ISSN : 0974 - 7532 Volume 10 Issue 2

Research & Reviews in

BioSciences

Regular Paper

RRBS, 10(2), 2015 [051-056]

Isolation and identification of the samples found in pathogenic bacteria from human placenta

Boutaïna Laassili[1], Mohammed Ouhssine[1], Hassan Aït Si Baha[2], Sultana Inekach[1] Mohammed Sobh[3], El Hassan Berny[1]

[1]Biotechnology Laboratory, Environment and Quality, Faculty of Sciences BP 133 14000, kenitra, (MOROCCO)

[2]Company Salawi Bea, (MOROCCO)

[3]Laboratory of Microbiology, Pharmacology, Toxicology and Environment Faculty of Sciences Ain Chock, University Hassan II BP 5366, Maarif, Casablanca 20100, (MOROCCO)

E-mail: ouhssineunivit@gmail.com

ABSTRACT

The purpose of this study was to identify pathogenic strains isolated from samples of human placenta. The analyzes were performed in our laboratory at the Faculty of Sciences of Kenitra.

We proceeded to the isolation and purification of 11 bacterial strains. The selection of the strains is based on the representation thereof in all colonies appeared on the surface of the petri dish. The pure strains were identified by a set of said testing prior tests. Following the identification is completed by using the API gallery and API 20 E 20 Strept. Reading of the results is done by reading software. The identification results 11 gave two bacterial strains including Escherichia coli, Salmonella choleraesuis 1 arizonae spp, Enterobacter asburiae 1, 1 Enterobacter cloacae, Citrobacter Youngae 1, 2 and Aeromonas hydrophila gr.2 gr1, 1 Enterobacter cloacae 1 Lactococcus lactis cremoris ssp and one Aerococcus Viridans 1.

All strains are identified pathogens. They are known by their pollution of the human digestive tract, they can cause urinary tract infections, diarrhea, gastroenteritis, septicemia, meningitis, peritonitis, pneumonia, brain abscesses, infections of the abdominal cavity, nosocomial infections, gastroenteritis, bacteremia. We can say that the placenta samples are rich in pathogenic bacteria, hence the need for hospital treatment of the waste before it is dumped in landfills.

KEYWORDS

Placenta;
Identification;
API 20 E gallery;
Gallery 20 api strept;
Pathogenic bacteria.

INTRODUCTION

The human placenta is an organ unknown because of difficult access and no ideal animal model. It is a complex organ that appeals to many biological knowledge and offers outstanding specialties fascinantes. Il is also central to many diseases of pregnancy[1].

The placenta is a transient member that allows the development of the fetus[2]. It is generally rounded or ovular whose average diameter is about 22 cm. Its weight is about 450 g and has a thickness of about

Regular Paper

2.5 cm at the center of the disc placentaire. Son role is to allow the exchange of substances in the blood of the mother and that of transport fœtus. Le components between the two partners is through different channels without ever setting contact. Ainsi, the embryo receives nutrients and oxygen and removes waste mom (carbon dioxide, urea....)[3].

These substances cross the blood-called placentaire. The membrane is an effective barrier against some pathogenic bacteria. By cons, drugs, alcohol, viruses and some parasites can pass through the blood of the fetus and cause, in some cases, malformations (developmental delay, mental retardation, physical abnormalities)[4].

All the above is for the placenta. However, postpartum evacuation makes a déchet. Il is considered a hospital waste[5] and should be destroyed[6]. It becomes a source of nuisance[7]. It is therefore necessary to treat it according to the different stages of treatment reserved for waste medical waste[8]. The placenta is a niche promoting the development of harmful pathogens to humans and other elements of the environment (air, soil, water, plant and animal).

It is in this context that this study. We will isolate, purify and identify pathogens associated with human placenta samples. The results of this activity we used basic data to predict upcoming article in the control options.

MATERIALS AND METHODS

Echantillonnage and isolation of bacteria

Prélèvement samples

A total of 12 samples of human placenta was taken from four clinics in the city of Kenitra. The sampling is carried out as recommended by the guide to good hygiene and aseptic microbiology laboratory practice.

The samples were then placed in a sterile labeled vial. The latter is immediately put in a cooler at 4 ° C and is then sent to the laboratory in an insulated container for analysis.

Preparation of samples

After vigorous homogenization of samples, we conducted a series of decimal dilutions of 10-1 to 10-6 with a sterile saline solution. From each dilution, we introduced 0.1 ml in a Petri dish containing the suitable culture medium for each type of bacteria. The dishes were incubated at 37 ° C and 44 ° C for 24 hours.

