gastroenterology, Hepatology and Infectious Diseases, Shebin El-Kom Teaching Hospital, Shebin El-Kom, Egypt
6-Professor of Cell Culture and Molecular Virology, Genetic Engineering and Biotechnology Research Institute University of Sadat City, Egypt

Abstract

Background: Ochrobactrum intermedium bacteria has some roles as an opportunistic organism in many cases of gastritis and dyspepsia. Human amniotic membrane is known to have an antimicrobial effect on several organisms.

Aim of Study: This study briefly aims to screening for a novel anti-bacterial treatment on some pathogenic gastrointestinal tract bacteria.

Material and Methods: Sixty-three gastric biopsies from dyspeptic patients were collectedfrom (Shebin El-Kom Teaching Hospital, Shebin El-Kom, Egypt). Selected isolate of O.intermedium from thirty positive isolates was molecular identified and phylogenic tree was carried out using 16S rRNA which was sequenced and compared with available 16S ribosomal sequences in the NCBI GenBank database (OR067155). Human Amniotic membrane (HAM) was freshly isolated from the placenta and HAECs have been enzymatically isolated using prewarm 0.05% trypsin/EDTA, then cells were seeded in standard culture medium DMEM/F12 (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% FBS and incubated at 37ºC. Cell counts was done using a hemocytometer, cell maintenance and viability was followed-up using trypan blue. Possible inhibitory activity of HAM against O.intermedium has been tested and recorded. Ochrobactrum genus have been confirmed in the human gastric niche based on PCR.

Results: Biopsies from patients with gastroesophageal reflux disease showed H.pylori existence in histopathology with also positive urease, catalase and oxidase tests, while PCR results showed O.intermedium without H.pylori. Human amniotic membrane had an inhibitory effect against O.intermedium.

Conclusion: This study demonstrates that Human amniotic membrane can be used as an antibacterial against O.intermedium in cases of gastritis or dyspepsia, especially in immunocompromised patients, O.intermedium should be considered as an opportunistic organism whether H.pylori exists or not.

Key Words: *HAECs – Helicobacter pylori – Human amniotic membrane – Non-ulcer dyspepsia – Ochrobactrum intermedium – Polymerase chain reaction.*

Correspondence to: Dr. Wafaa Elshabshiry, Animal Cell and Tissue Culture, Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City

Introduction

GLOBALLY, patients with gastric, duodenal ulcers and chronic gastritis are mainly infected by H. pylori. The organism plays a major etiological role in the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and distal gastric cancer **[1,2]**. It has been reported that 50% of adults in developed countries and 80%–90% of the population in developing countries are infected with H. pylori **[3,4]**. Along with the antigen test on stools, urea breath test and endoscopic and histopathological examination are considered the gold standards for diagnosis **[5]**. Previous studies have revealed that O.intermedium is an emerging pathogen that causes atrophic gastritis along with H.pylori **[6]**. O.intermedium also can cause presumed bacteremia in bladder cancer patients and liver abscesses after liv-

er transplantation **[7,8]**. But clinical manifestations and diseases caused by O.intermedium are poorly characterized. Brucella, Bartonella, Agrobacterium, and Ochrobactrum are only a few of the vast group of bacteria known as the alpha proteobacteria that can interact with eukaryotic cells. Ochrobactrum intermedium is an Alpha proteobacteria member that is a Gram-negative, aerobic, capsulating bacilli. It is the closest genetic relative of genus Brucella as evidenced by protein profiling, western blot, immunoelectrophoresis, amplified fragment length polymorphism, 16S rRNAgene and RecA gene sequence-based studies **[9]**. In gastric biopsies, several microorganisms other than Helicobacter pylori have previously been identified. The presence of O. intermedium along with H. pylori was reported earlier in a subject from North-India diagnosed with non-ulcer dyspepsia **[10]**. One unusual finding was the presence of significant fibrosis found during histological analysis of the gastric antral biopsy in the lamina propria of the gastric mucosa. Whether this fibrosis was caused either partially or totally by O. intermedium was not clear. Along with H. pylori, other Ochrobactrum species have also been linked, such as O. anthropi and moderate gastritis in squirrel monkeys **[11]**. Staphylococci, enterococci, and Gastrospirillum hominis **[12]** have also been linked to gastrointestinal problems **[13]**. In some cases, they have been isolated from antral biopsies from patients with or without H. pylori colonization **[14]**. Along with H. pylori, other Ochrobactrum species have also been linked, such as O. anthropi and moderate gastritis in squirrel monkeys **[15]**. Staphylococci, enterococci, and Gastrospirillum hominis **[12]** have also been linked to gastrointestinal problems.

