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Prevalence and Antimicrobial Susceptibility of *Mycoplasma hominis* and *Ureaplasma* Species among Women in Dakar

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Authors' contributions

This work was carried out in collaboration among all authors. Author ASN Conceptualized and designed the study and drafted the article. Authors ASN, MCS, CF, CS, ODB and PATG collected the data. Authors ASN, BK and MCS did data analysis. Authors MCS, MD and GL reviewed the article. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Genital mycoplasmas, which cause infections of the lower reproductive tract in women, are a major cause of morbidity and complications. The aim of this study was to determine the prevalence of mycoplasma infections and the antibiotic susceptibility profile among women in Dakar.

Material and methods: We conducted a retrospective descriptive study over a four-year period, from 02 January 2018 to 31 December 2021. The study population consisted of women referred to the microbiology laboratory at Military Hospital of Ouakam for genital mycoplasma testing. The Mycoplasma IES kit was used for mycoplasma testing and susceptibility testing in accordance with the manufacturer's recommendations.

Results: A total of 1431 patients were enrolled during the study period. The mean age was 32.6 ± 8.5 years. The overall prevalence of mycoplasma infection was 55.8% (798/1431). Of the 798 positive cultures, 52.8% were infected with *Ureaplasma urealyticum* (UU) and 17.9% with *Mycoplasma hominis* (MH). UU+MH co-infection was 31.08%. Analysis of the sensitivity profiles showed that *UU* was sensitive to most of the antibiotics used, while M*. hominis* strains showed low levels of resistance to tetracyclines (TET DOX, MIN), quinolones (OFX, LEV, SPA) and JOS. This resistance was much higher with macrolides (ERY, ROX, AZT), certain quinolones (ASP, CIP) and aminoglycosides (SPE). However, *M. hominis* strains were much more resistant to these molecules than *U. urealitycum* isolates.

Conclusion: Our study revealed a high rate of genital mycoplasma infection among women in Dakar. The high rates of resistance to certain molecules underline the importance of surveillance to prevent transmission of resistant strains and rational use of antibiotics.

Keywords: Prevalence; mycoplasma; sensitivity; antibiotics; women; Dakar.

1. INTRODUCTION

Mycoplasmas, including *Mycoplasma hominis* and *Ureaplasma* species, are known as the smallest free-living organisms without a cell wall (Longdoh et al.,2018). They are facultative anaerobic organisms that are generally found in the lower urogenital tract as commensal bacteria (Lee et al., 2020; Wang et al., 2023; Stavart et al., 2023). Colonisation varies with age, race, socio-economic status, sexual activity and hormonal status and increases during pregnancy (Matasariu et al. 2022). These organisms are implicated in a wide range of infectious diseases in adults and children (kiljevic et al., 2016). *Ureaplasma spp* species are more frequently isolated from women at 30% than M. hominis which is present at less than 10% (Grama et al. 2013). These bacteria are considered to be aetiological agents causing various urogenital diseases in women, such as cervicitis, cystitis, bacterial vaginosis, pelvic inflammatory disease, chorioamnionitis, postpartum fever, infertility, prematurity, intrauterine growth retardation and systemic neonatal infections (**Longdoh et al.,2018**; Lee et al., 2020). These infections of the lower reproductive tract in women are major causes of morbidity, but can also lead to complications and sequelae (Diadhiou et al., 2019). Hence the importance of good prevention and early management of these infections, particularly in women of childbearing age. In Senegal, most patients presenting with signs suggestive of STIs (urethral discharge and vaginal discharge syndromes) are often diagnosed presumptively using a syndromic approach. However, this approach can lack sensitivity and specificity in some settings and lead to mismanagement of several conditions, particularly during Mycoplasma infections (Barry et al. 2018). Because mycoplasmas lack a cell wall, they are naturally resistant to beta-lactams and glycopeptides (Wang et al., 2023). They are not as sensitive to sulphonamides or trimethoprim because they do not synthesise folic acid (Goutier-Bouchardon, 2018; Lee et al., 2020). This leads to a considerable reduction in the range of antibiotics available for treatment. Therefore, macrolides, quinolones, and tetracyclines are frequently used to treat infections caused by mycoplasmas and ureaplasmas (Wang et al., 2023). In addition, in recent years there has been overuse of antibiotics, with non-standard drugs, repeated infections and an increase in drug resistance in *U. urealyticum* (UU) and *M. hominis* (MH), leading to numerous difficulties in standardised clinical treatment (Ahmadi et al., 2016; Gu et al., 2020). Antibiotic susceptibility testing of mycoplasmas isolated from the urogenital tract is

