Antimicrobial Susceptibility Patterns of Coryneform Bacteria Isolated from Semen

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Abstract: *Background*: The susceptibility of coryneform bacteria has been studied mostly in invasive pathogens, less data are available concerning the mucosal or physiological fluid strains. At the same time they can serve as the source of various infections, besides the invasive infections also the local ones, for example, coryneforms of male genital tract have been associated with inflammatory prostatitis.

Material and Methods: A total of 62 coryneform isolates from human semen were tested for susceptibility to eight antimicrobial agents using the E test method.

Results: All strains were susceptible to ampicillin-sulbactam and only a few were resistant to penicillin G and TMP/SMX while nearly one third of strains were resistant or intermediate to doxycycline (35%) and norfloxacin (29%), and more than half to clindamycin (63%), nitrofurantoin (62%) and erythromycin (53%). The strains showing resistance to at least 3 antimicrobials belonged to *Corynebacterium* group F1, *Corynebacterium seminale* and *Cellulomonas/Microbacterium* sp. A distinct co-occurring macrolide and lincosamine resistance pattern was common.

Conclusion: Ampicillin-sulbactam, penicillin G and TMP/SMX revealed the highest activity against coryneforms isolated from semen that were frequently resistant or intermediate to several other antimicrobials. Norfloxacin revealed only moderate activity against prostatitis-associated *Corynebacterium* group G.

Keywords: Antimicrobial susceptibility, *Corynebacterium*, coryneform, semen, prostatitis.

INTRODUCTION

Coryneform bacteria are common inhabitants of the mucous membranes and skin. This colonization can lead to infection, especially in neutropenic patients and in patients undergoing surgical manipulations but also in immunocompetent subjects. In addition to the invasive infections these bacteria have been associated also with the local infections, for example, inflammatory prostatitis [1-4].

It is believed that the pathogenicity of coryneform bacteria has been underestimated and often underappreciated, despite increasing numbers of reports associating corynebacteria with human infections. Therefore a renewed interest in *Corynebacterium* and similar species has emerged during the last decades among clinicians and microbiologists alike [5, 6]. At the same time, there are few reports about their antimicrobial susceptibility. These studies have frequently indicated high resistance of coryneform bacteria to antibiotics [6, 7], and it is also proposed that corynebacteria can be the reservoir of drug resistance genes [8]. The susceptibility pattern of coryneform species has been researched mostly in association with emergence of multiresistant nosocomial pathogens, less data are available concerning the mucosal or physiological fluid strains.

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We evaluated the antimicrobial susceptibility of different coryneform species isolated from human semen.

MATERIAL AND METHODS

Study Group

The study was carried out between September 2003 and May 2005 at Tartu University Hospital. The study group included 109 men, of them 37 men were the participants of the prospective study of the etiopathogenesis of chronic prostatitis, and 72 men were the participants of the prospective study Environment and Reproductive Health (EU 6th FP project QLRT-2001-02911). In 50 out of 109 men, leukocytospermia was found, therefore, NIH IIIa category prostatitis (20 men) or NIH IV category prostatitis (30 men) was diagnosed in them according to the NIH Classification of the Prostatitis Syndromes as described [1]. The remaining 59 men had neither pelvic pain/discomfort complaints nor leukocytospermia; therefore, they served as a control group. The mean age in prostatitis patients was 28.5 (SE±0.62) years and 20.0 (±1.32) years in controls. All subjects were at least 18 years old. None of the men had received antimicrobial therapy within 3 months. Participation in the study was voluntary. Informed consent was obtained from the patients. The studies were approved by the Ethics Review Committee on Human Research of the University of Tartu.

Samples

Semen samples were collected by patients after they washed their glans penis with regular soap and water, and

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urinated. The samples were obtained by masturbation and were ejaculated into a sterile collection tube in a private room near laboratories. After ejaculation, the semen was incubated at 37°C for 25 to 45 minutes for liquefaction.

Isolation and Identification of Coryneform Bacteria

The samples were cultured quantitatively to detect anaerobic, microaerophilic and aerobic bacteria within 1 hour from collection. Freshly prepared blood agar and chocolate agar, Wilkins-Chalgren medium (Oxoid, Unipath, Basingstoke, UK) supplemented with 5% horse blood, Wilkins-Chalgren medium supplemented with 5% horse blood and GN supplement (Oxoid), MRS agar (Oxoid) and *Gardnerella vaginalis*-selective agar (Oxoid) were used. Aerobic (blood agar) and microaerobic (chocolate agar, MRS agar and *Gardnerella vaginalis*-selective agar in 10% CO_2 atmosphere) cultures were incubated at 37ºC for 1-3 days, anaerobic cultures (Wilkins-Chalgren media in an anaerobic glove box) for 3-5 days.