The human amniotic membrane (HAM) is derived from fetal ectoderm with a total thickness of 0.02 to 0.5mm. The amnion is smooth, glistering, and tough and has five layers **[8]**. It consists of cubical and columnar epithelial cells. According to several studies **[16-20],** AM has an antimicrobial impact against a variety of bacteria and fungi, including E. coli, S. aureus, P. aeruginosa, Streptococcus pneumoniae, Staphylococcus saprophyticus, and others. One of the tissues that generates and secretes antimicrobial peptides is the human amnion. Elafin is the other amnion antimicrobial peptide that act as anti-protease **[21]** and elastase inhibitor. Amniotic membrane contains natural antimicrobial molecules which are component of innate immune system so it acts as a safe guard against Gram negative and Gram-positive bacteria, viral and fungal infection **[21]**. This study attempts to clarify the extent of the danger of O.intermedium bacteria in indigestion and gastritis. As the genomic properties of O. intermedium are poorly characterized and, as a consequence uncertainty surrounds their function in human disease and health. A deeper knowledge of O.intermedium'sability to survive in acidic conditions may result from the elucidation of physiological characteristics and the identification of genes putatively involved in the various metabolic pathways. As well this work seeks to ensure and determine the extent and how the human amniotic membrane epithelial cells actually act as an antibacterial against some types of gut opportunistic bacteria including O.intermedium.

Material and Methods

Urease test kit was obtained from Oxoid England. Skirrow's Supplement (SR69) Oxoid England, Brain Heart Infusion (BHI) agar, and Brain Heart Infusion (BHI) broth. 2,3,5-Triphenyltetrazolium chloride (TTC) was obtained from Himedia India. Expired blood was obtained from Blood Bank of National Liver Institute, Shebin El-Kom. A sterile McCartney bottle holding 0.2g/L of cysteine and 20% glycerol in brain heart infusion (BHI) broth was immediately filled with biopsies that had been proven to be urease test positive. These bottles were then transported on ice to the laboratory within 60 minutes of collection for further examination. Standard cell culture medium DMEM/F12 (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% FBS, 100U/ml penicillin, 100µg/ml streptomycin (1X, Sigma Aldrich, USA) were used as standard culture media. Phosphate Buffer Solution (PBS) and Hank balanced salt solution (HBSS) were used as transportation buffers and washing buffers pre isolation. Trypsin EDTA (Thermo Fisher Scientific, USA, 0.05%) was used as a digestive enzyme to isolate the cells from the membrane.

1- Subjects and sampling:

A total of 63 gastric dyspeptic patients (36 males, 27 female) illustrated in Table (1) who were admitted to The General Authority for Teaching Hospitals and Institutes, Shebin El-Kom Teaching Hospital Department of Gastroenterology from December 2020 to September 2021, with ages average from 25–72 years, were included in this study. Gastric biopsy specimens were collected from the antrum and corpus of the stomach. No subjects had received treatment for H. pylori infection. This study was approved by the Research Ethics Committee of Genetic Engineering and Biotechnology Research Institute, Approval number (GEBRI-USCREC-202109) August 2021.

2- Inoculation of Gastric Biopsy:

The BHI broth containing the gastric biopsy was homogenized using the Glass Tissue Homogenizer for 30 seconds to produce an evenly distributed mixture of biopsy tissue in the broth. The homogenized biopsy was then inoculated onto the brain heart infusion blood-amended agar. The inoculation was done, by dipping a flamed inoculation loop that has cooled for some time into the Mc Cartney's bottle containing the homogenized biopsy tissue. The loop was used to streak the surface of an amended Brain Heart Infusion blood agar plate. The inocu-

lation loop was famed until they were red-hot after usage. In order to create microaerophilic conditions (80% N2, 10% CO_2, and 5% O_2) inside the container, the infected plates were then placed inverted into a Gaspak airtight container. The replicated inoculated plates together with the gas generating kit were incubated at 25ºC, 37ºC, and 42ºC for 3-7 days. Subculturing of Bacterial Isolates. Sparkling colonies could only be seen on the modified BHI blood agar cultivated at 37°C after 3–7 days of incubation. To create pure cultures of pathogenic microorganisms, a single sparkling colony was selected using a flamed inoculation loop, streaked on the surface of a freshly updated BHI blood agar plate, and then cultured under microaerophilic and aerophilic conditions at 37°C for an additional 3–7 days.

Table (1): Illustrate the demographic data of relation between age, sex secondary standard and Prevalence of O. intermedium infection among patients undergoing upper Gastro-intestinal endoscopy in Shebin El-Kom Teaching Hospital, Shebin El-Kom, Egypt.

Variables	O.intermedium positive		O.intermedium negative		Total	Chi-square	
	Number	Percentage	Number	Percentage		$\chi 2$	*p*-value
N. of patients	30	47.62%	33	52.38%	63		
Age:							
Older adults (51 – 75)	4	6.35%	21	33.33%	25	17.35	0.001
Adults (31 – 50)	15	23.81%	5	7.94%	20		
Middle age (18- 30)	11	17.46%	7	11.11%	18		
Gender:							
Male	20	31.75%	16	25.40%	36	2.12	0.145
Female	10	15.87%	17	26.98%	27		
Complications:							
Gastritis caused by O. intermedium	19	63.33%			19	2.13	0.144
Ulcers caused by O.intermedium	11	36.67%			11		

3- Microscopy and Biochemical Tests for Identification of pathogenicmicroorganism:

Bacterial isolates were identified based on colony morphology and physical characteristics in the presence of TTC dye. Biochemical tests, namely, gram staining reaction, oxidase, urease, and catalase tests.