therefore of vital importance (Kiljevic et al., 2016). In Senegal, few data are available on the frequency and antibiotic susceptibility of mycoplasmas. We therefore conducted this study, which. The aim was to determine the prevalence of mycoplasma infections (*M. hominis* and *U. urealyticum*) and the antibiotic susceptibility profile among women in Dakar.

2. MATERIALS AND METHODS

We conducted a retrospective descriptive study over a four-year period, from January 2018 to December 2021. The study concerned all women referred to the microbiology laboratory at Ouakam Military Hospital for genital mycoplasma testing. Two vaginal samples were taken from each patient using sterile swabs. A survey questionnaire revealed that the patients were initially diagnosed and had not used antibiotics in the two weeks prior to sample collection.

2.1 Research and Antibiotic Susceptibility Testing for Mycoplasma

Mycoplasma tests were performed using the Mycoplasma IES kit (Autobio Diagnostics Co., Ltd.; Zhengzhou-China) for urogenital mycoplasma in accordance with the manufacturer's recommendations. All reagents were used within the validity period and internal controls were performed. All samples were incubated at 37°C for 24 or 48 hours. *U. urealyticum* results were observed after 24 h and *M. hominis* results after 48 h. The Mycoplasma kit is based on the culture and biochemistry reaction. Urea can be broken down by urease for *U. urealyticum* and release NH3; arginine can be broken down by Arginase for *M. hominis* and release NH3. The NH3 then causes an increase in the pH of the liquid medium. The corresponding change in colour of the indicator is used to judge the result. A change in the colour of the medium from yellow to red indicated growth of Mycoplasma (+), while no change in colour indicated no growth of Mycoplasma (-).

In this study, several antibiotics were tested for their sensitivity, namely doxycycline (DOX), minocycline (MIN), josamycin (JOS) and tetracycline (TET), erythromycin (ERY) spectinomycin (SPE) acetylspiramycin (ASP), clarithromycin (CLA), roxithromycin (ROX), azithromycin (AZT), ofloxacin (OFX), ciprofloxacin (CIP), levofloxacin (LEV) and sparfloxacin (SPA). The sensitivity of the drugs was checked as follows: when the indicator well was in favour of UU or MH growth and the high and low concentration drug wells did not turn red, the drug was considered sensitive (S); when the low concentration well turned red and the high concentration hole remained unchanged, it was considered intermediate (I); when the high and low concentration holes turned red, it was considered resistant (R).

2.2 Statistical Analysis

Statistical analyses were performed using Epi Info software. Continuous variables were described as means with standard deviation. Normally distributed variables were compared with a t-test. Categorical variables were presented as percentages and Fisher's exact or chi-square tests were used for proportional evaluations. The association between associated risk factors and positivity was assessed by univariate logistic regression analysis. We defined four age groups (years) for the analysis, namely < 25 years, 25 - 35 years, 36 - 45 years, and 45 years and over. The chi-square test was used to compare two groups. The significance level was $p \leq 0.05$ for all statistical data.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 General characteristics of the study population

A total of 1431 patients were referred to the laboratory for mycoplasma testing during the study period. The mean age was 32.6 ± 8.5 years. The number of patients received increased over the years from 2018 (19.01%) to 2021. (30.05%) (IC=95%). The most representative age group was the 25-35 age group, which accounted for 47.7% (n=682), followed by the 35-45 age group with 29.3% (n=419) (see Table 1).

3.1.2 Prevalence of mycoplasma infections

For the 1,431 samples analysed, the study showed both single and mixed infections at population level. The overall prevalence was 55.8% (798/1431). Of the 798 positive cultures, the infection rate for UU was 52.8%, while that for MH was 17.9%. A mixed UU+MH infection rate of 31.08% was found. The UU infection rate was much higher than the *M. hominis infection rate* over the years. Infection with *M. hominis*

increased significantly over the years (p=0.0005), unlike infection with *U. urealitycum*, where there was no significant difference (see Table 1). (see Table 1).