Colonies with different morphology were Gram stained and examined microscopically. Primary identification of coryneform bacteria was performed by Gram stain morphology and catalase test. Coryneform bacteria were found from blood agar, chocolate agar, Wilkins-Chalgren medium and *Gardnerella vaginalis* selective agar. They were identified using the API Coryne biochemical identification system (bioMérieux, France) according to manufacturer's instructions with an exception of *Corynebacterium seminale* strains that were identified with beta-glucuronidase test on blood agar with MUG supplement (Oxoid), visualizing a positive reaction as a fluorescence near colonies under 254 nm ultraviolet light.

Bacterial Isolates

Coryneform bacteria were found in semen of 38 men with inflammatory prostatitis and 49 controls; altogether 148 coryneform strains were isolated from semen samples as described elsewhere [1]. Subsequently, 62 randomly selected isolates (36 from inflammatory prostatitis patients and 26 from controls) were included into susceptibility testing: *Corynebacterium seminale* (29 strains; of them 17 from prostatitis patients and 12 from controls), *Corynebacterium* group G (8; 7/1), *C. jeikeium* (7; 2/5), *C. striatum* (4; 4/0), *Dermabacter hominis* (4; 0/4), *Cellulomonas/Microbacterium* sp. (4; 2/2), *Corynebacterium* group F1 (2; 2/0), *Brevibacterium* sp. (1; 0/1), *Turicella otitidis* (1; 0/1), *Arthrobacter* sp. (1; 1/0), and *C. mucifaciens* (1; 1/0).

Susceptibility Testing

The E-test susceptibility testing method was chosen since it has shown a good correlation of MICs with both broth microdilution and agar dilution in tests with *Corynebacterium* spp. [7]. The E test strips (AB Biodisk) on cation adjusted Mueller-Hinton agar (Oxoid) supplemented with 5% of blood were used as described elsewhere [9] and following manufacturer's recommendations. Minimal inhibitory concentrations (MICs) for 8 antibacterial agents were determined. CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) interpretive criteria for corynebacteria were used for penicillin G, trimethoprim-sulfamethoxazole (TMP/SMX), doxycycline, erythromycin and clindamycin [10]. Because no CLSI interpretive criteria for corynebacteria exist as concerns ampicillin-sulbactam, norfloxacin and nitrofurantoin, breakpoints for staphylococci were used for these antibiotics as suggested elsewhere [5, 11, 12]. The strains were considered susceptible (resistant) if their MICs were as follows: penicillin G ≤1 (≥4) μg/mL, ampicillin-sulbactam ≤8/4 (≥32/16) μg/mL, TMP/SMX ≤2/38 (≥4/76) μg/mL, doxycycline ≤4 (≥16) μg/mL, erythromycin ≤0.5 (≥2) μg/mL, clindamycin ≤0.5 (≥4) μg/mL, norfloxacin ≤4 (≥16) μg/mL and nitrofurantoin ≤32 (≥128) μg/mL.

RESULTS

The minimal inhibitory concentrations and numbers of non-susceptible (resistant plus intermediate) strains are presented in Table **1**.

All tested strains were susceptible to ampicillin-sulbactam, one strain was resistant and 3 strains intermediate to penicillin G. Single strains were resistant to TMP/SMX (5%) and doxycycline (6%) but intermediate resistance was common to the latter (29%). More than half of coryneforms were resistant or intermediate to clindamycin (63%), nitrofurantoin (62%) and erythromycin (53%), somewhat less strains to norfloxacin (29%). Similar susceptibility pattern was characteristic to the most common species, *C. seminale*, most of its strains were resistant or intermediate to clindamycin, erythromycin, doxycycline and nitrofurantoin.

One *Cellulomonas/Microbacterium* sp. strain was resistant to 4 (erythromycin, clindamycin, penicillin G and nitrofurantoin), one *Corynebacterium* group F1 strain to 3 (erythromycin, clindamycin, doxycycline) and 3 *C. seminale* strains to 3 antimicrobials (erythromycin and clindamycin combined with norfloxacin, nitrofurantoin or TMP-SMX). In addition, 20 strains (12 *C. seminale*, 4 *Corynebacterium* group G, 2 *D. hominis*, 1 *C. striatum*, and 1 *Cellulomonas/Microbacterium* sp.) were resistant to 2 antimicrobials. The susceptibility of the strains originating from inflammatory prostatitis patients and those of controls were compared (data not shown) and no significant differences were found.

