Assessment the Activity of Some Enzymes and Antibiotic Substances Sensitivity on Pathogenic Bacteria Species

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Microbial and opportunistic pathogens cause serious problem to human host by releasing different compounds which are involved in colonization, invasion or in alteration of immune process. In this study we propose ourselves to analyze the virulence profile of microorganisms involved in urinary tract infections and bacterial vaginosis in order to compare the virulence mechanisms developed by these microorganisms. We assessed the virulence profile (motility, adhesion to HeLa cell line, biofilm formation, production of enzymes, antibiotic susceptibility) using phenotypic methods. The microbial strains isolated from urinary tract infections present a high ability to release amylases, caseinase, siderophore-like, half of them have present high motility and also were highly resistant to antibiotics, while the microbial strains isolated from bacterial vaginosis present a high ability to bind human epithelial cells and to release hemolyzin and DN-ase.

Keywords: urinary tract infections; bacterial vaginosis; virulence factors; Streptococcus; E. coli

Urinary tract infections (UTI) are the second most common type of human infections, and the most frequent type of healthcare-associated infection. The most often encountered etiology of UTI are represented by *Escherichia (E.) coli,* followed by *Staphylococcus (S.)* sp., *Klebsiella (K.)* sp. and *Enterobacter (E.)* sp. strains [1, 2]. Bacterial vaginosis (BV) usually occurs as a result of the replacement of normal vaginal microbiota (rich in *Lactobacillus* sp.) usually with anaerobic bacteria (e.g., *Ureaplasma, Mycoplasma, Prevotella, Gardnerella* sp. or uncultivable anaerobes). Some women experience transient vaginal microbial changes and most of BV were asymptomatic [3, 4]. Vulvovaginal infections are often caused by opportunistic species *Candida albicans* and use of nonsteroidal anti-inflammatory drugs can amplified the effect of antifungal drugs [5, 6]. *Streptococcus (S.) agalactiae* known also as group B Streptococcus (GBS) is a microorganism that often lives in the vagina of normally healthy women. GBS is one of the most common bacterial causes of life-threatening infection in newborns and can cause neonatal sepsis, meningitis, pneumonia, premature delivery. In 2002, the Centers for Disease Control and Prevention recommended screening of pregnant women in order to decrease the incidence of sepsis caused by GBS [7-9].

UTI and BV are the most common type of infection in women. It has been observed that the women suffering from bacterial vaginosis are at a greater risk of urinary tract infections. Therefore, the periodic evaluation of the antimicrobial susceptibility and virulence factors of these microorganisms is important to understand the evolution from commensalism to pathogenicity and for developing new strategies to control endogenous infections [10, 11].

The purpose of the present study was to comparatively evaluate the virulence potential of microbial strains isolated from symptomatic urinary tract and genital infections in women, by assessing: the adherence to cellular substratum, motility, biofilm formation, secretion of hydrolytic enzymes, hemolytic activity, production of Fe^{3+} chelating agents and their antibiotic resistance profiles [12].

Experimental part

Samples and growth conditions

For this study there were analyzed a number of 34 bacterial strains isolated from urine sample (14) and vaginal swabs (20) taken from patients with symptomatic urogenital infections (in 2010 from female patients in Bucharest). All bacterial strains were cryopreserved and stored in the microbial culture collection of the Faculty of Biology, University of Bucharest.

Strains identification. The strains were identified according to their morphological and biochemical characteristic by Biolog Microbial Identification System (Biolog System, USA(using GP/GN MicroPlates. Strains were compared with four control strains, i.e.: *E. faecalis* ATCC 29212, *S. aureus* ATCC 6538 and *E. coli* ATCC 10536.

Motility determination. For motility determination 5µL of overnight bacterial suspensions (10^8 cells /mL), re-suspended in phosphate buffered saline (PBS) were inoculated into semisolid Muller-Hinton (MH) broth containing 0.4% agar (v/v) and the plates were incubated for 24h at 37°C. After incubation the motility was determined by measuring the colony diameter. The assay was carried out in triplicate.