Isolement and purification

The bacteria were isolated from plates containing between 30 and 300 colonies. The well separated colonies were transplanted into a new solid culture medium. Incubation is carried out even at 37 ° C or 44 ° C for 24 hours.

The strains obtained after transplanting are transplanted a second time in a new culture medium. After five successive subcultures, the strain is presumed pure. The resulting colonies were examined macroscopically and microscopically bacteria are then characterized by Gram stain.

Conservation strains

For storage, the strains obtained are stored as needed either in an inclined tube test medium at 4°C (short term storage). Strains can also be placed in nutrient broth supplemented with glycerol for longer storage (long term storage). Conservation in this case is carried out at -20° C.

Identification bacteria

Morphological -Study

a- Macroscopic examination

It is made to note the size (diameter), pigmentation, consistency and appearance of isolated colonies.

b-Microscopic examination

The Gram staining method is most commonly used in medical bacteriology. It used to color bacteria and distinguish direct examination by their ability to bind gentian violet (Gram +) or fuchsin (Gram -). Gram stain allows us to examine the shape of the bacterial cell, the mode of cell association and character of associated Gram him (+ or -).

Biochemical -Tests

a- Catalase

Catalase is an enzyme existing in the high molecular weight all aerobic bacteria. It allows them to live in the presence of oxygen. In addition to the

cytochrome respiratory chain, there are in effect in a short aerobic bacteria chain accessory fixing hydrogen to oxygen resulting in oxygenated water (hydrogen peroxide), known for its high toxicity bacteria. Catalase allows the decomposition of hydrogen peroxide into water and oxygen according to the reaction:

2H2O2 →H2O + O2

The search for this enzyme is performed simply by contacting a colony with a drop of hydrogen peroxide (H2O2). Abundant gassing indicates the presence of catalase. Lactic acid bacteria are catalase negative[9].

b- Search oxidase

The search for this enzyme is to put in a test tube, a "Ox" disc and soak with a drop of distilled water. Then take a portion of the colony to study and spread it on the disc. After about 10 minutes a dark violet color appears on disk and then turns black: This behavior indicates that the test oxidase (+)[10]

Identification of bacteria by API 20 E and API 20 Strept galleries

Gallery API 20 E

API 20 E is a standardized system for the identification of Enterobacteriaceae and other Gram-negative bacilli not tedious, with 21 miniaturized biochemical tests.

API 20 E gallery contains 20 microtubes containing dehydrated substrates. The vials were inoculated with a bacterial suspension that restores the tests. The reactions occurred during the incubation period are reflected in changes in color or spontaneous revealed by the addition of reagents. Reading the results is done by using a mapping software [11].

Gallery 20 Strept API

API 20 Strep is a standardized system that combines 20 biochemical tests. It allows to make a diagnosis for most species of streptococci, enterococci and the most common related germs.

The API 20 Strep gallery comprises 20 microtubes containing dehydrated substrates for the detection of enzymatic activity and fermentation of sugars. Each tube was inoculated with a dense suspension of a pure culture. The reactions occurred during the incubation period are reflected in changes in color or spontaneous revealed by the addition of reagents.

Fermentation tests were inoculated with an enriched medium (containing a pH indicator) which rehydrates sugars. Fermentation of carbohydrates results in acidification, resulting in a spontaneous change in the colored indicator. Reading the results is done by using a mapping software[12].

RESULTS AND DISCUSSION

Morphological tests

a-macroscopic observation

After isolation of microorganisms found in the placental samples, we first performed a macroscopic observation based on the observation of the follow-

TABLE 1 : Morphological characteristics isolated from samples of human placenta colonies

Code of strain	size	Colony appearance	Pigmentation	consistency
B1	Small colonies (1 - 2 mm diameter)	Smooth	Greyish	Fat colonies
B2	Small colonies (1 - 2 mm diameter)	Smooth	Greyish	Fat colonies
B3	Small colonies (2 mm diameter)	Smooth irregular outline	White	Fat colonies
B4	Large colonies (5 mm diameter)	Smooth irregular outline	White	Fat colonies
B5	Large colonies (5 mm diameter)	Rough irregular outline	Greyish	Creamy colonies
B6	Large colonies (4 mm diameter)	smooth	yellowish	Creamy colonies
B7	Large colonies (5 mm diameter)	smooth	white	Creamy colonies
B8	Small colonies (2 mm diameter)	smooth	white	Creamy colonies
B9	Small colonies (1 mm diameter)	smooth	Greyish	Fat colonies
B10	Small colonies (1 mm diameter)	smooth	white	Fat colonies
B11	Small colonies (1 mm diameter)	smooth	white	Fat colonies

