UMAB PoLSMER
Online https://www.asjp.cerist.dz/en/PresentationRevue/871
ISSN: 2800-101X, **E-ISSN**: 2992-0949
Volume 2, Number 2, July 2024

RESEARCH ARTICLE

Impact of antibiotics on *Lactobacillus reuteri* from human vagina

Bechelaghem Nadia[1,,2♠] and Arabi Abed[3]

[1]Higher school of agronomy, Mostaganem, 27000. Algeria. [2]Microbiology and plant biology laboratory, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, 27000. Algeria. [3]Laboratoire Agrobiotechnologie, Ressources génétiques et Modélisation, Faculty of natural and life sciences, Abdelhamid Ibn Badis University, Mostaganem 27000, Algeria.

♠n.bechelaghem@esa-mosta.dz

Received: March 25, 2024/ **Accepted:** June30, 2024/ **Published:** July 15, 2024

ABSTRACT

The main goal of this study was to review the impact of antibiotics on vaginal lactobacilli specifically the strain *Lactobacillus reuteri* isolated from healthy Algerian women in order to avoid the overuse of antibiotics which have a detrimental effect on it. Twenty-four vaginal lactobacilli isolated and identified in previous study were tested for their susceptibility to eight antibiotics from different class commonly used to treat vaginal disorders. The disc diffusion method in MRS agar was performed. The results suggest that all of the twenty-four isolates of *L. reuteri* have variable sensitivity to antibiotics used. All of them were more sensitive to imipenem, moderate sensitivity to clindamycin, chloramphenicol and rifampicin, and less sensitive to ciprofloxacin, penicillin and gentamicin. On the other hand, all were resistant to vancomycin.Obtained data allow to conclude that therapy with vancomycin does not affect the number of *L. reuteri* in the vagina. However, treatment with clindamycin, chloramphenicol, rifampicin, ciprofloxacin, penicillin and gentamicin can promote a decrease of this species of lactobacilli in the vagina. In fact, treatment with imipenem should be avoided as it has a destructive effect on all *L. reuteri* isolates. It should be emphasized that the varying susceptibility of these friendly bacteria should be taken into account when choosing schemes antibacterial treatment.

Keywords: Antibiotic, susceptibility, vaginal, *Lactobacillus reuteri*, infections

INTRODUCTION

The female genital tract is one of the primary locations usually dominated by *Lactobacillus* species known as Doderleïn bacilli (Redondo-Lopez *et al*., 1990). The presence of lactobacilli is an important sign of a healthy vagina which inhibits the growth of pathogenic microorganisms. In general, vaginal lactobacilli offer protection against various diseases including, bacterial vaginosis, vaginal candidiasis, and recurrent urinary tract infections. This protection is provided by the production of lactic acid, hydrogen peroxide, bacteriocins and biosurfactants (Dasari *et al*., 2014). Lactobacilli also protect the vaginal ecosystem through competitive exclusion, coaggregation, immunomodulation and signaling which can lead to down regulation of toxin production in pathogens (Kaewsrichan *et al*., 2006; Reid *et al*., 2011; Borges *et al*., 2014).

Vaginal lactobacilli are very sensitive to antibiotics, in particular to macrolides and tetracyclines often prescribed in gynecological infections, but also to most major families of molecules. This directly leads to the imbalance of the vaginal ecosystem from the start of any antibiotic therapy. Only metronidazole and quinolones seem to have little activity on this Doderleïn microbiota (Bannatyne and Smith, 1998 ;Hillier *et al*., 2000). By their bactericidal action, antibiotics administered locally (vaginally) or systemically lead to a reduction or even eradication of vaginal lactobacilli. This situation is favorable to the proliferation of opportunistic microorganisms such as *Candida*. The risk of developing vulvovaginal candidiasis after antibiotic therapy is all the more important as the antibiotic therapy is broad-spectrum and the duration of treatment is long. It is estimated that the percentage of vaginal yeast infections that follow taking an antibiotic is around 28% to 33% (Pirotta and Garland, 2006 ; Sobel, 2007 ; Amouri *et al*., 2010).

The main objective of this work is to assess the impact of antibiotics on the population of vaginal lactobacilli in women. And as there are few studies on the effect of antibiotics on the strain *L. reuteri* dominated in Mostaganem city, in western of Algeria, we found it interesting to target the antibiotics affecting this last strain.