HeLa cell adhesion assay. HeLa cells were grown until they reached 70% confluence in 6-well plates using Dulbecco's modified Eagle medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) at 37 ^{0}C, in a humidified atmosphere with 5% CO_2. The bacterial strains were grown in BHI broth for 20h at 37^{0}C, then were centrifuged for 10 min at 10.000 rpm and the pellet was re-suspended in PBS to a final density of 10^8cells/ml, 500 µL of microbial strains suspension were used in order to infect the HeLa cells for 2 h at 37^{0}C. Bacterial adhesion was

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detected using optic microscopy by counting the number of bacterial cells attached to the epithelial cells in five randomized microscopic fields [13].

Biofilm development on the inert substratum. Biofilm formation assay was performed using the microtiter assay. Cell pellet obtained after centrifugation was washed twice with PBS and 200 µL of cell suspension (10^5 cells/mL) were added in 96 wells polystyrene plates and incubated for 48h at 37^0C. After incubation the wells were washed three times with PBS in order to remove the non-adherent cells and fixed with 95% methanol. After 5 min, 250 µL of crystal violet solution (2mg/mL) were added in each well and incubated for 15 min at room temperature. After staining wells were washed with PBS four times and re-suspended with 240 µL of 33% acetic acid. The biofilm formation was evaluated by measuring the absorbance of stained suspension at wavelength 490 nm. Assays were carried out in triplicate and were repeated three times [13].

Soluble virulence factor assays. For the production of enzymes implicated in virulence, 10µL of overnight bacterial suspension (10^8 cells/ mL), re-suspended in PBS were inoculated onto the surface of culture media with different specific substrates. Enzyme activity was determined according to previously published data [13]. Ratio values exceeding 1 indicated the presence of the investigated enzymatic activity.

Detection of caseinase was performed by growing bacteria strains on Luria-Bertani (LB) broth supplemented with milk casein (10%). The positive reaction consisted in the appearance of a white precipitate around the colony.

Hemolysin activity was evaluated using MH medium supplemented with 5% defibrinated sheep blood. For hemolytic activity the plates were incubated for 24h at 37°C. The positive reaction consisted in the appearance of clear area around the culture spot.

Production of extracellular dezoxyribonuclease was quantified on DN-ase test agar (Difco, USA) supplemented with 0.01% toluidine blue solution (v/v). The DNA hydrolysis was indicated by the occurrence of a pink area around the culture spot.

Detection of amylase was performed by growing bacterial strains on LB plates supplemented with 10% sterile starch. After incubation the plates were stained with Lugol solution. The amylase activity was corresponding to the colorless area around the culture spot.

Production of siderophore-like was analyzed by growing the bacterial strains on Bile Esculin Agar (BEA). The plates were evaluated at 24h after incubation, the positive reaction being observed as a black pigmentation around the culture spot.

Antibiotic susceptibility test. Drug susceptibility was determined using the disk diffusion method, according to CLSI 2011 recommendation. Antibiotics disks were purchased from BioMerieux (Bio-discs, France). As a control for antibiotics activity *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 were used in this experiment.

Results and discussions

Strains identification

The Biolog System used in this study for the identification of bacteria, based on biochemical tests was able to identify all strains with a similitude coefficient of over 76%. The strains isolated from UTI were identified as *E. coli* (12 strains), *K. oxytoca* (1) and *E. aerogenes* (1), and in vaginal infections were identified as *Streptococcus* (*S.*) *agalactiae* (13 strains), *Micrococcus* (*M.*) *luteus* (4) and one strain of *S. xylosus, S. bovis* and *E. faecalis.*

Motility determination

The ability to be motile represent an advantage for bacterial strains involved in UTI, it enables them to easily progress towards the upper urinary tract and reach to the kidneys, thus making the infection more difficult to treat. The microbial strains exhibiting a bacillary morphology isolated from UTI were analyzed in order to determine their motility. The motility was specific to each strain, five of *E. coli* strains being highly motile, while the motility of the rest of the *E. coli, K. oxytoca* B27 and *E. aerogenes* B35 strains was poor (figs. 1 and 2).