4- Histopathological examination:

Histology was performed on all gastric biopsies using hematoxylin-eosin (H&E) and Loeffler's methylene blue stains to visualize the pathogenic organism. At the same time, specimens were histologically evaluated according to the Sydney system of classification *[21]*. Parameters like inflammation, atrophy, activity, H.pylori, intestinal metaplasia, and lymphoid aggregates/lymphoid follicles were taken into account while making the histological interpretation.

5- PCR amplification and sequencing of 16s rRNA gene:

The selected Bacterial isolate was identified using 16S rRNA gene partial sequencing method *[23]*. Using a genomic DNA extraction kit (Intron, Biotechnology, Korea), genomic DNA was isolated from the chosen isolate. PCR amplification of 16S ribosomal DNA (16S rDNA) was performed with a set of universal primers; 27F-Forward primer 5'AGA GTT TGA TCC TGG CTC AG3' (20 mer) and 1492R-Reverse primer 5'CTA CGG CTA CCT TGT TAC GA3' (20 mer). The PCR amplification was performed using the thermal cycler (Thermo Fisher Scientific, USA) programmed for 35 cycles as follows; initial denaturation at 95ºC for 5min, denaturation at 94ºC for 35sec., followed by annealing at 57.5ºC for 30sec., then extension at 72ºC for 1min, and finally the extension was at 72ºC for 10 min. The produced amplicons of the selected isolate (PCR product) was tested for its quality by electrophoresis using agarose gel (1%) and compared with 1kb DNA ladder (Intron Biotechnology, Korea). The electrophoresis run was performed in 1xTris-Borate-EDTA (TBE) buffer at 80V for 40min in Bio-Rad submarine (8x12 cm), stained with ethidium bromide then DNA banding patterns of 16S gene amplicons were visualized using a UV-transilluminator (Thermo Fisher scientific, USA) under UV light. PCR products were purified by using gene JETTM genomic DNA purification kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. Then sequenced using forward and

reverse primers with ABI 3730xl DNA sequencer. Nucleotide BLAST facility was employed in order to evaluate the degree of DNA similarity. Nucleotide bases obtained after sequencing were identified and compared with similar sequences retrieved from the GenBank database within the National Center for Biotechnology information (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank/index.html) using nucleotide Basic Local Alignment Search Tool (BLAST) Gene Sequences in the database (http://www.ncbi. nlm.gov/BLAST/). After depositing the nucleotide sequences of the 16S rRNA genes to GenBank with accession number OR067155, the phylogenetic analysis of sequences was created using Alignment Search Tools (BLAST).

6- *Preparation of amniotic membrane:*

The preparation of amniotic membrane and cells according to the method described by **[20,23-25]**. Placentas were obtained from Harmel Hospital in Sadat City, Minoufya, Egypt from healthy women with a normal pregnancy and normal delivery without any complications that could affect the quality of cells and membrane. This procedure always carriedoutunder strict sterilized conditions.

- Isolation of HAM from the placenta:

The freshly isolated placenta which was transferred from Harmal hospital in Sadat city to GEBRI-USC cell culture laboratory in a sterile container containing ice-cold Hanks' balanced salt solution (calcium- and magnesium-free HBSS) supplemented with 1% penicillin/streptomycin solution (Sigma Aldrich, USA) to cover up the placental surface. The placenta was then transferred to a sterile container (under horizontal laminar flow hood) where the amniotic membrane was faced upwards. The amniotic membrane was manually separated from the chorion layer, starting from the outer edge of the amniotic membrane and continuing toward the umbilical cord **[23,24]**. Then the membrane was washed several times with ice-cold PBS to remove blood clots and cell debris as shown in Fig. (1).

- *Establishment of the HAECs from the amniotic membrane:*

HAECs were separated from the amniotic membrane by using enzymatic method:

Amnion free from blood was cut into small pieces around 5cm long and transferred with sterilized forceps to two new 50ml falcon conical tubes. To each tube 20ml pre-warm 0.05% trypsin/EDTA (Thermo Fisher Scientific, USA), and then incubated at 37°C for 15mins with gentle shaking (first digestion). Then the obtained cells from this step were discarded to remove blood clots and other cell debris. Afterward, the membrane pieces were transferred into two new 50ml falcon conical tubes containing 20ml of 0.05% trypsin/EDTA (Thermo Fisher Scientific, USA) and incubated at 37°C for 30mins with gentle shaking (second digestion) as described by **[24,25]**. This step was repeated and the third digestion was carried out. The second and the third digestions were neutralized to inhibit trypsin activity by adding 20ml DMEM F12 augmented with 10% FBS, and finally centrifuged at 300×g for 5mins at 4°C. The cell pellets were then re-suspended in 10ml standard complete culture medium and mixed together. The cell number was counted using Thoma hemocytometer and cell viability was determined using trypan blue. It is suggested to check the status of the HAECs after each digestion step to determine whether further digestion steps were required to separate the majority of cells from the membrane or not. It is suggested that at the end of each digestion, the membrane pieces be gently shacked using sterilized forceps in the tubes containing the trypsin enzyme to separate all the epithelial cells in case they are still loosely attached to the membrane **[23,24]**. This technique has undergone several modifications in GEBRI-USC lab. The simplified procedure used for primary culture is briefly described in Fig. (2). The tissue in balanced salt solution (PBS) is finely cut into pinhead-sized pieces by a sterilized scalpel and washed by settlings. The balanced salt solution is then removed. The tissue pieces are transferred into tissue culture T flasks 25cm^2. After the addition of the appropriate growth medium, incubation is carried out for 3-5 days at 37°C. Then themedium is changed at weekly intervals until a substantial outgrowth of cells is observed.