MATERIALS AND METHODS

The lactobacilli population's origins

The twenty-four isolates of *L. reuteri* tested were isolated from healthy women of reproductive age between 18 and 45 years old. Vaginal specimen collection was performed aseptically by a midwife with sterile swabs, then transported directly to the medical analysis laboratory, bacteriology department at EPH Aïn Tedeles, Mostaganem city (Algeria). A phenotypic study was conducted at the research laboratory

of microbiology and plant biology at Mostaganem University, while a genotypic study took place at the Microbiology Laboratory in the Faculty of Veterinary Medicine at Mustafa Kemal University, Antakya, Turkey. The isolates are stored at -80°C in de Man, Rogosa and Sharpe (MRS) medium with 30% glycerol.

Lactobacilli reactivation

The twenty-four lactobacilli isolates were reactivated on MRS broth for 48 hours at 37°C under anaerobic conditions. These latter were inoculated on MRS agar and incubated anaerobically at 37 °C for 48h.

Antibiotics susceptibility testing

The antibiotic resistance/sensitivity profile of lactobacilli isolates was tested using disk diffusion method (Ocaña *et al*., 2006). Eight discs of antibiotics of different classes often used in the treatment of genital infections were chosen (Table1).

Table 1. Antibiotics tested on lactobacilli.

Antibiotic class	Antibioticstested
βlactams	Penicillin (P)(10 IU)
Glycopeptides	Vancomycin (VA) (30µg)
Aminoglycosides	Gentamycin (CN) (120 µg)
Lincosamides	Clindamycin (DA) (2µg)
Phenicoles	Chloramphenicol (C) (30µg)
Quinolones	Ciprofloxacin (Cip) (5µg)
Carbapenem	Imipenem (IPM) (10µg)
Other	Rifampicin (RA) (5µg)

Inoculum preparation

From a pure culture on MRS medium, a few well-isolated colonies were taken using a Pasteur pipette and inoculated into 5ml of MRS broth to activate them for 18h at 37°C under anaerobic conditions. The concentration of this inoculum of 10^8 CFU / ml corresponds to 0.5 Mc Farland was adjusted using a JENWAY 6715 UV / Vix type spectrophotometer, adjusted at 650 nm for an optical density of 0.1. The inoculum can be adjusted by adding either culture if it is diluted, or MRS broth if it is too concentrated (Rahal, 2005).

Seeding

A suspension of *Lactobacillus* equivalent to 0.5 McFarland was inoculated into Petri plates containing the MRS medium with a sterile cotton swab and then left to dry before depositing the antibiotic disks. The latter should press with a sterile bacteriological forceps to ensure its application. Once applied, the disc should not be moved (Rahal, 2005). Incubation was done at 37°C for 48 h under anaerobic conditions. The results were read by measuring the zones of inhibition of the growth of the lactobacilli tested.

RESULTS AND DISCUSSION

First of all, it was important to know which antibiotics that have detrimental effect on *L. reuteri* the prevalent species in Mostaganem city western of Algeria to avoid there over use. In fact, on MRS agar, the effect of the eight antibiotics on the twenty-four lactobacilli was investigated. Based on the data presented in Table 2, a significant variation in the sensitivity of the species *L. reuteri* to the antibiotics tested was observed. All isolates of this species were sensitive to P, RA, CN, C, CIP, D and IPM.The largest zones of inhibition noticed by the antibiotic IPM reached up to 54 mm in diameter on *L. reuteri* (L1 and L21), which shown that the latter species was too sensitive to this antibiotic. The latter was also sensitive to DA, C and RA with zones of inhibition between (30-46mm) and was not very sensitive to P, CIP and CN with zones between (15 – 30mm). While these two species are resistant to VA.

Testore *et al*. (2002) reported that fifty lactobacilli isolates taken from 30 cervical samples of healthy women were tested for sensitivity to 41 antibiotics, it was found that all isolates were sensitive to the imipenem therefore, our results are in agreement with these results.In another study performed by Egervärn (2009), 56 isolates of *L. reuteri* tested, were sensitive to gentamicin and intrinsically resistant to vancomycin. The same results were obtained in our study.

Melkumyan *et al*. (2015) found that antibiotic resistance and sensitivity varied depending on the lactobacilli type and strain. Several investigations have confirmed the variation in antibiotic susceptibility among lactobacilli species. This distinction emphasizes the necessity of typing lactobacilli in order to choose the best antibiotic treatment. Grouping antibiotics according to bacterial resistance *in vitro* helps define which drug is used to treat infections in women of childbearing age, is the least destructive to normal vaginal microflora and, therefore, the best choice to preserve the vaginal environment.