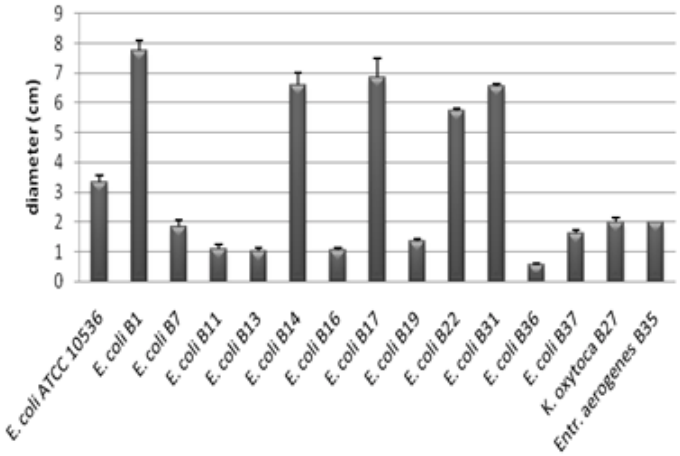

Fig. 1. Motility determination of the Gram negative bacilli strains

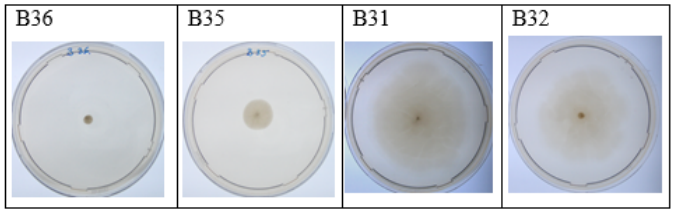

Fig. 2. Aspect of motility plates of different strains isolated from UTI

Biofilm formation

The ability of microbial strains to adhere and to further develop biofilms on different tissues or implanted medical devices makes them more resistant to antimicrobial therapy [14]. Among the Gram negative strains isolated from UTI, *K. oxytoca* B27 exhibited the highest capacity to develop biofilm, while the other strains showed a poor adhesion capacity to the polystyrene substratum (fig.3). Concerning the strains isolated from the genital swabs, *S. xylosus* B32 and *E. faecalis* B20 exhibited a high ability to adhere to polystyrene, as compared to *Micrococcus* strains who presented weak adhesion ability (fig. 4).

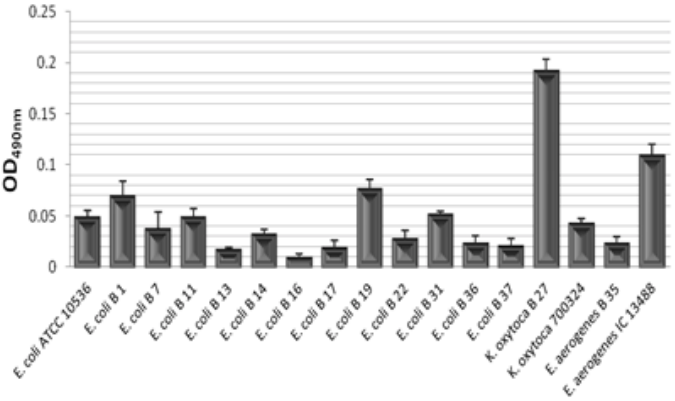

Fig. 3. Quantification of the 48h biofilm developed by the Gram negative strains isolated from UTI

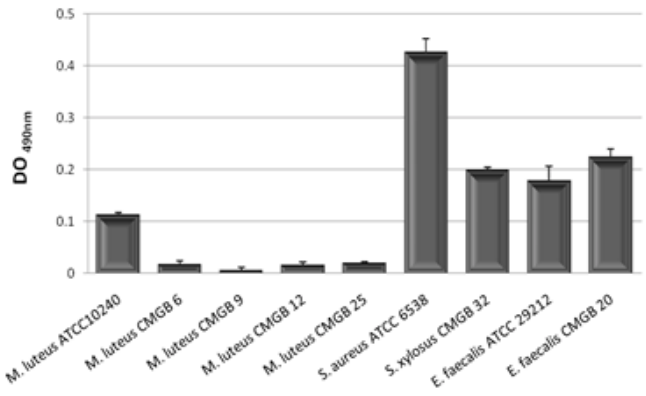

Fig. 4. Quantification of the 48h biofilm developed by the Gram positive strains isolated from vaginal infections