3.1.3 Distribution of *Ureaplasma urealyticum* infection

The overall prevalence of *U. urealyticum* was 52.8% [95% CI 50-55]. By study period, the frequency was highest in 2021 at 57.7% (n=248), and lowest in 2019 at 49.9% (n=164). However, the difference was not statistically significant (p=0.1). By age group, patients aged under 25 were more affected with 54.38%, followed by those aged between 25 and 35 (53.01%) and those aged 35-45 (51.5%), but the difference was not statistically significant (p = 0.9).

3.1.4 Distribution of *Mycoplasma hominis* infection

The prevalence of *M. hominis* infection was 17.9% [95% CI 16-20]. By study period, the highest rate of infection was observed in 2021, at 22.3% (n= 96), while in 2020 the lowest was 11.8%. The difference was statistically significant (p<0.001). Patients aged between 25 and 35 were the most affected, with 18.8%, followed by those aged under 25 (18.4%), while those aged over 45 were the least affected (13.2%). This difference was not statistically significant (p = 0.6).

3.1.5 Analysis of antibiotic susceptibility profiles

Analysis of the antimicrobial susceptibility profiles of the 798 positive cultures showed that over 70% of *U. urealyticum* isolates showed good susceptibility to MIN, DOX, SPA and LEV (84%, 81.5%, 81% and 71%) respectively. Over 60% sensitivity was found for JOS (68%) and CLR (61%), while TET and OFX showed sensitivity rates of over 45%. On the other hand, resistance rates of over 25% were found for ASP (50%), CIP (48%), SPE (41%), ROX (31%), AZT (31%) and ERY (29%), as shown in Fig. 1.

M. hominis isolates showed good sensitivity of greater than or equal to 70% for MIN, DOX and SPA respectively (74%, 71% and 70%). Sensitivity rates of over 40% were found for LEV, JOS, TET and OFX. High resistance rates of over 40% were observed for ERY (82%), ROX (63%), ASP (62%), CLA (54%), AZT (48%), SPE (47%) and CIP (42%), as shown in Fig. 2.

In summary, M. *hominis* and *U. urealiticum* strains showed low levels of resistance to TET, LEV, JOS, DOX, OFX, MIN and SPA. This resistance was much higher with ERY, ROX, ASP, CLA, AZT, SPE and CIP. This resistance was much more pronounced for M. hominis strains, ranging from 40% to 82%.

Table 1. Characteristics of the study population and prevalence of infection (N=1431)

	% (n)	95% CI
Study period		
2018	19.01 % (272)	17 - 21
2019	22.99 % (329)	21 - 25
2020	27.95 % (400)	26 - 30
2021	30.05 % (430)	28 - 32
age group (years)		
< 25	15.16 % (217)	13 - 17
25 - <35	47.7 % (682)	45 - 50
35 - <45	29.28 % (419)	27 - 32
> 45	8.13% (118)	7 - 9
Ureaplasma urealyticum		
Absence	47.16% (675)	45 - 50
Presence	52.83% (756)	50 - 55
Mycoplasma hominis		
Absence	82.11 % (1162)	80 - 84
Presence	17.9 % (259)	16 - 20