Regular Paper

TABLE 2 : Microscopic characteristics isolated from samples of human placenta colonies

Code of strain	Form	Gram Type
B1	bacille	-
B2	bacille	-
B3	cocci	-
B4	cocci	-
B5	cocci	-
B6	bacille	-
B7	cocci	-
B8	bacille	-
B9	bacille	-
B10	cocci	+
B11	cocci	+

TABLE 3 : Biochemical characteristics isolated from samples of human placenta colonies

Code of strain	catalase test	oxydase test
B1	+	-
B2	+	-
B3	+	+
B4	+	-
B5	+	+
B6	-	-
B7	+	-
B8	+	-
B9	+	-
B10	-	-
B11	-	-

ing characteristics: size, colony appearance, texture and pigmentation. The results are shown in TABLE 1.

b-microscopic Observation

It is based on the embodiment of the Gram stain. It gives an idea of the shape, the mode of association of bacterial cells and the type of bacteria on Gram studied. The results are shown in TABLE 2.

Biochemical tests

The isolated bacterial strains were identified by using the API gallery. The identification of strains is based on the evaluation of the biochemical characteristics on the case. The results are shown in TABLE 3.

Identification of bacteria isolated from the placenta

The results of the identification of bacteria isolated from the placenta are shown in TABLE 4. The identification tool we used is the API gallery 20 E. The latter consists of 20 microtubes ready and can achieve 21 biochemical tests. It will allow us to identify the Gram (-) of the family Enterobacteriaceae. It also identifies the Gram (-) not tedious.

From the results reported in TABLE 4, we can emphasize that the identification of the bacterium B1 profile corresponds to Escherichia coli, the bacterium B2 is gr1 Escherichia coli, the bacterium Aeromonas hydrophila B3 is Gr.2, B4 is the bacterium Citrobacter Youngae, the bacteria is Enterobacter cloacae B5, B6 is the bacterium Aeromonas hydrophila gr.1, the bacterium Enterobacter asburiae B7 is the bacterium Salmonella choleraesuis B8 is spp arizonae, the bacterium is B9 Citrobacter Youngae.

A second group of microorganisms was identified by a second gallery. It is the API 20 Strep gallery. This also includes 20 microtubes containing dehydrated substrates. The identification is based primarily on the detection of enzymatic activities, assimilation and fermentation of sugars. The results

Regular Paper

TABLE 4 : Identification of the bacteria isolated from the human placenta by the API results galleries 20 E

	Strains								
Tests	B1	B2	B3	B4	B5	B6	B7	B8	B9
ONPG	+	+	+	+	+	+	+	+	+
ADH	-	+	+	-	+	-	+	+	-
LDC	+	+	+	-	+	-	-	+	-
ODC	-	-	-	-	-	+	+	+	-
CIT	-	-	-	-	+	+	+	+	+
H2S	-	-	-	+	+	+	-	+	+
URE	-	-	-	-	-	-	-	-	-
TDA	-	-	+	-	+	-	-	-	-
IND	+	+	+	-	+	-	-	-	-
VP	-	-	-	-	-	+	-	-	-
GEL	-	-	+	-	+	-	+	+	-
GLU	+	+	+	+	+	-	+	+	+
MAN	+	+	+	+	+	+	+	+	+
INO	-	-	-	-	-	-	-	-	-
SOR	+	+	-	+	-	+	+	+	+
RHA	+	+	-	+	+	+	-	+	+
SAC	+	+	+	-	+	+	+	+	-
MEL	+	+	-	-	-	-	-	-	-
AMY	-	-	-	+	+	+	+	+	+
ARA	+	+	-	+	+	+	+	+	+
OX	-	-	+	-	+	-	-	-	-

ONPG: Ortho-nitrophenyl-βD Galactopyranosidase, ADH: Arginine deiminase, LDC: Lysine decarboxylase, ODC: ornithine decarboxylase, CIT: trisodium citrate, H2S: sodium thiosulfate, URE: urea, ADD: tryptophan deaminase, IND: indole, VP: Voges Proskauer, GEL: Gelatin, GLU: D-glucose, MAN: D-Mannitol, INO: inositol, SOR: D-sorbitol, RHA: L-rhamnose, SAC D-sucrose, MEL: D-melibiose, AMY amygdalin ARA: L-arabinose, Ox: Oxidase.

are shown in TABLE 5.