HeLa cell adhesion assay

Data regarding the ability of pathogenic strains to attach to human cervical carcinoma cell line HeLa are presented in table 1. Overall, the microbial strains isolated from vaginal infections exhibited a higher capacity to colonize the cellular substratum than those isolated from UTI (the average 16.4 to 8.5). The highest capacity of adherence to vaginal cell line was observed for *Micrococcus* strains followed by *Enterococcus* and *Staphylococcus* strains. For *Enterobacteriaceae* strains the number of adherent cell was relatively low comparing with the other species, excepting a few *E. coli* strains, i.e. B11, B13 and B17 which presented an average of adherent cells over 10 cells (table 1). Microscopic examination allowed not only to quantify adherent cells, but also to analyze the adhesion pattern inside each group of microorganisms. The *Micrococcus*

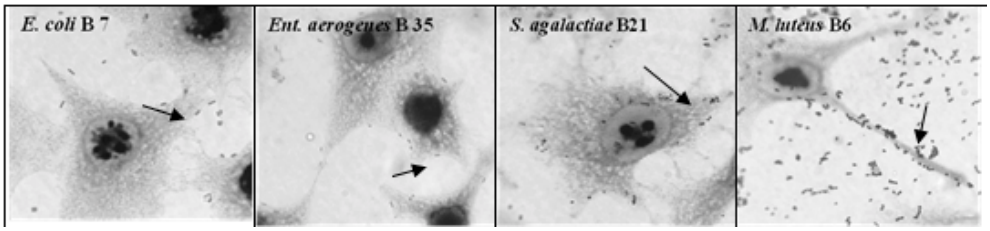

Fig. 5. Optic microscopy images showing the adherence patterns of different bacterial strains to HeLa cell line (Giemsa staining, x 100) from left to right: diffuse, diffuse localized

Table 1
BACTERIAL ADHERENCE ONTO EPITHELIAL CELLS

	Bacterial strains	No. adherent cells	Std. dev.	*Bacterial strains*	No. adherent cells	Std. dev.
Vaginal infections	*S. agalactiae B2*	18.8	2.9	*S. agalactiae B29*	2.4	1.3
	S. agalactiae B3	10.6	2.7	*S. agalactiae B30*	5.2	1.9
	S. agalactiae B4	14.2	1.9	*S. agalactiae B34*	18.0	2.2
	S. bovis B5	9.0	2.7	*E. faecalis* ATCC 29212	39.4	5.1
	S. agalactiae B8	9.6	2.3	*E. faecalis* B20	26.6	3.7
	S. agalactiae B10	1.4	1.7	*S. aureus* ATCC 6538	44.8	5.5
	S. agalactiae B21	20.6	2.7	*S. xylosus* B32	19.8	2.8
	S. agalactiae B23	5.8	1.9	*M. luteus* B6	63.0	5.9
	S. agalactiae B24	1.0	0.7	*M. luteus* B9	39.2	3.0
	S. agalactiae B26	2.0	1.6	*M. luteus* B12	31.4	3.2
	S. agalactiae B28	2.2	1.9	*M. luteus* B25	27.0	3.2
UTI	*E. coli* ATCC 10536	9.4	2.5	*E. coli* B19	7.6	2.4
	E. coli B1	2.6	1.1	*E. coli* B22	7.0	2.4
	E. coli B7	1.6	1.1	*E. coli* B31	1.8	1.5
	E. coli B11	20.4	3.7	*E. coli* B36	5.4	1.8
	E. coli B13	12.2	2.6	*E. coli* B37	16.2	3.4
	E. coli B14	4.8	1.5	*K. oxytoca* B27	4.0	1.6
	E. coli B16	9.6	3.0	*E. aerogenes* B35	4.6	1.8
	E. coli B17	12.0	2.9			

sp., *Staphylococcus* sp. and *Enterococcus* sp. strains presented a localized adherence pattern in which aggregated bacteria are attached to the cellular or inert support in a stacked-brick arrangement, while *Streptococcus* and *Enterobacteriaceae* group exhibited a diffuse pattern in which bacteria adhered uniformly to the whole cell surface (fig.5).