Table 2. Inhibition zones (in mm) of the eight antibiotics tested on the 24 isolates.

	Antibiotic							
L. reuteri isolates	DA	CIP	VA	C	RA	P	CN	IMP
L1	35	15	R	28	34	10	20	54
L3	30	15	R	30	36	R	20	40
L4	40	19	10	40	40	18	23	34
L5	40	16	R	40	30	10	25	46
L6	45	15	10	40	40	15	20	47
L7	38	15	R	42	40	15	20	44
L10	42	19	R	40	40	15	20	33
L12	45	18	R	45	40	20	25	47
L13	39	20	R	40	44	20	20	30
L14t	45	20	R	40	40	23	24	46
L14b	35	16	R	35	40	20	20	38
L15	40	18	R	38	37	20	24	48
L16	40	18	R	40	35	20	24	45
L17	40	36	R	35	38	25	25	50
L18	40	18	R	35	35	20	20	48
L19p	40	15	R	35	30	20	20	44
L19g	46	20	R	40	40	20	27	50
L20	40	19	R	40	40	25	25	50
L21	40	30	R	45	45	20	27	54
L22	42	16	17	40	39	20	27	47
L23	40	15	R	30	38	25	20	47
L24	37	15	R	35	35	15	20	40
L27	35	15	R	35	34	12	20	47
L30	34	15	R	36	39	20	18	40

All drugs were active against lactobacilli and it is only possible to indicate those which are less active or those which should be avoided because they are too active (for example, imipenem) (Testore *et al*., 2002). In addition, even within the classes of antibiotics, it is possible to choose between less or more active drugs. Clindamycin and metronidazole have been

found to be effective therapies for bacterial vaginosis, although their efficacy may be restricted by detrimental effects on normal vaginal microbiota development (Simoes *et al*., 2001). Clindamycin has raised particular concern because it has a spectrum of *in vitro* activity that covers lactobacilli (Bayer *et al*., 1978). However, studies with both clindamycin and metronidazole suggest that high concentrations of these drugs, such as those obtained with intravaginal treatment for bacterial vaginosis, may inhibit the growth of *Lactobacillus* species (Simoes *et al*., 2001; Aroutcheva *et al*., 2001).

It has been reported that strains develop intrinsic resistance to antibiotics could be useful and can be administered with antibiotics to restore habitat after antibiotic treatment (Cebeci and Gürakan, 2003; Gueimonde *et al*., 2013). Thus, it was observed that the microbiota could regenerate in a shorter time (Cebeci and Gürakan, 2003).

Even when antibiotic therapy is required, maintaining the normal human environment remains a goal for microbiologists and clinicians, and a greater understanding of the normal microflora and its antibiotic sensitivity is required (Testore *et al*., 2002).

CONCLUSION

This research was done to validate *in vitro* the influence of antibiotics on the vaginal lactobacilli population in women, especially the predominate species *L. reuteri* in Mostaganem province, Algeria. According to the findings of this study, vancomycin had no effect on isolates of *L. reteuri* in the vagina, which can be prescribed to treat vaginal infections. However, imipenem was excessively active so should be avoided since it will eradicate the vaginal microbiome. The varying sensitivity of vaginal lactobacilli species (in our case *L. reuteri*) should be taken into account when choosing antibacterial treatment regimens. However, this choice helps to preserve the health of the vaginal ecosystem by respecting lactobacilli as one of the probiotic bacteria. This study deserves to be consolidated by the use of different classes of antibiotics, in each class several types of antibiotics even those prescribed to treat other infections other than gynecological in order to choose the least active within the same class.

ACKNOWLEDGEMENTS

This research was conducted under the auspices of the Ministry of Higher Education and Scientific Research of Algeria, project no. 160011, with financial support from Abdelhamid Ibn Badis University, Mostaganem, Algeria, and the Medical Analysis Laboratory of Dr. CHAIBADRAA, Aïn-Tedeles, Mostaganem, Algeria. Furthermore, the study received sponsorship from Mustafa Kemal University, Hatay, Turkey, where molecular identification was performed. The authors express their gratitude to the women who participated in this study by providing their samples.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS: BN conceived the presented idea, developed the theory, and wrote the manuscript with support from BI. The project was initiated with encouragement from Dr. Chaibadraa.

