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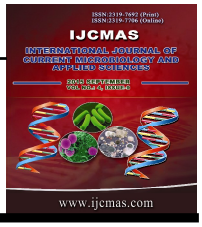

Original Research Article

Prevalence of *Escherichia coli* and Specific Pathogenic Bacteria (SPB) in the Risk of Preterm Delivery in Women with Pathological Pregnancy in Medical Biology Center of Analysis at Abass Ndao's Hospital in DAKAR (Senegal)

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Keywords

Preterm, Indeed pathogenic *Escherichia coli*, Specific pathogenic bacteria, Pregnant women, Hospital, Dakar

A B S T R A C T

From January 2011 to January 2013, we conducted a study on a sample of 809 women hospitalized for complications during pregnancy (GP) with risk of premature delivery or abortion to determine the prevalence of *Escherichia coli*, and specific pathogenic bacteria (BPS), namely *Mycoplasma* and *Chlamydia trachomatis*. We have used a methodology to three different techniques to research each seed while doing the correlation with the records of women admitted to the GP. In addition, information on age, gravidity, the history and other clinical data were obtained through the use of a questionnaire. The results we obtained reveal bacterial infections with *E. coli* and *Mycoplasma* 7.34% to 37.5%. These *Mycoplasma*, *Ureaplasma urealyticum* and *Mycoplasma hominis* were associated in 12.5% of cases and observed in isolation, each for 25% (4/16) infections. Furthermore, the prevalence of *Chlamydia* was 33.3%. Given these relatively high rates and variability of results across studies, these infections deserve the attention of public health authorities and because they endanger the health of women in pregnancy and the newborn. In addition, the asymptomatic nature of *Mycoplasma* or *Chlamydia trachomatis* should push health personnel (doctors and gynecologists) to integrate research necessarily those specific pathogenic bacteria in the balance sheet, not even once during pregnancy.

Introduction

Genital infections are becoming more prevalent in the female population, especially among women in sexual activity or pregnancy.

They can be caused by bacteria such as *Escherichia coli* and specific pathogenic bacteria (SPB) namely *Mycoplasma* and *Chlamydia trachomatis*. They are a major

women's health problem worldwide, particularly in Senegal, as they are causing enormous damage such as premature and premature birth abortion. Studies have proven that they have most often infectious causes rank first (Goldenberg *et al.*, 2000). According to Andrews *et al.* (2000), women with *Chlamydia* are at three times higher risk of premature delivery. These cases may have non-infectious causes such as premature rupture of the membrane, fall, dizziness, tiredness, cervical opening, placenta praevia, maternal hypertension. It is in this context that we were interested in the balance sheet of infections caused by *Escherichia coli* and specific pathogenic bacteria in women hospitalized pathological pregnancy (GP) (coming for antenatal clinics) at Abass NDAO hospital's in Dakar. The general purpose of our study was to assess the impact of genital infections in the risk of preterm delivery.

Materials and Methods

Equipment for sampling and analysis of vaginal secretions – specula sterile single-use disposable surgical gloves, lamp, a gynecological examination table with a sheet of disposable chair collection if necessary; swabs sterile, sterile, Seeder, rack packs; hemolysis tubes and test tubes, gas lighters or lighter; blades and sterile plates, Bunsen burner, saline, sterile pipettes Pastors, optical microscope, sterile dry tubes, immersion oil, records of benches (name, date of sampling, results ...), plastic pipette Disposable Tips, pencil or felt, aluminum foil, oven, Backgrounds cultures (Sabouraud, Chapman, cooked blood agar (GSC) or chocolate agar (MHSC) and eosin methylene blue (EMB) Muller Hinton (MH), mini-gallery: Urea, Indole, Kligler Hajna (KH), Mannitol-mobility nitrate, citrate Simmons (CS) and the CLED medium used only for urinalysis. According bacteria sought, the culture media are specific for *Neisseria gonorrhoeae*, *Mycoplasma.* For *Chlamydia trachomatis*, culture is performed on cellular environments. The agents used for Gram dyes – gentian violet, iodine solution, alcohol at 95°C and fuchsin; reagents for additional tests to identify bacteria – hydrogen peroxide to catalase, oxidase discs, D-Galactosidase or orthonitrophényl ONPG, Erlich's reagent Kovacs Indole-... are the reagents used for the identification of *Mycoplasma* and *Chlamydia trachomatis.*