M. hominis/ U. urealyticum 31.08

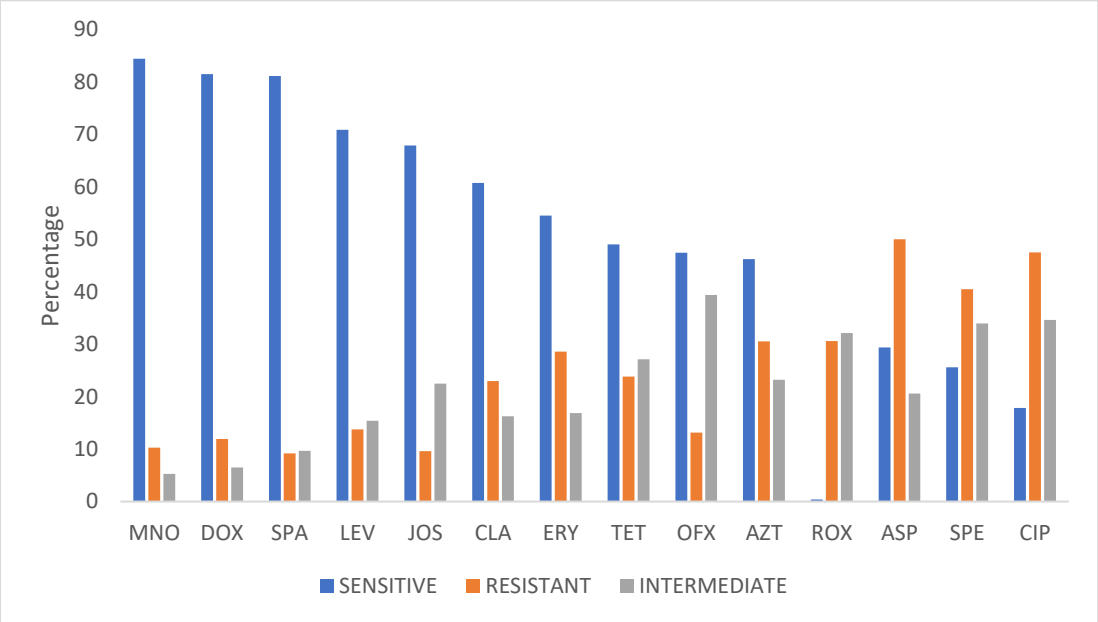

Fig. 1. Susceptibility profile of *U. urealyticum* strains

MIN= Minocycline, DOX= Doxycycline, SPA= Sparfloxacin, LEV= Levofloxacin, JOS= Josamycin, CLA= Clarithromycin, ERY= Erythromycin, TET= Tetracycline, OFX= Ofloxacin, AZT= Azithromycin, ROX= Roxithromycin, ASP= Acetylspiramycin, SPE= Spectinomycin (SPE), CIP= Ciprofloxacin