- *Maintenance of the test cells:*

Isolated HAECs were cultured in 25cm^2 tissue culture flasks using a standard culture medium (DMEM F12) and then they were incubated at 37°C. The plating productivity of the cells was determined after 2 days of incubation.

7- *In vitro evaluation of HAECs epithelial cells effect on the identified pathogenic bacterial isolates using disc diffusion agar bioassay:*

- *Preparation of the bacterial inoculum:*

Before testing, bacterial inoculums were prepared by Subculturingof bacterial isolates freshly. After 72h of incubation 3 individual identical colonies picked from each agar plate as shown in Fig. (7) by sterile inoculating loop then transferred into a tube with 0.9% saline solution suspended trying to adjust the turbidity of each bacterial suspension in the saline solution equivalent to the 0.5 McFarland standard (bioMèrieux, France) 1-2 × 10-8 CFU/ml (0.08-0.1 at 600nm).

- *Disc diffusion bioassay:*

Volume of 0.1ml of bacterial test isolates suspensions were added to 15ml of Molten Mueller-Hinton Agar (Oxoid, England), mixed well, poured into sterile Petri-dishes and allowed to stand for 10mins. Sterile filter paper discs of 6mm diameter

(Himedia, India) were impregnated with 2 groups of 50 and 100µL of (mixture of human amniotic epithelial cells resuspended in standard complete culture medium) used to detect the presence or absence of HAECs antibacterial activity on test isolates. At last the tested agar plates were incubated for 48h at 37ºC.

8- *In vitro detection of (HAM) antibacterial activity as a tissue on the identified pathogenic bacteria:*

Sterile Pipette blue tip cut by scalpel exposed to flame was used to cut the amniotic membrane tissue purified by enzymatic method to detect the diameter of amniotic membrane tissue (6mm diameter). Inhibition zones of HAM antibacterial activity effect as a tissue were calculated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

1- *Prevalence of Ochrobactruminter medium:*

Primary culture of 63 gastric biopsies (GB) showed growth of unknown pathogenic microorganism in 52 GB, subculturing showed growth in 30 (47.6%) GB as in pie Chart (1). O.intermedium strains were successfully isolated from gastric biopsies from patients with gastritis and molecularly identified by PCR. Biochemical tests, gram staining reaction was negative coccobacilli to rods in Fig. (4), urease, oxidase, catalase and motility were positive. Pure cultures of O.intermedium in TTC dye incorporated medium were obtained as Creamy, smooth, nonhemolytic sparkling colonies in Fig. (7). These patients had complaints about acute or chronic gastritis. Genotyping was performed on 4 O.intermediumstrains.

Fig. (1): Freshly obtained placenta post-delivery, Separation of amnion from chorion, and amniotic membrane separated from the placenta washed and sterilized, Amniotic membrane under inverted microscope.

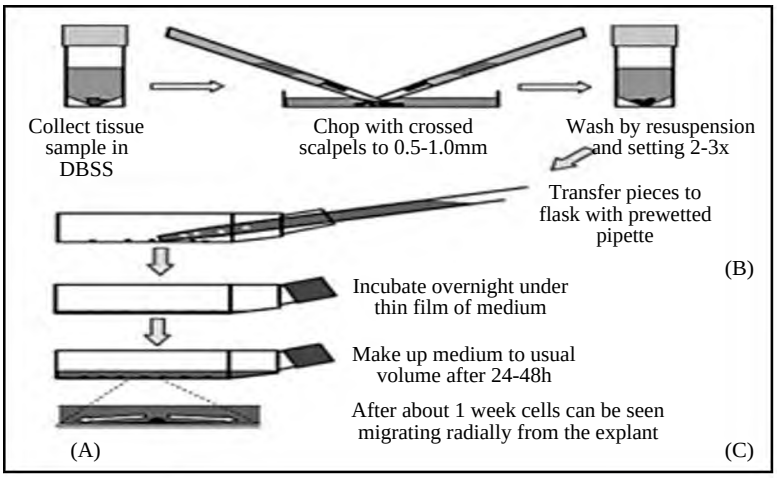


Fig. (2): Preparation of HAECs primary cell culture separated from the amniotic membrane as described in Freshney's Culture of Animal Cells: A Manual of Basic Techniqueand Specialized Applications 8th Edition (2021).

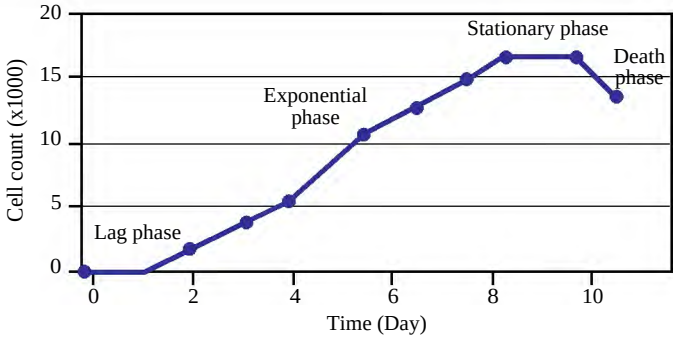


Fig. (3): Growth curve of HAECs.