To collect and study the urinary secretions: Vial, 10ml pipette tips yellow (about 200µl), saline and centrifuge were used.

Method

The terms of the review: Abstinence at least 24 hours, no personal hygiene, no antibiotics or observe a therapeutic window than 5 days before the removal and off rules.

Sampling

On the first day: The vaginal swab is done with two sterile swabs: at the ectocervix and the endocervix by putting the patient in the lithotomy position while noting the organoleptic characteristics (odor, abundance and color).

Microscopic examination

State fees: This review provides a hand to the quantitative cytology: that is to say, count each type of germ, and secondly to the qualitative cytology: that is to say the note presence of all the elements seen (epithelial cells, especially red blood cells and leukocytes as the absence of leukocytes from the first day does not allow us to continue the work in the case of a vaginal swab (PV) if the patient is not treated with antibiotics, the "Clue-Cells", yeasts, hyphae and possibly *Trichomonas*). Another

preparation is done directly on the blade with endocervical swab which is used for Gram staining.

Gram staining: The preparation is dried, attached to the burner flame and then stained with Gram. After staining, the reading is done under a microscope at a magnification of x100. This examination after staining is used to classify the bacterial flora in type I, II, III and VI, the diagnosis of bacterial vaginosis *Gardnerella vaginalis* by highlighting the "clue cells".

Culture: All vaginal swabs were seeded onto the following media: Sabouraud if there yeast fresh, Chapman if Gram +ve cocci, and eosin methylene blue to confirm the presence of *Enterobacteria*.

For *Chlamydia*: it is a test cassette, the presence of two red bands indicates a positive test while only one band (control) indicates a negative test. By against *Mycoplasma*, research is done using a test well (ten wells for a single search) that can detect the presence of *Mycoplasma hominis* and *Ureaplasma urealyticum* or both simultaneously. The advantage of this test is that it is associated with susceptibility. So we have the germ in question and sensitive antibiotics, resistant or intermediate. After inoculation, the culture media were incubated in an incubator at 37 ° C for 24 hours.

On the second day: The Wanted germs are isolated and identified. In culture dishes where absence of growth, all the boxes will be discarded except Sabouraud medium to be presented to the oven for another 24 hours. By pushing against where with few leukocytes detected in the fresh state, there is no infection and bacteriological search stops if the clinic supports. Otherwise, there is infection. Colonies found in the boxes will be Gram stained and then examined microscopically to determine the type of germ in question (coccis or bacilli) and then re-isolate on Mueller Hinton medium for pure strains. The reading will be the third day.

On the third day: After staining and observation of the germ in question if they are Gram-negative bacilli (B-), we put a small door in the mini-galleries with specific cultures (CS, KH, MM; Urea Indole). If CS, H2S, TDA, VP and Urea are negative (CS -; Urea -, H2S -, TDA -, VP -) and Mannitol, Mobility, glucose, lactose, ONPG, Indole are positive (Man+, Mob + Glu +, Lact +, ONPG+ and IND+), we say that this is *E. coli*.

In the case of gram-positive cocci (C +) we catalase by hydrogen peroxide and suspension of the colony, positive, driving a Staph latex test which gives positive *Staphylococcus aureus* and *Staphylococcus* spp to negativity. If catalase is negative, *Streptococcus* grouping (A, B, C, D, F, G) is carried out; susceptibility testing will be carried out after this step.