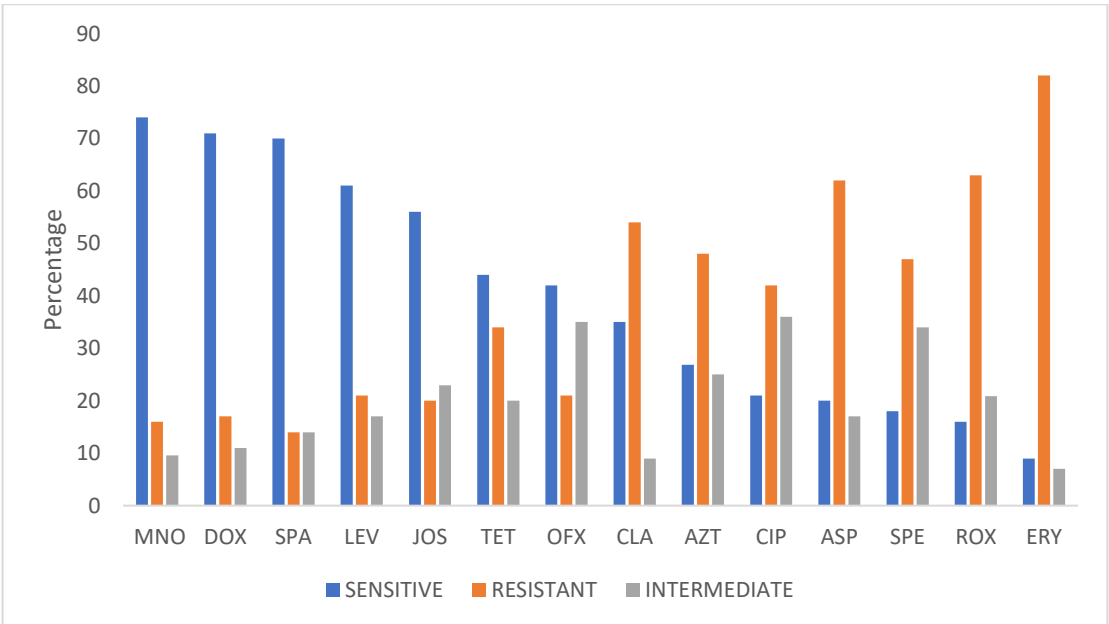

Fig. 2. Susceptibility profile of *M. hominis* strains

MIN= Minocycline, DOX= Doxycycline, SPA= Sparfloxacin, LEV= Levofloxacin, JOS= Josamycin, CLA= Clarithromycin, ERY= Erythromycin, TET= Tetracycline, OFX= Ofloxacin, AZT= Azithromycin, ROX= Roxithromycin, ASP= Acetylspiramycin, SPE= Spectinomycin (SPE), CIP= Ciprofloxacin

3.2 Discussion

Infections of the female reproductive tract have a huge impact on women's health, including their reproductive health (Holali Ameyapoh et al., 2021). The mycoplasmas that can cause these reproductive tract infections mainly include *Ureaplasma urealyticum* and *Mycoplasma hominis*. These lower reproductive tract infections in women are common in clinical

medicine and constitute one of the most important causes of morbidity (Diadhiou et al., 2019). The aim of our study was to assess the prevalence and antibiotic susceptibility of *M. hominis* and *U. urealyticum* in female genital samples. This retrospective study of 1431 samples from women in Dakar showed an overall prevalence of Mycoplasma infection of 55.8%. Other studies have found lower prevalences ranging from 37% to 45% (Longdoh et al.,2018, Shao et al., 2021; Zheng et al., 2020; Lee et al., 2020; Luo et al., 2022, Liu et al., 2024). A study conducted in Cameroon revealed a prevalence of Mycoplasma of 48% (Njunda et al., 2011). A higher prevalence (80%) was noted in South Africa (Redelinghuys et al., 2015). In West Africa, one study found a similar prevalence in Senegal of 54.8% (Tine et al., 2019). However, another study showed a lower prevalence of 42% in Senegal and 44% in Togo (Diadhiou et al., 2019; Holali Ameyapoh et al., 2021).

Of the 798 positive cultures, the infection rate for UU was much higher (52.8%) than that for MH, which was 17.9%. According to the results of several studies, the prevalence *of Ureaplasma* species in patients was higher than that of *M. hominis*. The prevalence of Ureaplasma and M. hominis species was 33.47% and 0.36% respectively (Zheng et al., 2020); 74.4% and 5.3% (Shao et al., 2021) in China; 78.2% and 3.1% in South Korea (Lee et al., 2020). A study carried out in Senegal found similar results, where the rate of UU infection (27.5%) was much higher than MH infection (14.5%) (Diadhiou et al., 2019). However, other studies had shown different results where the MH infection rate was significantly equal to or higher than UU in Senegal (Tine et al., 2019) and China (Zeng et al., 2016). The high rate of Mycoplasma infection (55.85%) found in our study is particularly due to a single UU infection, which was not consistent with some studies found in the literature (Skiljevic et al., 2016; Qing et al, 2017), indicating differences in the distribution of Mycoplasma in different areas that could be explained by the variability of diagnostic methods from one study to another, the types of sample used (urine, cervical swab and vaginal discharge), the use of antibiotics and also sexual behaviour (Lee et al., 2020; Zheng et al., 2020).

This particularly high prevalence among women in our study can be explained by women's physiology, which is more conducive to Mycoplasma infection, which can cause pyelonephritis, vaginitis, pelvic inflammatory disease and other illnesses affecting women's quality of life. Infections with *M. hominis* and *U. urealyticum* were more frequent in women aged under 25 and between 25 and 35 in the case of women aged under 45. These results have been found in other studies in China (Wang et al., 2016; Zheng et al., 2020; Shao et al., 2021; Luo et al., 2022) and Senegal (Tine et al., 2019). The high prevalence in this age group is due to the fact that it is sexually active and of childbearing age, which may be a predisposing factor for infection (Grama et al., 2013; Zheng et al., 2020).

In terms of antibiotic susceptibility, the majority of genital mycoplasmas were most sensitive to Minocycline, Doxycycline, Sparfloxacin, Levofloxacin and Josamycin. However, most strains were resistant to Acetylpyramycin, Ciprofloxacin, Roxythromycin, Azithromycin and Erythromycin (Adelman ET AL., 2013; Gautier-Bouchardon et al., 2018).