Soluble virulence factors assay

Many virulence factors are involved in the ability of bacterial pathogens to cause disease, one of them being the ability to release enzymes/ compounds [15]. Hemolytic activity is considered an important virulent factor, which is frequently evaluated in pathogenic strains. This activity was highlighted for all *Streptococcus* and *Staphylococcus* strains while for the rest of the strains the presence of hemolysins were not detected (table 2**).** Phospholipases

Table 2

THE PRODUCTION OF ENZYMES / COMPOUNDS INVOLVED IN VIRULENCE OF PATHOGENIC STRAINS

	Bacterial strains	Hemolysine		Siderophore-like		DN-ase	Caseinase		Amylase	
		Pz	Std. dev.	Pz	Std. dev.		Pz	Std. dev.	Pz	Std. dev.
Vaginal infections	*S. agalactiae B2*	1.21	0.03	1.00	-	+	1.45	0.02	1.00	-
	S. agalactiae B3	1.31	0.07	1.00	-	-	1.00	-	1.00	-
	S. agalactiae B4	1.24	0.04	1.00	-	+	1.39	0.04	1.00	-
	S. bovis B5	1.22	0.04	1.00	-	-	1.43	0.03	1.00	-
	S. agalactiae B8	1.30	0.02	1.00	-	+	1.00	-	1.00	-
	S. agalactiae B10	1.28	0.06	1.00	-	+	1.37	0.01	1.00	-
	S. agalactiae B21	1.31	0.05	2.00	0.03	+	1.40	0.02	1.00	-
	S. agalactiae B23	1.27	0.02	1.00	-	+	1.00	-	1.00	-
	S. agalactiae B24	1.48	0.01	1.00	-	-	1.00	-	1.00	-
	S. agalactiae B26	1.32	0.03	1.00	-	-	1.00	-	1.00	-
	S. agalactiae B28	1.26	0.02	1.00	-	+	1.44	0.06	1.00	-
	S. agalactiae B29	1.16	0.02	1.00	-	-	1.00	-	1.00	-
	S. agalactiae B30	1.16	0.05	1.00	-	+	1.00	-	1.00	-
	S. agalactiae B34	1.41	0.03	1.00	-	-	1.20	0.01	1.00	-
	E. faecalis ATCC 29212	1.00	-	2.69	0.02	-	1.10	0.01	1.44	0.03
	E. faecalis B20	1.00	-	2.50	0.09	-	2.05	0.07	1.38	0.04
	S. aureus ATCC 6538	2.37	0.01	1.00	-	-	1.55	0.08	1.16	0.08
	S. xylosus B32	1.62	0.02	1.00	-	+	1.25	0.05	2.50	0.05
	M. luteus B6	1.00	-	1.72	0.13	-	1.65	0.02	1.59	0.01
	M. luteus B9	1.00	-	1.00	-	-	1.51	0.01	1.74	0.03
	M. luteus B12	1.00	-	1.19	0.03	-	1.73	0.05	1.68	0.02
	M. luteus B25	1.00	-	1.40	0.06	-	1.50	0.04	1.79	0.01