The culture of ECBU requires two successive days: On the first day, we need 5ml of whole urine in a haemolysis tube after eliminating the first streams. Then centrifuged bed fresh for note the number of white blood cell and epithelial cells per field prior to a dilution of 1/10000 of urine that is to say to 10 ul of urine in 1ml of sterile physiological saline and inoculate 10 ul of dilution on the CLED medium to be incubated for 24 hours at a temperature of 37° C; playback will be at the second day. On the second day, the observation of the CLED medium has 2 Case possibilities: If there is growth, it is necessary to count the number of colonies (> 10) associated with the number of leukocytes (> 5 leukocytes per field), it is concluded that it is a urinary tract infection; If there is no growth and / or

leukocyte count is less than 5 per field, There are absence of urinary tract infection.

Secondly, to follow the evolution of these pathological pregnancy in women having a comparative approach at the hospital registers. Furthermore, we sought to determine the prevalence of those at risk of abortion or premature birth, whether the targeted bacteria, other germs or having a non-infectious cause.

Results and Discussion

Our study population consists of 809 women hospitalized for complications during pregnancy (GP) Gynecology at Abass NDAO the hospital between January 2011 and December 2013. Ages ranging from 15 to 49 are divided into age groups of 10 years. The age of some patients has not yet been mentioned. Table 1 shows that the study population consists mostly of women aged 20–49 years who are of childbearing age. Women with 20–29 years and 30–39 years are the most numerous with respective percentages of 34.36% and 18.17%. The table II shows that among the 809 patients, reasons for consultation were most often: the threat of premature birth (31.78%), premature rupture of the membrane (14.5%) and pregnancy-induced vomiting (10.06%). The table 3 shows that among hospitalized patients, women most prone to this threat are paucipares having at least four children with 37.57% followed by those whose status does has not been specified (36.7%) and those who have never had children with 21.36%. In our study (Table 4), the Paucigeste are most exposed with 34.49% followed by primigravidae (20.77%). It shows a very high proportion of those whose status is not clear (36.71%). According to the age, among the 809 women hospitalized GP, we had 109 research requests from *E. coli*; 16 applications of *Mycoplasma* and *Chlamydia* of 12 applications. In the case of *E. coli*, only 8 women had a positive test. Women 20–29 are more infection with a rate of 50% *E. coli*. The women of 15–19 years and 30–39 represent the same percentage (25%). These three age groups in full genital activity are infected. No cases were recorded between 40 and 49 (Table 5). In the case of *Mycoplasma*, we have 6 positive tests. Always women aged 20–29 are more infection with a rate of 50% (Table 6). For Chlamydia, only 4 cases are always positive with the edge 20–29 which represents the majority 50% (Table 7). The prevalence of infections due to targeted germs: *E. coli*: 7.34% (8 positive cases / 109) (Figure 1); *Chlamydia trachomatis*: 33.3% (four positive cases / 12) (Figure 2) and *Mycoplasma* prevalence is 37.5% (6 positive cases / 16). The prevalence of infection *Ureaplasma urealyticum* alone is the same as that of *Mycoplasma hominis* alone: 25% (4 positive / 16). Both germs were associated in equal prevalence of 12.5% (Table 8).

For *E. coli*, our results differ from those Giullane *et al.* (2003) reported a lower value than ours 0.5% (1 in 212 women) of women in premature risk or premature rupture of the membrane. They are also contrary to those of Scharg et al in 2006 with a very high value of 49% (64 premature sur132) were born before 33 weeks as well as a newspaper of the Tunisian society of Medical Science published in 2008, *E. coli* was the predominant germ in preterm found with a prevalence of 38.5%.

This difference in prevalence in these studies could be explained by variability on sample sizes, different sensitization on the pathogenesis of this bacterium. Failure further investigations especially in the case of our study because among women hospitalized in GP, there is a small part that makes this examination the rest is either not requested by the midwife or control is

requested but not made by means of faults (poverty) or it is the lab that has no reagents. These reviews should be mandatory for pregnant women, they suffer from an immune defense caused not only by the high rate of hormones but also venous stasis (urine incompletely).