Our results showed that *U. urealyticum* infection was sensitive to most antibiotics, such as Minocycline, Doxycycline, Josamycin, Sparfloxacin, Levofloxacin, Josamycin, Clarithromycin and Tetracycline. *M. hominis* strains were sensitive to Minocycline, Doxycycline, Sparfloxacin, Levofloxacin, Josamycin and Tetracycline respectively. Similar results were found in the study by Jang et al in 2019. Our study showed that Mycoplasma strains had low levels of resistance to TET, LEV, JOS, DOX, OFX, MIN and SPA. This resistance was much higher with ERY, ROX, ASP, AZT, SPE and CIP. However, *M. hominis* strains were much more resistant to these molecules than isolates of Ureaplasma species. These results were confirmed by other studies in 2020 in China by Gu et al and in South Korea by Lee et al. In fact, *M. hominis* has several drug resistance genes, natural resistance to most macrocyclic ester-based drugs and cross-resistance to quinolones (quinolones (Jang et al., 2019). However, the high resistance of *M. hominis* strains to Spectinomycin was not found in another study carried out in China, where 77.8% of *M. hominis* strains were sensitive to Spectinomycin (Zheng et al., 2023). In a study carried out by Shao in China, 70% resistance to ciprofloxacin was found in *U. urealyticum* isolates. These results differ from thosfound in our study, where 48% of U. urealyticum strains were resistant to ciprofloxacin. In this study, more than 80% of M. *hominis* strains were resistant to Erythromycin, which was in line with our results where 82% of isolates were resistant

to Erythromycin. On the other hand, the high resistance rate of over 80% to Roxithromycin, zithromycin and Clarithromycin was higher than our results, where resistance to these molecules was less than or equal to 60%. (Shao et al 2021).

However, the low rate of resistance of *U. urealyticum* to quinolones such as Sparfloxacin and Levofloxacin (9% and 13% respectively) was found in other studies in the United States where the rate of resistance to Levofloxacin in *U. urealyticum* was relatively nil (0%) (Valentine-King and Brown, 2017). These results were not consistent with other studies (Wang et al., 2016; Zeng et al., 2016; Yang et al., 2020; Ma et al., 2021; Song et al., 2022). It should be noted that levels of fluoroquinolone resistance differed significantly from one country to another. In Italy, 77.1% of *Ureaplasma spp*. were resistant to Ciprofloxacin and 26.3% of isolates were resistant to Ofloxacin (Foschi et al., 2018). Another study carried out in China found a levofloxacin resistance rate of 82.43% in *U. urealyticum* (Yang et al., 2020). This wide variability in quinolone resistance may be linked to the strategy or tendency to use antibiotics in different regions. (Song et al., 2022).

In summary, our results showed that the most effective compounds against *U. urealyticum* and *M. hominis* were tetracyclines (Minocycline, Doxycycline) and Josamycin. Our results are comparable to those found in other studies (Gu et al., 2020; Zheng et al., 2020; Shao et al., 2021; Zheng et al., 2023).

4. CONCLUSION

Our study of the retrospective analysis of the prevalence and antibiotic susceptibility of *U. urealyticum* and *M. hominis* strains showed high rates of genital mycoplasma in women in Dakar. *U. urealyticum* infection was much more common (52.8%), followed by *U. urealyticum* / *M. hominis* co-infection (31.08%) and *M. hominis* infection (17.9%). The study also showed that these infections were much more prevalent in young women under the age of 40. Early diagnosis and effective treatment are essential in the fight against these infections. The high rates of resistance to certain molecules underline the importance of surveillance to prevent the transmission of resistant strains and the rational use of antibiotics.

Tetracyclines and josamycin remain the most promising antibiotics, with exceptional activity against *U. Urealyticum* and *M. hominis.*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

This study was hospital-based research conducted in normal conditions under the Declaration of Helsinki. Ethical permission was obtained from the hospital authorities. Information collected during the study was analyzed using the participant's identification code to ensure confidentiality. Patient consent was also obtained prior to sample collection.

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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