	E. coli ATCC 10536	1.00	-	1.00	-	-	1.74	0.05	2.18	0.06
	E. coli B1	1.00	-	2.49	0.18	-	1.80	0.06	2.88	0.04
	E. coli B7	1.00	-	1.00	-	-	1.36	0.01	1.70	0.02
	E. coli B11	1.00	-	2.46	0.17	-	1.36	0.10	1.70	0.01
	E. coli B13	1.00	-	1.15	0.05	-	1.53	0.05	2.00	0.05
	E. coli B14	1.00	-	1.52	0.03	-	1.91	0.01	1.69	0.20
	E. coli B16	1.00	-	1.73	0.11	-	1.64	0.05	1.75	0.05
	E. coli B17	1.00	-	1.40	0.15	-	1.73	0.05	1.90	0.07
	E. coli B19	1.00	-	1.74	0.09	-	1.84	0.02	2.00	0.01
	E. coli B22	1.00	-	2.23	0.12	-	2.08	0.02	2.03	0.12
	E. coli B31	1.00	-	1.21	0.03	-	2.00	0.05	1.78	0.05
	E. coli B36	1.00	-	1.00	-	-	1.00	-	2.35	0.21
	E. coli B37	1.00	-	1.00	-	-	1.66	0.03	1.92	0.01
	K. oxytoca B27	1.00	-	2.30	0.01	-	1.45	0.04	1.07	0.02
	E. aerogenes B35	1.00	-	2.60	0.05	-	1.61	0.08	1.60	0.01

(Pz- protease zone; values > 1.0 and + means positive reaction; value 1.00 and -means no activity)

and lipase are involved in the invasion process, by their ability to disrupt cellular membranes. These two enzymes were produced only by *S. xylosus* B32 (data not presented). The ability of pathogenic microorganisms to acquire iron from the environment during infection is another very important virulence factor. One of the mechanisms involved in iron obtaining by pathogenic microorganisms is represented by the production of specific ligands called siderophores which are chelating iron from host or from environment [14]. All Gram negative strains excepting *E. coli* B7 produced siderophore-*like* compounds. Regarding the Gram positive strains only strains belonging to *Microococcus* and *Enterococcus* genera were able to synthesize siderophore-*like* compounds. The presence of extracellular DN-ase activity helps pathogenic strains to fight against neutrophil extracellular traps produced by leukocytes which are composed from DNA and histones [15]. DN-ase activity could not be quantified like the other enzyme because of the weak colour signal around the colony. The results revealed that DN-ase was produced by half of *S. agalactiae* strains and by *S. xylosus* B32 strain. Amylase and caseinase are considered indirect virulence factors and the presence of these two enzymes confers to microbial strains the opportunity to colonize various ecological niches [16]. All Gram negative and Gram positive bacteria excepting *Streptococcus* strains included in this study presented these enzymes. Some *Sreptococcus* strains presented caseinase but neither of them do not produced amylase.

Antibiotic susceptibility profiles

The antibiotic susceptibility pattern of *Entero-bacteriaceae* strains isolated from UTI revealed the production of Extended-Spectrum β-Lactamase (ESBL) in two, *E. coli* strains, *i.e.* B11 and B36, exhibiting resistance to third and fourth generation cephalosporins. There were identified also five *E. coli* strains resistant to fluoroquinolone. Instead all isolates exhibited susceptibility to carbapenems (imipenem). Most of the *Streptococcus* strains present intermediate phenotype to penicillin. It was observed a high resistance to ampicillin and cefepime (six strains) and to fluoroquinolone (three strains). *Micrococcus* species presence resistance to: rifampin, oxacilin, penicilin and erytromycin instead all strains were sensitive to trimetoprim-sulfametoxazol. n*S. xylosus B32* and *E. faecalis B20* strains have not shown clinical resistance to any of the tested antibiotics (data not presented).

Bacterial resistance to antibiotics has become a major source of concern for public health. The emergence of resistant bacteria has accelerated in recent years, mainly as a result of increased selective pressure [17]. Antibiotics currently represent the most commonly prescribed treatment for UTI, and patient who had suffer from recurrent infection, having three or more infections a year, may be prescribed antibiotics prophylactically. This study include the most important virulent factors of bacterial, cell wall associated factors (biofilm formation, motility, adherence to HeLa cell line), soluble secreted enzymes (protease, phospholipase, lipase, DN-ase, amylase), Fe^{3+} accumulation and antibiotic resistance [12, 18].