For *Chlamydia trachomatis*, our results are similar to those of Rours *et al.* (2011a) with a prevalence of 25% (76/304), detected by PCR in placental tissues of women ≤ 32 weeks. However, our results are contradictory to those of a lot of studies with lower frequencies, the article published in Brazil in 2008, *Chlamydia* trachomatis infection during pregnancy was 4.8% (Silveira *et al.*, 2009). This same prevalence was found in the United States on women aged 18–26 years (Miller *et al.*, 2004).

Our results are always adverse to those of another study by Kataoka and al in 2006 (Kataoka *et al.*, 2006), the prevalence of *Chlamydia trachomatis* was 3.2%; as well as the article on infectious diseases Rours *et al.* (2010a) 35 and SA (7.4%) were significantly <SA preterm before 32 (14.9%) associated with *Chlamydia trachomatis*; another study Rours *et al.* (2011b) in another article, *Chlamydia trachomatis* was detected by PCR in placental tissues of women ≤ 32 weeks with a prevalence of 25% (Jane Hitti *et al.*, 2010).

These results differ from those of Croatian study in 2004 (Matovina *et al.*, 2004) on miscarriages between 4 and 19 SA, *Chlamydia trachomatis* prevalence was 0.9% in placental tissues examined; those of a study conducted in South Africa in 2006 (Odendaal and Schoeman, 2006), relative prevalence of *Chlamydia* was 2.20%. They are contradictory to still another study done in the Netherlands in 2011 (Rours *et al.*, 2011b), *Chlamydia trachomatis* had a prevalence of 4% for women who have recently given birth prematurely, detected by the test method instead of urine released or other placentas.

This disparity may be several explanations by variability in the study population; an increase of this plague misunderstood by its asymptomatic nature because many women live without knowing especially women in pregnancy that have acid flora in favor of pathogenic bacteria. It could be due to a non - sexual abstinence or multiple partners.

For *Mycoplasma*, our results are similar to those of an article by Taylor-Robinson Lamont in 2010, *M. hominis* had a prevalence of 24% and those found in Czech in 1985 (Kapatais-Zoumbos *et al.*, 1985) on 225 women with premature rupture membranes, 28% were colonized with *M. hominis* but differ to those obtained *U. urealyticum* 68% were positive. Our results are contrary to those of a study done at Brussels in 2009 (Lee *et al.*, 2009), premature births are 53.6% with colonization by *U. urealyticu*m; so those of another study in 1988 (Ndiaye *et al.*, 1988), *U. urealyticum* was present in 96%, but it was found that in 32% of women who do not have rupture of the membrane.

They also deviate from a study done in Japan in 2010 (Mc Gowin *et al.*, 2010) on the culture of placentas from pregnancies with preterm birth less than 32 weeks, the prevalence *Ureaplasma* is 83%. In addition to the different sensitivities and specificities adopted in the various study centers, which is likely to give variable results, the degree of risk of sexual exposure on maternal health could thus affect this diversity.

Table.1 Study population by age

Years	Number	Percentage (%)
15-19	**43**	**5,30**
20 -29	**278**	**34,36**
30 -39	**147**	**18,17**
40 -49	**18**	**2,25**
Not specified	**323**	**39,92**
Total	**809**	**100**