REFERENCES

1. Amouri, I., Abbes, S., Sellami, H., Makni, F., Sellami, A., Ayadi, A. (2010). La candidose vulvovaginale : Revue. vulvovaginal candidiasis, A review. *J Med Mycology* 20(2): 108-15. https://doi.org/10.1016/j.mycmed.2010.01.005.
2. Aroutcheva, A., Simoes, J.A., Schott, S., Faro, S. (2001): The inhibitory effect of clindamycin on *Lactobacillus* in vitro. *Infect Dis Obstet Gynecol* 9:239-44.
3. Bannatyne, R., Smith, A. (1998): Recurrent bacterial vaginosis and metronidazole resistance in *Gardnerella vaginalis*. *Sex Transm Infect*; 74:455-662.
4. Bayer, A.S., Chow, A.W., Concepcion, N., Guze, L.B. (1978): Susceptibility of 40 lactobacilli to six antimicrobial agents with broad gram-positive anaerobic spectra. *Antimicrob Agent Chemother* 14:720-2.
5. Borges, S., Silva, J., Teixeira, P (2014): The role of lactobacilli and probiotics in maintaining vaginal health. *Arch Gynecol Obstet*, 289: 479-89.
6. Cebeci, A., Gürakan, C. (2003): Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiol*, 20(5):511-518.
7. Dasari, S., Naidu, R., Shouri, D., Wudayagiri, R and Valluru, L. (2014): Antimicrobial activity of *Lactobacillus* against microbial flora of cervico vaginal infections. *Asian Pac J Trop Dis*, 4(1): 18-24.
8. Egervärn, M. (2009): Antibiotic Resistance in *Lactobacillus reuteri* and *Lactobacillus plantarum*. Doctoral Thesis. Swedish University of Agricultural Sciences Uppsala.
9. Gueimonde, M.,Sánchez, B., Clara, G. de los Reyes-Gavilán and Margolles, A. (2013): Antibiotic resistance in probiotic bacteria. *Front microbiol* 4, 202-6.
10. Hillier, S., Holloway, M., Rabe, L. (2000): Antimicrobial agent susceptibility of *Lactobacillus crispatus*, *L. jensenii*, *L. gasseri*, and other *Lactobacillus* species. In: 39thInterscience conference on antimicrobial agents and chemotherapy, *ASM's annual meeting on infectious diseases. American Society for Microbiology, Washington.*
11. Kaewsrichan, J., Peeyananjarassri, K, and Kongprasertkit, J. (2006): Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. *FEMS Immunol Med Microbiol*, 48:75–83.
12. Melkumyan, A.R, Priputnevich, T.V., Ankirskaya, A.S., Murav'eva, V.V., Lubasovskaya, L.A. (2015): Effects of antibiotic treatment on the *Lactobacillus* composition of vaginal microbiota. *Bull Exp Biol Med*, 158: 766–8.
13. Ocaña,V., Clara, S., Nader,Macıas, M.E. (2006): Antibiotic susceptibility of potentially probiotic vaginal lactobacilli. *Infect Dis Obstet Gynecol* Article ID 18182, 1-6.
14. Pirotta, M.V., Garland, S.M. (2006): Genital *Candida* species detected in samples from women in Melbourne, Australia, before and after treatment with antibiotics. *J Clin Microbiol*; 44(9)**:** 3213-7.
15. Rahal, K. (2005). Standardisation de

l'antibiogramme en médecine humaine. 4ème édition.

16. Redondo-Lopez, V.R., Cook, L and Sobel, J.D. (1990): Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis* 12:856- 872
17. Reid, G, Younes ,J.A, Van der Mei, H.C, Gloor, G.B, Knight, R, Busscher, H.J. (2011). Microbiota restoration: natural and supplemented recovery of human microbial communities. *Nat Rev Microbiol*. Jan, 9 (1):27-38. https://doi.org/10.1038/nrmicro2473
18. Simoes, J.A., Aroutcheva, A.A., Schott, S., Faro, S. (2001): Effect of metronidazole on the growth of vaginal lactobacilli in vitro. *Infect Dis Obstet Gynecol*, 9:41-5.
19. Sobel, J.D. (2007): Vulvovaginal candidosis. The Lancet 369:1961-1971.
20. Testore, G.P., Sarrecchia, C., Zupi, E., Sordilo, P., Valli, E., Bove, F., and Andreon M. (2002): Antibiotic susceptibility of lactobacilli isolated from the cervix of healthy women. *Microbecol health dis*, *14*(1)**:**14-18.