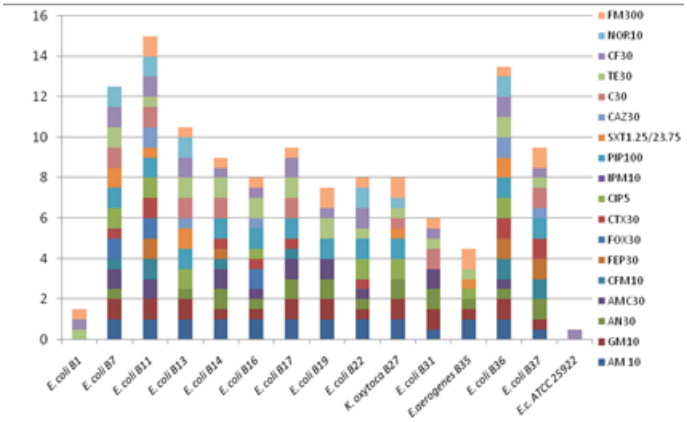

Fig. 6. Susceptibility to antibiotics of Enterobacteriaceae group isolated from UTI R-resistance; S-sensitive; I- intermediate; Ampicillin (AM10); Gentamicin (GM10); Amikacin (AN30); Amoxicillin – Clavulanic acid (AMC30); Cefuroxime (CFM10); Cefepime (FEP30); Cefoxitine (FOX30); Cefotaxime (CTX30); Ciprofloxacin (CIP5); Imipenem (IPM10); Piperacillin (PIP100); Trimethoprim/Sulfamethoxazole (SXT1.25/23.75); Ceftazidime (CAZ30); Chloramphenicol (C30); Tetracycline (TE30); Cefalotine (CF30); Norfloxacin (NOR10); Nitrofurantoin (FM300)

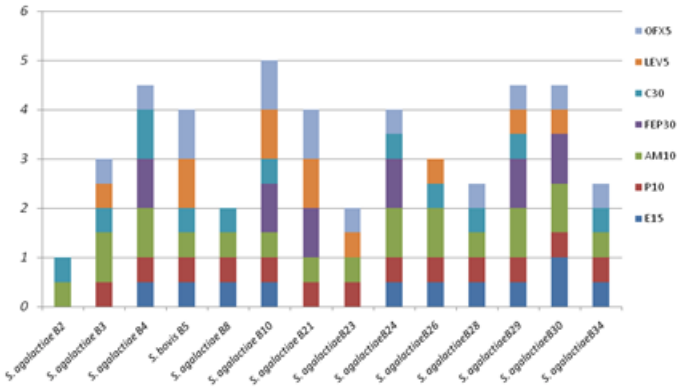

Fig. 7. Susceptibility to antibiotics of Streptococcus group R-resistance; S-sensitive; I- intermediate; Erythromycin (E15); Penicillin G (P10); Ampicillin (AM10); Cefepime (FEP30); Chloramphenicol (C30); Levofloxacin (LEV15); Ofloxacin (OFX5)

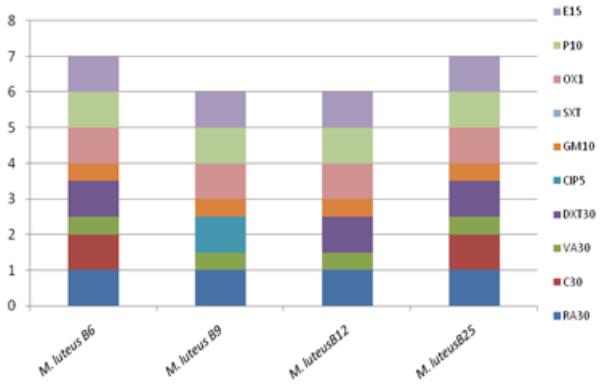

Fig. 8. Susceptibility to antibiotics of *Micrococcus* strains R-resistance; S-sensitive; I- intermediate; Rifampin (RA30); Chloramphenicol (C30); Vancomycin (VA30); Doxycicline (DXT30); Linezolid (LZD10); Ciprofloxacin (CIP5); Gentamicin (GM10); Trimethoprim/Sulfamethoxazole (SXT1.25/23.75); Oxacillin (OX 1); Penicillin G (P10); Erythromycin (E15)

It is known that flagella play an important role in virulence, it helps the cell to run away from dangerous compounds, to find more favorable environment and is also implicated in adhesion and invasion process. The motility test results revealed that half of the *E. coli* strains were highly motile probably this is one of the reason why *E. coli* continue to be the most frequent species isolated from UTI [1]. The predominant pathogen species isolated from urine sample remain *Escherichia coli* which is also responsible for asymptomatic bacteriuria and for recurrent cystitis [19]. Surveillance data show that resistance in *E. coli* is consistently highest for antimicrobial agents; a retrospective analysis of *E.coli* from urine specimens collected from patients during 1997–2007 showed an increasing resistance trend for ciprofloxacin, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanic acid [20].