Table.2 Study population by reason for consultation

Admission Reason	Number	Percentage (%)
Pre eclampsia	70	7,9
Preterm labor (PL)	**281**	**31,78**
Malaria syndrome	49	5,5
Infectious syndrome	32	3,6
Scarred uterus	3	0,3
Premature rupture or fluid flow (trace amnion)	**129**	**14,5**
Vomiting of pregnancy	**89**	**10,06**
Metrorrhagia	49	5,5
Severe anemia	9	1,01
Dead egg retention	47	5,3
Hyper blood pressure (hypertension)	15	1,69
Threatened abortion	54	6,1
Large ovarian cyst or myoma	13	1,47
Decidual hematoma	4	0,45
Toxemia of pregnancy	3	0,3
Hypertensive heart disease	5	0,56
Drop	1	0,1
Cervical opening	4	0,45
Dizziness	2	0,2
Placenta previa	15	1,6
Fetal tachycardia	2	0,2
Insulin-dependent diabetes (IDDM)	8	0,9
Total	884	

Table.3 Study population according to gender

Parity	Number	Percentage (%)
Nulliparous	**172**	**21,3**
Pauciparous	**304**	**37,6**
Multipare	36	4,4
Not specified	**297**	**36,7**
Total	**809**	**100**

Table.4 Study population according to gestity

Gesture	Number	Percentage (%)
Gravida	168	20,77
Paucigravida	279	34,49
Multigravida	65	8,03
Not specified	297	36,71
Total	809	100

Table.5 Distribution of infection with *E. coli* results by age

Age (Years)	**Infection to *E. coli* (+)**		**Infection to *E. coli* (–)**	
	Number	**Percentage (%)**	**Number**	**Percentage (%)**
15-19	2	25	6	5,9
20-29	4	50	37	36,6
30-39	2	25	25	24,75
40-49	0	0	16	15,8
Not specified	0	0	16	15,8
Total	8	100	101	100

Table.6 Distribution of results to *Mycoplasma* infection by age

Age (year)	Infection to *Mycoplasma* (+)		Infection to *Mycoplasma* (–)	
	Number	Percentage (%)	Number	Percentage (%)
15-19	0	0	1	10
20-29	3	50	3	30
30-39	2	33,3	4	40
40-49	1	16,7	1	10
Not specified	0	0	1	10
Total	**6**	100	**10**	**100**

Table.7 Distribution of *Chlamydia* infection results by age

Age	Infection to *Chlamydia trachomatis* (+)		Infection to *Chlamydia trachomatis* (–)	
	Number	Percentage	Number	Percentage
15-19	0	0	0	0
20-29	2	50	3	37,5
30-39	1	25	4	50
40-49	1	25	1	12,5
Total	4	100	8	100

Table.8 *Mycoplasma* results

Number	*Mycoplasma* result			
16	Negative		Positive	
	Number	(%)	Number	(%)
	10	62,5	**6**	**37,5**