2- Histological examination of the gastric biopsies for the presence of pathogenic bacteria:

In all diagnostic and prevailed cases, inflammation was observed in all biopsies. Intestinal metaplasia (IM) was present only in (9 cases) with ulcerative dyspeptic (UD) biopsies. Only NUD cases had H. pylori and/or non-H. pylori (Ochrobactruminter medium) prevalence. Lymphoid aggregates and lymphoid follicles (LA/LF) were present in Non ulcerative dyspeptic (NUD) patients, whereas LA/LF were variable in biopsy specimens from other gastricdiseases.

3- Molecular Identification and phylogenic tree of selected isolate:

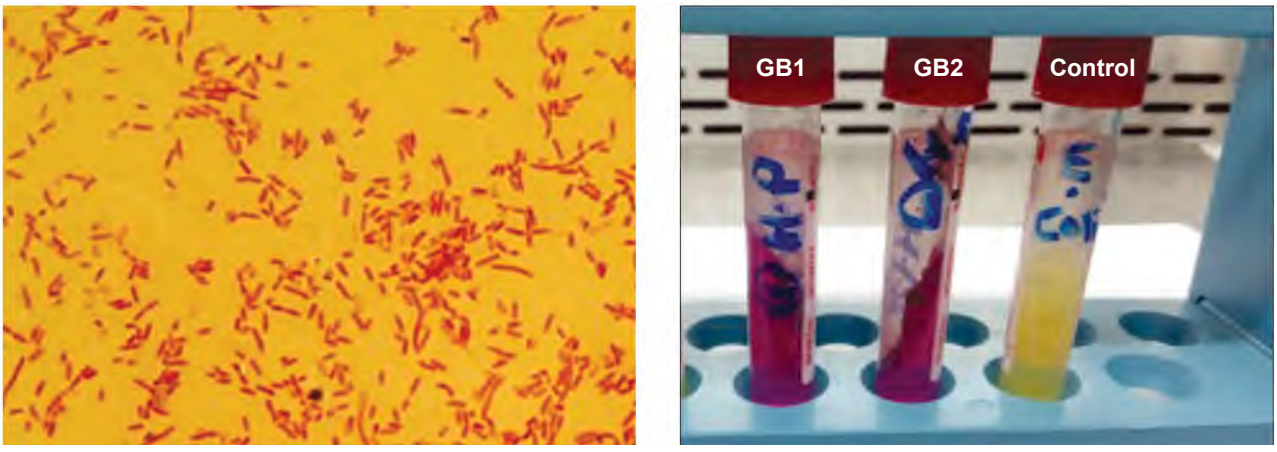
Sequencing of the highly conserved 16S rRNA gene is one of the most commonly used techniques for identifying microorganisms; it is a powerful, simple, and fast method for determining evolutionary and phylogenetic relationships among microorganisms *[2]*. Sequencing of 16S rRNA gene has been employed to facilitate the differential identification among the genus Ochrobactrum *[31]*. PCR amplification of the DNA samples from the selected isolate generated a PCR product of expected size (975 bp). The analysis of 16S rRNA gene of the Bacterial isolateEGY1 was sequenced and compared with available 16S ribosomal sequences in the NCBI GenBank database website (http://www.ncbi.nlm.nih.gov/GenBank/index.html) using BLAST. The NCBI database showed highest percentage of similarity being 97.05% with Ochrobactrum intermedium strain NBRC 1268 Under accessionno. OR067155. Gene bank nucleotide database using the blast-n algorithm revealed significant matches with hi max score of 1982, zero e-value, and 99.54% nucleotide identity for isolate EGY1. The phylogenetic tree Fig. (5) shows high genetic relationship between the Egyptian Bacterial Ochrobactrum intermedium isolate EGY1 and Ochrobactrum intermedium strain NBRC 1268; which strongly confirm its identity as Ochrobactrum intermedium.

4- Isolation of human amniotic epithelial cells (HAECs):

The isolated HAECs' initial plating efficiency on culture dishes was at least 80% after 48 hours. Human amniotic epithelial cells (HAECs) as previously described in materials and methods were isolated from the human amniotic membrane (HAM) which was separated from freshly obtained placenta post-delivery. The resulted cells as illustrated in figure (6a, 6b) the proliferation of HAECs could be observed in different intervals and follow-up after 6-12 days.

5- Disc diffusion bioassay and tissue of HAM antibacterial activity:

Thehuman amniotic membrane isolated from placenta showed possible inhibitory activity against all tested strains for O.intermedium as shown in Fig. (8A,B). Using a mixture of human amniotic epithelial cells resuspended in standard complete culture medium showed antibacterial activity zone varying from 10 to 16mm, but when used as a tissue it formed enlargement of inhibition zonein diameter of approximately 20mm.



(A) (B)

Fig. (4): (A) Gram stains of O. chrobactrum intermedium prepared from colonies grown on blood agar plates, (B) Urease-positive bacteria grown on two gastric biopsies compared with E.coli as a negative control.

