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Mobiluncus species in gynaecological and obstetric infections: antimicrobial resistance and prevalence in a Turkish population

Hrisi Bahar [a], Müzeyyen Mamal Torun [a], Fahri Öçer [b], Bekir Kocazeybek [a,*]

[a] *Istanbul University, Department of Microbiology and Clinical Microbiology, Cerrahpaşa School of Medicine, 34303 Cerrahpaşa, Istanbul, Turkey*

[b] *Istanbul University, Department of Obstetrics and Gynecology, Cerrahpaşa School of Medicine, Istanbul, Turkey*

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Abstract

The purpose of this study was to determine the prevalence and antimicrobial resistance of *Mobiluncus* species isolated from specimens collected from Turkish women with gynaecological infections. *Mobiluncus* species were isolated on enriched Schaedler agar and RLK agar plates under anaerobic conditions. The MICs of various antibiotics were evaluated using an agar dilution procedure. The prevalence of *Mobiluncus* species isolated from vulvo-vaginal abscesses, endometrial smears, salpingitis and bacterial vaginosis was 2%, 4.7%, 3.8% and 49%, respectively. *Mobiluncus* isolates were only resistant to metronidazole (81% resistance). The isolation rate of *M. curtisii* was higher than *M. mulieris* in Turkish women with bacterial vaginosis, vulvo-vaginal abscesses, endometritis or salpingitis.

Keywords: *Mobiluncus* species; Antimicrobial resistance; Vulvo-vaginal abscesses; Endometrial smears; Salpingitis; Bacterial vaginosis

1. Introduction

The association of the curved rods of *Mobiluncus* spp. with vaginal fluid has been recognised since the turn of the century [1]. These bacteria were thought to be potentially pathogenic for man as they were isolated either alone or together with other anaerobes, from breast and umbilical abscesses. *Mobiluncus* spp. have also been found in a blood culture from a woman with liver cirrhosis and in the chorioamnionic membranes of a placenta at preterm delivery [2–6]. Multivariate analysis of vaginal flora has shown that *Mobiluncus* spp. occur in large numbers in bacterial vaginosis (BV) and may therefore serve as an indicator organism for this clinical syndrome [1,7]. Women with BV were also found to be at high risk of having bartholinitis, skenitis, upper genital tract infection (UGTI), postpartum and postabortal endometritis, chorioamnionitis and infections following gynaecological surgery or diagnostic procedures [8–11].

Mobiluncus curtisii and *M. mulieris* have been frequently observed in wet mounts or Gram stained smears of vaginal fluid of patients with BV [12]. The fastidious nature of these strictly anaerobic organisms meant they were not isolated using conventional culture techniques. Smith and Moore [13] first isolated them by using cold enrichment and a selective medium, RLK agar. Although metronidazole treatment of women with BV successfully eliminated the symptoms and signs of vaginal discharge, high re-occurrence rates of the disease were reported due to re-infection by sexual contacts and this increased the risk of many BV related infections [14,15]. Effective antibiotics to eliminate pathogenic anaerobes as *Mobiluncus* species need to be defined [15–19].

We determined the antimicrobial resistance and the prevalence of *Mobiluncus* species isolated from Turkish women with vulvo-vaginal abscess, endometritis, salpingitis and BV in order to evaluate their importance in gynaecological and obstetric infections.

* Corresponding author. Tel.: +90 212 414 30 75.
E-mail address: bekirkcz@superonline.com (B. Kocazeybek).

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M. mulieris strains resistant to metronidazole whereas in our study, 6 of 24 *M. mulieris* strains were resistant. May be this resistance could be attributed to the preferential and empirical use of metronidazole to treat common parasitic infections in our country. All of our strains were found susceptible to penicillin G, cefoxitin, imipenem, sulbactam + ampicillin and clindamycin.

We concluded that there was a high prevalence of metronidazole-resistant *Mobiluncus* species in our population and the isolation rate of *M. curtisii* were higher than those of *M. mulieris* in Turkish women with BV, vulvo-vaginal abscesses, endometritis and salpingitis.

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Table 1
Prevalence of *Mobiluncus* species in specimens taken from Turkish women with vulvo-vaginal abscesses, endometritis, salpingitis and BV

Infection	Specimens (*n*)	*M. curtisii* strains, *n* (%)	*M. mulieris* strains, *n* (%)
Vulvo-vaginal abscess	Pus (102)	2 (2)	–
Salpingitis	Pus (26)	1 (3.8)	–
Endometritis	Endometrial smear (42)	2 (4.7)	–
Bacterial vaginosis	Vaginal discharge (194)	70 (36.1)	25 (12.8)

with *A. prevotii*, *P. bivia*, *P. disiens* and *P. asaccharolytica* (Table 1).

The antimicrobial susceptibility of Mobiluncus isolates is shown in Table 2.