Figure.1 *E. coli* result

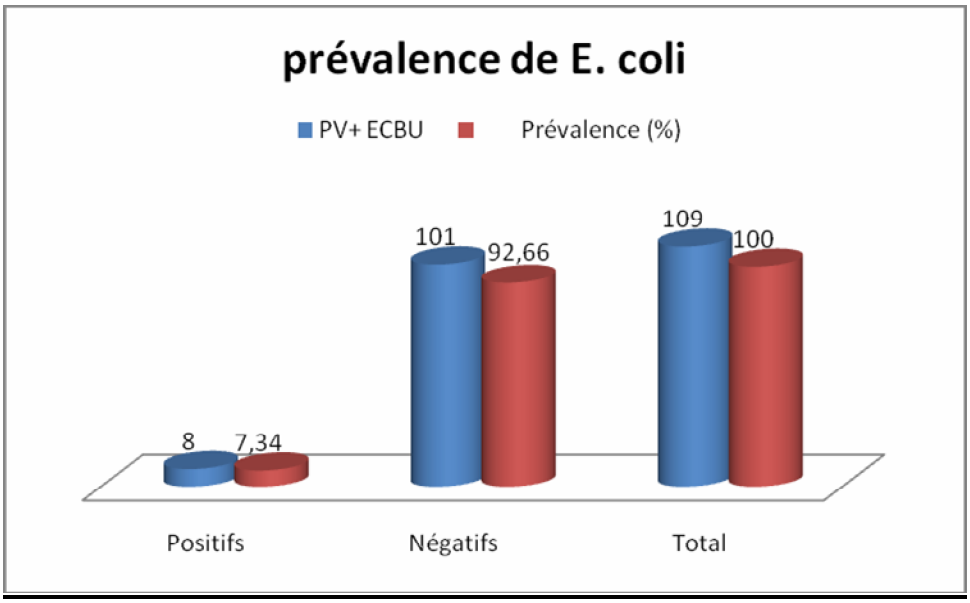
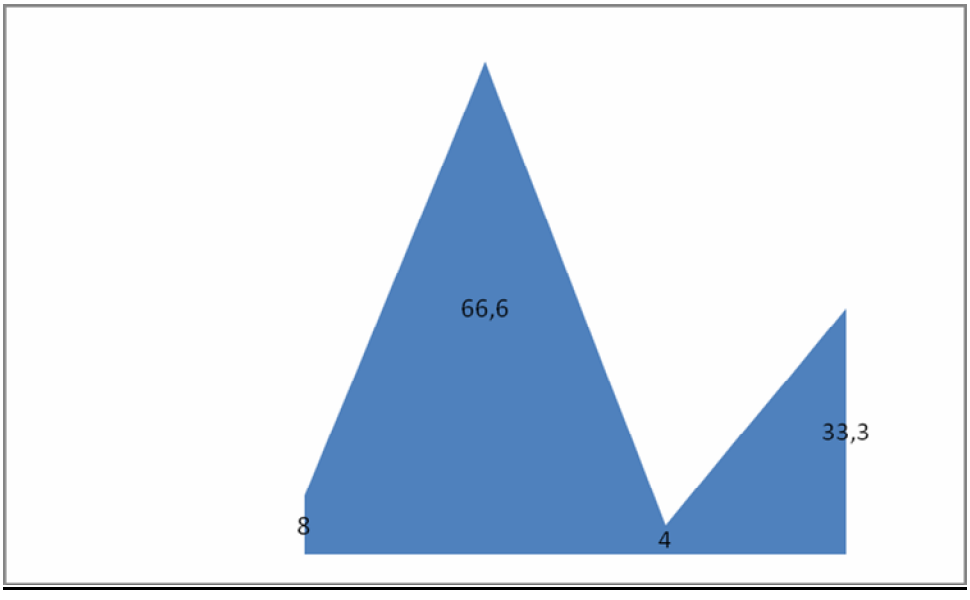


Figure.2 Result of *Chlamydia*



From the perspective of age, we have found, in our study, that among patients hospitalized GP presenting risks (infectious or not), age ranges were between 20–29 years and 30–39 years, the most frequent were between 20 and 29 years for all women infected. What to suggest that these agents are more common in women of reproductive right. Others had an age range 13–19 years. This reveals how early adolescent pregnancy. Thus according to our study, we can say that the most risky are those women in advanced reproductive state. However we have not made the correlation of parity of our patients who have complementary and our targeted reviews of bacteria even other clinical factors such as gestity, the appearance of the cervix, reasons for consultation, the appearance of losses and others.

In conclusion, the results suggest that the threat of premature birth and abortions remain a major concern in women's health. Indeed the majority of cases are non-infectious causes so unexpected (HTA, Fall, dizziness, oligohydramnios ...) but they can be infectious. This is the case of the targeted bacteria in this study: *E. coli* and specific pathogenic bacteria. The latter occupy a very important place in these conditions and especially the SPB as *Mycoplasma*: 37.5%, *Chlamydia:* 33.3% and *E. coli*: 7.34%. Compared to age, this scourge is more common in women full reproductive status of an age of 20-29ans. At last of our study, it is important to know from our targeted bacteria to be able to support it in order to reduce this scourge.

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