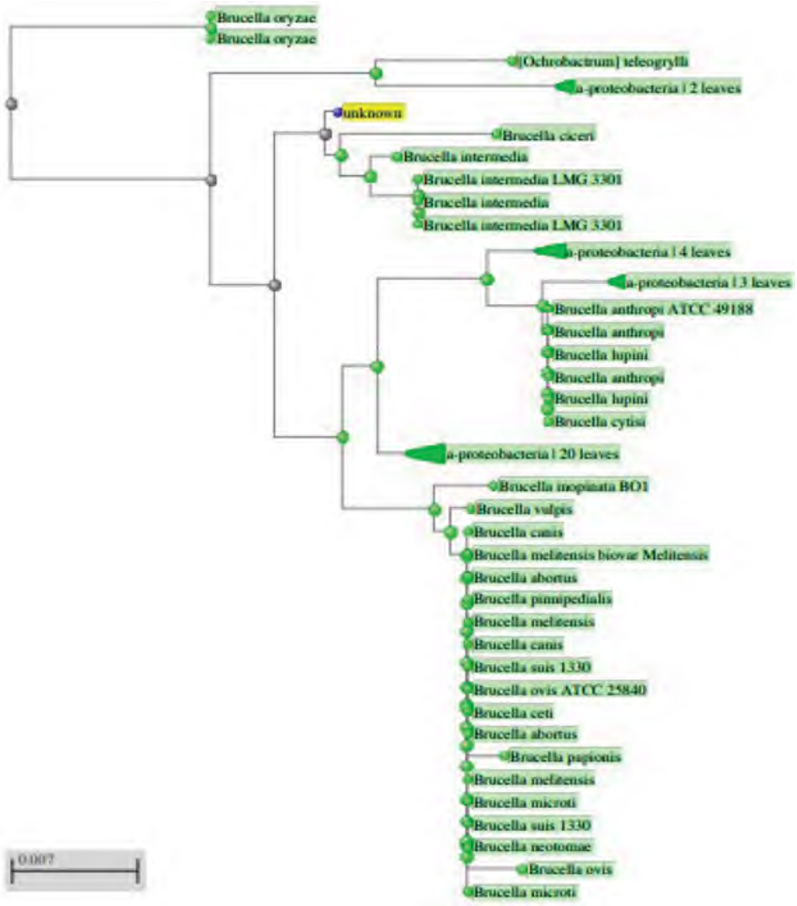


Fig (5): Phylogenetic structure of the strain Ochrobactrum inermedium along with the genus Brucella. The tree based on partial 16S rRNA gene sequences obtained using neighbor joining with Maximum Composite Likelihood method (MEGA package). GenBank accession numbers are given with the species name. Bar, 0.0070 substitutions per site

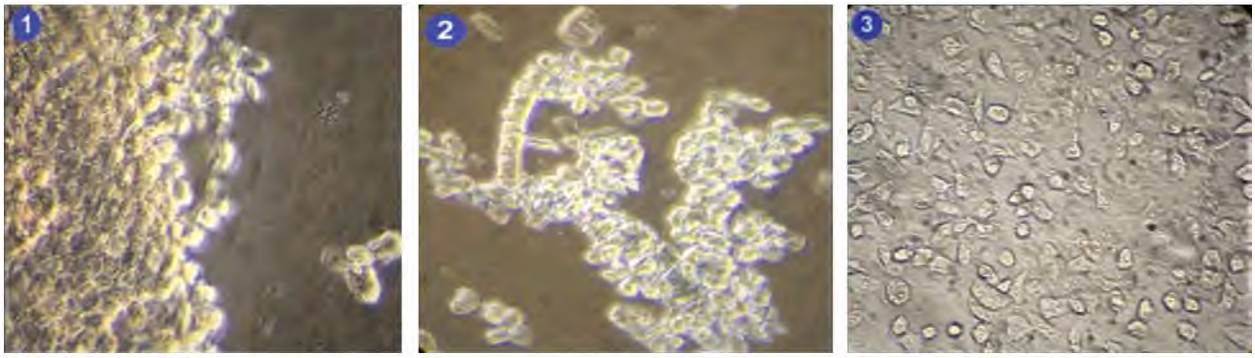


Fig. (6A): Showed different proliferation stages of HAECs isolated method. Magnification 400x.

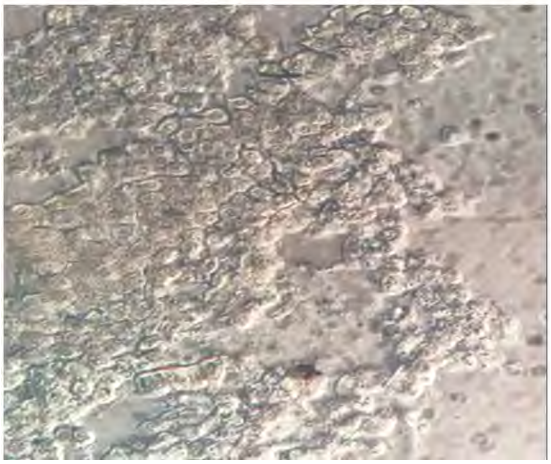


Fig. (6B): HAECs isolated after enzymatic method modification in the GEBRI lab. Magnification 400x.