The antimicrobial MICs for the control strain ATCC 35241 were: penicillin 0.015 mg/l, ampicillin + sulbactam 0.06 mg/l, cefoxitin 0.06 mg/l, imipenem 0.015 mg/l, clindamycin 0.06 mg/l, metronidazole 128 mg/l. All isolates were susceptible to all antibiotics except for metronidazole. Eighty-one isolates were resistant to metronidazole.

4. Discussion

There have been several reports on the polymicrobial nature of obstetric and gynaecological infections [6,8,10,11]. Obligate anaerobes, which are usually mixed with facultative bacteria, were found as potential pathogens in these infections. *P. bivia*, *P. disiens*, peptostreptococci and *B. fragilis* group bacteria were common pathogen anaerobes particularly in closed space infections such as tubo-ovarian and vulvo-vaginal abscesses [8–11].

BV, the most common vaginal infection, is characterised by depletion of vaginal lactobacilli with increased *G. vaginalis Mobiluncus* spp., *Prevotella* spp., *Peptostreptococcus* spp. and *Mycoplasma* spp. [1,7,8,12,13,26]. *Mobiluncus* species have been used as an indicator for BV because of their frequent isolation from vaginal smears of affected patients [1,7,8,12,13,26]. Recent studies reported that BV associated bacteria could ascend to the upper genital tract and give rise to serious infections such as cervicitis, salpingitis, endometritis, postoperative infections, urinary tract infections, UGTI, chorioamnionitis, postpartum endometritis and also preterm delivery and premature rupture of the membranes [6,8,10,11].

Larsson et al. [30] looked for *Mobiluncus* species in vaginal discharges from women admitted their department for a first-trimester abortion and found a correlation between the presence of *Mobiluncus* in vaginal discharge and the incidence of UGTI. Faro et al. [31] found a relationship between BV and postoperative infections in both obstetric and gynaecological settings. Newton et al. [23] and Watts et al. [24] showed that BV was a risk factor for puerperal endometritis. Finally, Hillier et al. [32] determined the role of BV and bacterial vaginosis-associated microorganisms in endometritis. Reoccurrence of BV and related infections after metronidazole treatment depends on many factors [14]. There are speculations that the antimicrobial resistance of pathogenic anaerobes plays an important role in these infections and could be one of the causes of reoccurrence [7,14,16,19,20]. The antimicrobial susceptibilities of *Mobiluncus* spp. and their resistance rate to metronidazole have been documented in various populations [17–19]. It was also reported that 4% of *Mobiluncus* species were clindamycin resistant [17,19]. For the sensitive strains, the MIC 90 was ≤0.06 mg/l [17]. In our study all of our strains were sensitive to clindamycin and the MIC 90 was 0.125 mg/l. Several investigators showed that metronidazole had little effect on *Mobiluncus* species and a metronidazole resistance of about 30–70% was reported for these isolates [16–19]. In many studies MIC 90 of metronidazole was determined 128 mg/l [17,19]. In this present study the 100% of *M. curtisii* strains and the 24% of *M. mulieris* strains were found to be resistant to metronidazole. The resistance rate was 81%. Metronidazole had a MIC_{50} of 32 mg/l and a MIC_{90} of 128 mg/l for *M. curtisii* strains and an MIC50 of 4 mg/l and an MIC90 of 64 mg/l for *M mulieris* strains. Unlike previous studies, Turkish strains of *Mobiluncus* species were found to be highly resistant probably due to the intrinsic metronidazole resistance of *M. curtisii* that affected 75% of our strains. Spiegel [20] reported 5 of 10

Table 2
The MICs (mg/l) of six antimicrobial agents against 75 strains of *Mobiluncus curtisii* and 25 strains of *Mobiluncus mulieris* isolated from different gynaecological and obstetric infections

Antimicrobial agent	*M. curtisii* (*n*: 75)					*M. mulieris* (*n*: 25)				
	Range (mg/l)	MIC 50 (mg/l)	MIC 90 (%)	S (%)	R	Range (mg/l)	MIC 50 (mg/l)	MIC 90 (%)	S (%)	R
Penicillin	0.06–0.5	0.125	0.250	100	–	≤0.03–0.125	0.06	0.125	100	–
Ampicillin–sulbactam	0.125–0.250	0.125	0.250	100	–	0.125	0.125	0.125	100	–
Cefoxitin	0.125–4	1	4	100	–	0.125–2	0.125	2	100	–
Imipenem	≤0.03–1	0.250	1	100	–	≤0.03–1	0.03	0.5	100	–
Clindamycin	0.06–0.125	0.06	0.125	100	–	0.06–0.125	0.06	0.125	100	–
Metronidazole	32–>128	32	128	–	100	1–128	4	64	76	24

S: sensitive; R: resistant.

2. Materials and methods

2.1. Patients

From May 1999 to May 2002, we collected the following specimens: pus from vulvo-vaginal abscess of 102 patients, pus from tubes of 26 patients with salpingitis, endometrial smear specimens from 42 patients with endometritis and vaginal discharge from 194 patients with BV all of whom were admitted to the Department of Obstetrics and Gynaecology of Istanbul University, Cerrahpaşa Medical Faculty. The cases with vulvo-vaginal abscess, endometritis or with salpingitis had a history of frequent recurrent BV.