All tested bacteria are able to bind epithelial cells receptors. The Gram positive bacterial strains isolated from vaginal infections, belonging to Micrococcus, Enterococcus, Staphylococcus genera and some *Streptococcus* strains present a high capacity of adhesion comparing with Gram negative strains isolated from UTI. Microscopically observations showed typical chains of *Micrococcus* along epithelial cells, instead Enterobacteriaceae group and *Streptococcus* strains are randomly dispersed on the cell surface (fig. 5). Microbial communities play an important role in promotion homeostasis in the vagina and in preventing colonization of pathogenic bacteria. Some studies have shown increased antimicrobial properties of some lactic acid bacteria species against pathogenic strains belonging to *E. coli* species [21]. The imbalance of the microbial population in the human vagina lead to symptoms associated with bacterial vaginosis which is associated with increased risk for acquiring sexually transmitted infections and developing obstetrical complications. Among bacteria which form biofilms *Staphylococcus sp., Enterococcus sp.* and *Klebsiella sp.* strains presented significant values.

Each enzyme selected for this study present an important role in pathogenesis process. Amylase and caseinase are involved in nutrient acquisition [22]. *S. xylosus* B32 strain was the only pathogenic bacteria that produce various enzymes and showed a high capacity of adherence to epithelial cells and inert substrate represented by polystyrene instead present susceptibility to antibiotics. *Micrococcus* strains have an enzymatic profile similar with Enterobacteriaceae group; instead they showed a high rate of adherence to epithelial cells.

It is well known that a part of streptococcal strains are able to cross placenta and reach the fetus causing serious infections. Our results revealed that streptococcal strains presented hemolysins, DN-ase and a part of them also caseinase. Streptococcal strains have also the ability to

bind epithelial cell, trait which confers them the opportunity to colonize human vaginal tract [23]. Instead strains belonging to *Enterobacteriaceae* group presented mobility, the ability to attach to epithelial cell and to form biofilm, the ability to biosynthesis siderophore-like compounds, caseinase and amylase. The antibiotic resistance was very high for two of the enterobacteriaceae strains *E. coli* B 11 and *E. coli* 36, who present high resistance to antibiotics. The increasing use of carbapenem for treatment of diseases caused by *Enterobacteriaceae* expressing extended spectrum-β-lactamase lead to the selection of spread carbapenemase-producting pathogens [24]. Carbapenem resistance has escalated in medically important species such as *E. coli*, and may lead to almost untreatable community-acquired infections. Significant antimicrobial activity against some *E. coli* strains was recorded by use of specific essential oils extract [25].

The β-hemolytic Gram-positive *S. agalactiae* is often encountered in the gastro-intestinal and the genital tract of healthy women as part of normal flora. We have noticed 3 strains of *S. agalactiae* and 5 *E. coli* strains resistant to floroquinolone. Floroquinolones are characterized as broad-spectrum antibacterial drugs active against both Gram-positive and Gram-negative bacteria species. Due to the overuse of these drugs, many bacteria species produced resistance to them and these resistant strains of pathogen species are now a common threat worldwide [26].

Conclusions

The results revealed that the virulence of these strains is very complex and it is associated with a combination of several factors. Evaluation of these factors showed that each strain present a unique and complex virulence profile. The bacterial strains involved in vaginal infection present a high ability to attach to epithelial cells while almost half of bacterial strains isolated from UTI were highly motile and present the ability to attach to epithelial cells. Strains isolated from UTI present a high resistance to antibiotics, in partly due to the over use of antimicrobial compounds.

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