Fig. (7): O. intermedium sparkling colonies grown on prepared blood agar (BHI agar incorporated with TTC dye + 5% human expired blood).

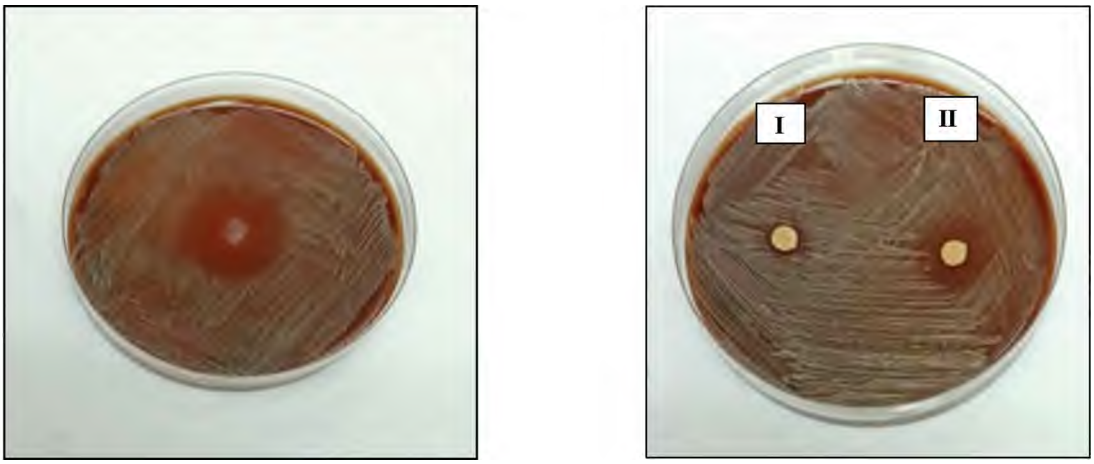
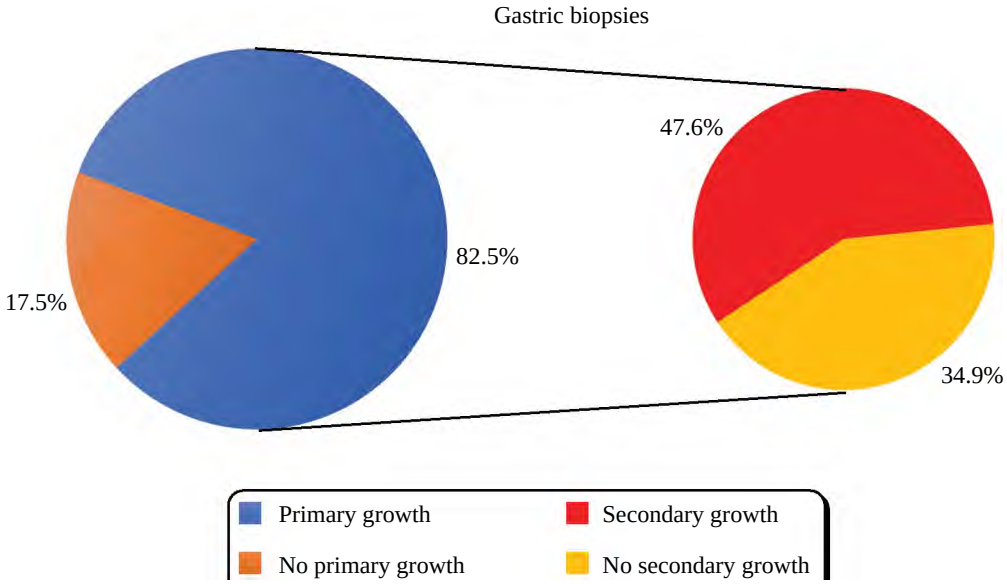


Fig. (8): (A) Effect of 0.5 cm in diameterpiece from Amniotic membrane tissueon O. intermedium growth. (B) Effect of Human amniotic membrane epithelial cells (HAECs) on O.intermedium: I: Effect of 50µl (HAECs) mixture/disc diffusion (antimicrobial susceptibility test) on O. intermedium. II: Effect of 100µl (HAECs) mixture/disc diffusion on O.intermedium.



Pie Chart (1): Showing results of gastric biopsies cultured on primary and secondary enriched blood agar media.