2.2. Sampling method

Pus from vulvo-vaginal abscesses and fallopian tubes of patients with salpingitis was collected by aspiration or after surgical incision and was transferred to the laboratory in sealed plastic syringes within half an hour of collection. Endometrial tissue smears from patients with endometritis were obtained using swabs after incision of the endometrium, and vaginal discharge of patients with BV were obtained from the posterior vaginal fornix using Dacron tipped swabs. Specimens were transferred to the laboratory in Cary and Blair medium (BBL Microbiology System, Cockeysville, MD Maryland, USA) within half an hour of collection [21–24].

2.3. Culture methods

For anaerobic bacteria isolation, each sample was inoculated on anaerobe blood agar, phenylethyl alcohol blood agar, kanamycin–vancomycin blood agar, Bacteroides bile aesculin agar (BBL Microbiology System, Cockeysville, MD Maryland, USA) and RLK medium developed by Smith and Moore for selective isolation of *Mobiluncus* spp. The latter medium consisted of colistin-nalidixic acid agar (BBL Microbiology System, Cockeysville, MD Maryland, USA) with 0.6% yeast extract, 2% peptone and 5% sheep blood. Forty-eight mg/l of tinidazole and 20 mg/l nalididixic acid were added to the medium after autoclaving. Cultures were incubated in anaerobic jars (Oxoid U S A Inc., Columbia MD) using Anaero-Gen (Oxoid and Mitsubishi Gas Company) for at least 7 days. Colonies were Gram stained to obtain organisms with the characteristic curved rod shape [13,22,25].

2.4. Identification of Mobiluncus species

Colonies of different forms on anaerobic media were described, Gram stained and subcultured to (1) two chocolate agar plates, one of which was incubated at 37 °C for 48 h and the other under CO_2 atmosphere for 72 h to verify aerobic or aerotolerance characteristics of the colony; (2) to an anaerobe blood agar plate to obtain pure cultures of each colony [25]. The initial identification of *Mobiluncus* species was made based on the motility, metronidazole resistance, catalase production, nitrate reduction, hippuric acid hydrolysis, α-D-galactosidase activity and carbohydrate fermentation characteristics [13–26].

M. curtisii was metronidazole resistant, a short curved rod and hydrolysed arginine and hippuric acid, and had α-D-galactosidase activity. It did not ferment glycerol or trehalose. *M. mulieris* was metronidazole sensitive, a long curved rod, did not hydrolyse arginine and hippuric acid and did not have α-D-galactosidase activity. They fermented glycerol and trehalose. Bacterial identification was performed using API 32 ID (Bio Merieux SA, Marcy L'Etoile, France) and Sceptor anaerobic ID Panels (Becton Dickinson) [19]. *M. curtisii* ATCC 35241 = NCTC 11656 strain, *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 strains were used as controls [27].

2.5. Agar dilution procedure

The reference agar dilution procedure for susceptibility testing of the anaerobic bacteria was performed following the NCCLS reference standard M11-A6 [28,29]. The following antimicrobial agents were tested: penicillin G, ampicillin + sulbactam, cefoxitin, imipenem, clindamycin and metronidazole. Plates were prepared using Wilkins-Chalgren agar (Oxoid) and inoculated with bacteria on the day of preparation. The plates were incubated in an anaerobic jar. A plate without antibiotics and strains of *Mobiluncus curtisii* ATCC 35241 = NCTC 11656, *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls.

3. Results

Gram stained smears showing Gram-negative or Gram-variable curved rods were taken to contain *Mobiluncus* morphotypes. Such curved rods were observed in 11 (10.7%) of 102 specimens from patients with vulvo-vaginal abscesses, in 4 (15.3%) of 26 specimens from patients with salpingitis, in 3 of 42 endometrial smears (7.1%) from patients with endometritis and in 150 of 194 specimens of vaginal discharge (77.3%) from patients with BV. *M. curtisii* grew from 2% (two strains) of pus specimens from the 102 patients with vulvo-vaginal abscess. One of these strains was co-isolated with *Anaerococcus prevotii* and the second was co-isolated with *Peptostreptococcus anaerobius* and *Porphyromonas asaccharolytica*. *M. curtisi* was isolated once from pus specimens from 26 cases of salpingitis. This strain was co-isolated with *A. prevotii* and *Prevotella bivia*. Two isolates of *M. curtisii* were isolated from 42 specimens of endometrial smears from patients with endometritis (4.7%). One of these strains was isolated together with *P. anaerobius* and the second with *P. bivia* and A. *prevotii*. The vaginal discharge specimens from 194 patients with BV grew 70 isolates of *M. curtisii* (36.1%) in mixed culture with A. *prevotii*, *P. bivia* and *P. disiens* and 25 (12.8%) isolates of *M. mulieris* together