Susceptibility pattern of *Corynebacterium* group G is given in Fig. (**1**), indicating that all investigated strains of this group were susceptible to ampicillin-sulbactam and penicillin G, majority of strains were susceptible to TMP/SMX and doxycycline, half of strains to erythromycin and norfloxacin, quarter of strains to clindamycin and none to nitrofurantoin.

DISCUSSION

Since our strains originated from the male genital tract we have tested their susceptibility mainly to antibiotics commonly used in andrological practice. According to our previous study [1], two coryneforms – rather common *Corynebacterium* group G and rather uncommon genus *Arthrobacter* – were found to be associated with prostatitis. Of them, *Arthrobacter* resisted to nitrofurantoin and clindamycin but *Corynebacterium* group G showed relatively high resistance to many antibacterial agents. The latter microor-

Table 1. Susceptibility of Coryneform Bacteria Originating from Human Semen to 8 Antimicrobial Agents

Organism (No. of Isolates)	Antimicrobial Agent	MIC (μg/mL)			Number (%) of Non-Susceptible Strains**
		Range	50% *	90% *	
Corynebacterium seminale (29)	TMP/SMX ***	<0.0002...>32	0.38	1	1 (3%)
	Doxycycline	0.094...24	6	12	18 (62%)
	Erythromycin	<0.015...>256	1.5	32	18 (62%)
	Penicillin G	0.003...1.5	0.023	0.25	1 (3%)
	Ampicillin-sulbactam	<0.016...4	0.023	0.38	0 (0%)
	Nitrofurantoin	1.5...>512	48	96	16 (55%)
	Norfloxacin	0.064...24	1.5	12	6 (21%)
	Clindamycin	<0.016...>256	>256	>256	18 (62%)
Corynebacterium group G (8)	TMP/SMX	0.25...50	0.44	50	1 (13%)
	Doxycycline	0.125...24	0.38	24	1 (13%)
	Erythromycin	0.023...16	0.625	16	4 (50%)
	Penicillin G	0.064...0.5	0.172	0.5	0 (0%)
	Ampicillin-sulbactam	0.09...0.5	0.19	0.5	0 (0%)
	Nitrofurantoin	96...>256	112	>256	8 (100%)
	Norfloxacin	0.19...12	1	12	4 (50%)
	Clindamycin	0.38...3	1.5	3	6 (75%)
C. jeikeium (7)	TMP/SMX	0.125...2	0.75		0 (0%)
	Doxycycline	0.19...1	0.25		0 (0%)
	Erythromycin	0...12	0.047		2 (29%)
	Penicillin G	0.047...1.5	0.19		1 (14%)
	Ampicillin-sulbactam	0.064...1.5	0.094		0 (0%)
	Nitrofurantoin	3...96	6		3 (43%)
	Norfloxacin	0.19...1.5	0.38		0 (0%)
	Clindamycin	0.1...4	1.5		6 (86%)
C. striatum (4)	TMP/SMX	0.094...0.5			0 (0%)
	Doxycycline	0.064...0.38			0 (0%)
	Erythromycin	<0.016…3			1 (25%)
	Penicillin G	0.016...0.38			0 (0%)
	Ampicillin-sulbactam	0.023...0.38			0 (0%)
	Nitrofurantoin	24...>512			2 (50%)
	Norfloxacin	0.25...0.38			0 (0%)
	Clindamycin	0.004...3			2 (50%)
Corynebacterium group F 1 (2)	TMP/SMX	0.125...3			1 (50%)
	Doxycycline	4...24			1 (50%)
	Erythromycin	1.5...>256			2 (100%)
	Penicillin G	0.094...0.125			0 (0%)
	Ampicillin-sulbactam	0.125...0.125			0 (0%)
	Nitrofurantoin	48...48			2 (100%)
	Norfloxacin	0.5...12			1 (50%)
	Clindamycin	>256...>256			2 (100%)

(Table 1) contd…..