Discussion

Gastric mucosal damage occurs mainly in patients with H.pylori. Some cases in this study, results investigated a group of 63 patients with gastritis and ulcers using endoscopy and gastric biopsies. Our finding is that both H.Pylori and O.Intermedium have several genes for biosynthesis of cytosolic urease for its survival in the acidic environment of stomach lumenis agreed with many studies **[14,26].** Some cases (25%) that had peptic ulcers, dyspepsia and gastroesophageal reflux disease showed H.pylori existence in histopathology with also positive urease, catalase and oxidase tests, while PCR results showed O.intermedium with no H.pylori that disagreed with many studies **[27].** The current study, on some cases with gastritis or gastric ulcers showed O.intermedum existence with no H.pylori in PCR results that disagreed with many studies **[27].**Many researchers stated that O. intermedium displayed a high level of resistance to several antimicrobial agents notably to beta lactam antibiotics **[22,29],** which represent the most commonly prescribed antimicrobial agents. Further studiessuggesting that Antibiotic resistance is frequently associated with resistance to environmental xenobiotics **[22,27].** In addition to the confirmed data about the intestinal metaplasia could be a marker for chronic gastritis due to H. pylori infection **[2].** The promising antimicrobial effects demonstrated by HAM and HAECs added to the other biological properties in this current study results indicate that the human amniotic membrane isolated from placenta proved possible inhibitory activity against all tested strains for O.intermedium when amixture of human amniotic epithelial cells resuspended in standard complete culture medium showed antibacterial activity zone varying from 10 to 16mm, but when used as a tissue it formed enlarged inhibition zone. Some patients with ulcerative dyspepsia had intestinal metaplasia in their biopsies, while these results demonstrate that several bacteria other than Helicobacter pylori have been detected earlier in gastric biopsies such as O.intermedum (unpublished data). The presence of O.intermedium along with H. pylori was reported earlier in a subject from North-India diagnosed with non-ulcer dyspepsia. Previous studies **[10,12,25]** reported that high yield of the viable cells was accompanied with contamination with other cell populations. The present investigation, however, demonstrated a highly viable HAECs at high yield and minimal MSC contamination. The mean viability of isolated HAECs in this study was 87% compared with previously reports with a range from 83% to 99% **[25].** Regarding the precedent stated results of HAM and HAECs concerning their properties already demonstrated such as the production of anti-inflammatory agents suchas hyaluronic acid, the suppression of pro-inflammatory cytokines, anti-fibrotic propertiesdue to the negative regulation of transforming growth factor (TGF-β), low antigenicity, and immunomodulatory properties as a result of the factor secreted by epithelial cells, which, during pregnancy inhibit the migration of macrophages and natural killer cells in order toprevent a maternal immune attack that could support the conclusion and the current obtained results.

Conclusion: The current study showed that the human amniotic membrane can be considered and used as an antibacterial against H. pylori and O. intermediumin cases of gastritis or indigestion, especially in immunocompromised patients, and O. intermedium should be considered an opportunistic organism whether H. pylori is present or not.

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مدى انتشار Ochrobactrum Intermedium فى عينات خزعة المعدة لمرضى التهاب المعدة وعسر الهضم وتأثير الغشاء الامنيوسى البشرى كمضاد للبكتيريا

البكتيريا الوسيطة O.chrobactrum لها بعض الأدوار ككائن حى انتهازى في العديد من حالات التهاب المعدة وعسر الهضم. من المعروف أن الغشاء الأمنيوسى البشرى له تأثير مضاد للميكروبات على العديد من الكائنات الحية. فى هذه الدراسة تم جمع ثلاثة وستين خزعة من المعدة من مرضى عسر الهضم من (مستشفى شبين الكوم التعليمى، شبين الكوم، مصر). تم تحديد العزلة المختارة لـ O. intermedium من ثلاثين عزلة إيجابية وتم إجراء شجرة النشوء والتطور باستخدام الرنا الريباسى 16S الذي تم تحليل التتابع التسلسلى له ومقارنته بتسلسلات الريبوسوم 16S المتوفرة في قاعدة بيانات بنك الجينات NCBI تحت رقم (GenBank OR067155). تم الحصول علي عزل الغشاء الأمنيوسى البشرى (HAM) اثناء عمليات الولادة حديثًا من المشيمة من مستشفي هارمل بمدينة السادات تحت ظروف محكمة التعقيم وتم عزل HAECs إنزيميًا باستخدام ٠,٠٥٪ من التربسين / EDTA ، ثم تم زرع الخلايا فى وسط الاستزراع القياسى DMEM / F12 (Thermo Fisher Scientific، Gibco، الولايات المتحدة الأمريكية) مكملًا بـ ١٠٪ FBS وحضنت عند ٣٧ درجة مئوية. تم إجراء تعداد الخلايا باستخدام شريحة العد ، وتمت متابعة الخلايا وقدرتها على البقاء باستخدام صبغة التريبان الأزرق. تم اختبار وتسجيل النشاط المثبط المحتمل لـ HAM ضد O. intermedium. تم تأكيد وجود جنس O.chrobactrum فى المعدة البشرية اعتمادا أظهرت النتائج ان الخزعات من المرضى الذين يعانون من مرض الالتهاب الجرثومى المعدى المريئى وجود الملوية البوابية (الجرثومة المعدية Pylori.H) فى التشريح الهستوباثولوجى مع إيجابية اختبارات اليورياز والكاتلاز والأكسيداز، فى حين أظهرت نتائج PCR وجود الملوية البوابية المتوسطة بدون الملوية البوابية (الجرثومة المعدية). اثبتت النتائج أيضاً انه كان للغشاء الامنيوسى البشرى تأثير مثبط ضد O.chrobactrum. الوسيط.

الاستنتاج: أوضحتالدراسة أنه يمكن استخدام الغشاء الامنيوسي للإنسان كمضاد للبكتيريا ضد البكتيريا الحلزونية الوسيطة في حالات التهاب المعدة أو عسر الهضم، وخاصة في المرضى الذين يعانون من ضعف المناعة، ويجب اعتبار البكتيريا الحلزونية المتوسطة كائنًا انتهازيًا سواء كانت الملوية البوابية موجودة أم لا.