Reading the identification recorded in TABLE 5 profile, we were able to withdraw from the use of the identification software B10 is bacteria Lactococcus lactis ssp cremoris B11 and that the bacterium is one Aerococcus Viridans.

All identified strains is pathogenic species. This allows us to say that the human placenta is a source of contamination. It may be the cause of the transmission of serious diseases. People at risk will be the staff of the hospital area (midwives), responsible for the cleaning of maternity staff responsible for the collection of waste and garbage at the landfill.

The risk is even more serious than the amount of placenta extracts is important. It is therefore recommended to make awareness among the medical staff. Provide ongoing training on good hygiene practices to anyone coming into contact with this type of waste. It is also recommended to adopt an appropriate means of processing placentas.

CONCLUSION

In conclusion, we can say that the placenta is certainly a very important organ given its role throughout the period of the pregnancy of the woman. After that, the body becomes a source of hospital waste and a variety of pathogenic bacteria. These can be a great danger to human health on the one hand, and the environment in which the waste is dumped placenta other.

Pathogenic bacteria found for the first time in this scientific work have been identified step by step starting from the microscopic observation to use the complete name recognition API system. The miniaturized and standardized equipment of conventional biochemical techniques for identification, us allowed

Regular Paper

TABLE 5 : Results of identifying isolated from human placenta by the API 20 Strep bacteria galleries

	Strains	
Tests	B10	B11
VP	+	-
HIP	-	+
ESC	-	+
PYRA	-	-
αGAL	+	+
βGUR	-	-
βGAL	-	-
PAL	-	-
LAP	-	-
ADH	-	-
RIB	-	+
ARA	-	-
MAN	-	+
SOR	-	+
LAC	+	+
TRE	+	+
INU	-	-
RAF	-	-
AMD	-	+
GLYG	-	-

VP: Voges Proskauer HIP: hippuric acid, ESC: esculin PYRA: puroglutamique-β-naphthylamide acid αGAL: 6-bromo-2-naphthyl-αDgalactopyranoside, βGUR: naphthol ASBI-glucuronic acid, betagal 2-naphthyl βD-galactopyranoside, PAL: alkaline phosphatase, LAP: leucine aminopeptidase, ADH: Arginine deiminase, RIB: D-ribose, ARA: L-arabinose, MAN: D-mannitol, SOR: D-sorbitol, LAC: D-lactose, TRE D-trehalose, NUI: inulin, RAF D-raffinose, AMD starch GLYG: glycogen.

to be with pathogenic species including Escherichia coli, Salmonella spp arizonae choleraesuis, Enterobacter asburiae, Enterobacter cloacae, Citrobacter Youngae, Gr.2 Aeromonas hydrophila, Enterobacter cloacae, Lactococcus lactis ssp cremoris and Aerococcus Viridans 1 and Aeromonas hydrophila gr.1.

We can say that the placenta samples are rich in pathogenic bacteria, hence the need for hospital treatment of the waste before it is dumped in landfills.

REFERENCES

[1] D.Evain-Brion, A.Malassiné; The human placenta, Lavoisier Edition, 198 **(2010)**.

[2] V.Tsatsaris, A.Malassiné, T.Fournier, K.Handschuh, J.P.Schaaps, J.M.Foidart, D.Evain-Brion; The human placenta.

[3] www.futura-sciences.com/magazines/nature/infos/dico/d/botanique-nutriment-793.

[4] www.futura-sciences.com/magazines/matiere/infos/dico/d/chimie-alcool-640.

[5] Ann Eckout; spokesman for the AFMPS "Federal Agency for Medicines and Health Products."

[6] Philip Henry; Minister of the walloon environment, Arrested on Hospital Waste Activity June 30, **(1994)**.

[7] www.bebesetmamans.com "Born in being connected to the placenta."

[8] Patrick Leprat; "Infectious waste medical waste", National Engineering School of Limoges, Limoges University, Larpent et al., **9**, **(1990)**.

[9] Delarras Camille; Practical microbiology laboratory for analysis or sanitary control, Lavoisier Edition, 128,129,269,271 **(2007)**.

[10] API 20 E, Biomerieux, "Identification of Enterobacteriaceae and other Gram-negative bacilli System" Reference 20100/20160.

[11] API 20 Strep, Biomerieux, "Identification and Related Streptococaceae germs System" Reference 20600.