Organism (No. of Isolates)	Antimicrobial Agent	MIC (µg/mL)			Number (%) of Non-Susceptible Strains**
		Range	50% *	90% *	
C. mucifaciens (1)	TMP/SMX	0.25			0 (0%)
	Doxycycline	0.125			0 (0%)
	Erythromycin	<0.016			0 (0%)
	Penicillin G	0.047			0 (0%)
	Ampicillin-sulbactam	0.094			0 (0%)
	Nitrofurantoin	12			0 (0%)
	Norfloxacin	0.125			0 (0%)
	Clindamycin	1.5			1 (100%)
Cellulomonas/ Microbacterium sp. (4)	TMP/SMX	0.012...0.5			0 (0%)
	Doxycycline	0.125...12			2 (50%)
	Erythromycin	0.023...32			2 (50%)
	Penicillin G	0.016...>32			2 (50%)
	Ampicillin-sulbactam	0.047...1.5			0 (0%)
	Nitrofurantoin	16...192			1 (25%)
	Norfloxacin	0.125...8			2 (50%)
	Clindamycin	0.05...>256			2 (50%)
Dermabacter hominis (4)	TMP/SMX	0.19...>32			1 (25%)
	Doxycycline	0.094...0.25			0 (0%)
	Erythromycin	1.5...3			4 (100%)
	Penicillin G	0.19...0.38			0 (0%)
	Ampicillin-sulbactam	0.38...0.75			0 (0%)
	Nitrofurantoin	48...96			4 (100%)
	Norfloxacin	12...24			4 (100%)
	Clindamycin	0.047...0.38			0 (0%)
Brevibacterium sp. (1)	TMP/SMX	0.008			0 (0%)
	Doxycycline	0.047			0 (0%)
	Erythromycin	0.25			0 (0%)
	Penicillin G	0.047			0 (0%)
	Ampicillin-sulbactam	0.125			0 (0%)
	Nitrofurantoin	>512			1 (100%)
	Norfloxacin	2			0 (0%)
	Clindamycin	2			1 (100%)
Turicella otitidis (1)	TMP/SMX	0.032			0 (0%)
	Doxycycline	0.19			0 (0%)
	Erythromycin	0.25			0 (0%)
	Penicillin G	0.047			0 (0%)
	Ampicillin-sulbactam	0.125			0 (0%)
	Nitrofurantoin	>512			1 (100%)
	Norfloxacin	2			0 (0%)
	Clindamycin	2			1 (100%)

(Table 1) contd…..

Organism (No. of Isolates)	Antimicrobial Agent	MIC (µg/mL)			Number (%) of Non-Susceptible Strains**
		Range	50% *	90% *	
Arthrobacter sp. (1)	TMP/SMX	1			0 (0%)
	Doxycycline	0.75			0 (0%)
	Erythromycin	0.047			0 (0%)
	Penicillin G	0.094			0 (0%)
	Ampicillin-sulbactam	0.25			0 (0%)
	Nitrofurantoin	>512			1 (100%)
	Norfloxacin	4			0 (0%)
	Clindamycin	0.75			1 (100%)

* 50% and 90% stand for MIC50 and MIC90, respectively.

** Non-susceptible strains include resistant and intermediate strains. Since ampicillin-sulbactam, nitrofurantoin and norfloxacin are not represented in the CLSI interpretive criteria entry for *Corynebacterium sp*., the interpretive criteria for *Staphylococcus sp*. have been used for those three antimicrobial agents, instead.

*** TMP/SMX: trimethoprim-sulfamethoxazole

ganism was often resistant to norfloxacin that is commonly used for treatment of male genital tract infections. Since treatment of prostatitis usually does not aim for a particular target, susceptibility of possible pathogens may give valuable information for choosing an antibiotic.

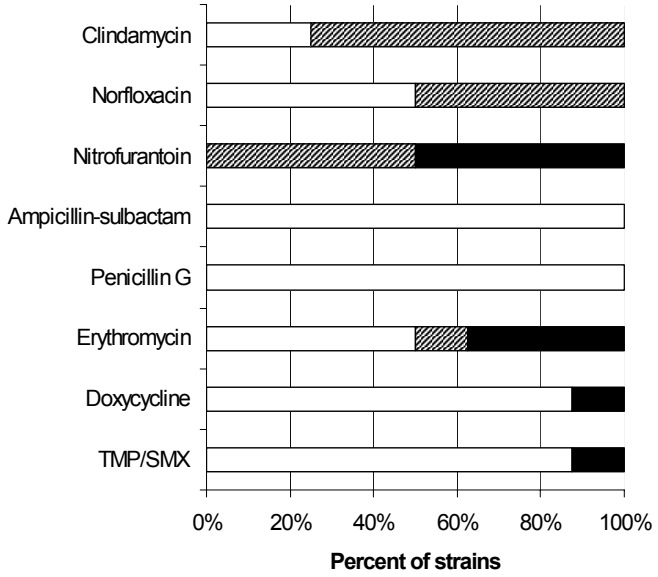

Fig. (1). Susceptibility pattern of *Corynebacterium* group G. White – susceptible; striped – intermediate; black – resistant.

Our resistance data principally agreed with the previous data, although there exists more information about invasive than mucosal strains. According to a rule of the thumb, mucosal strains are usually more susceptible than the invasive ones but there are exceptions like pneumococci [13]. In general, the susceptibility patterns of coryneform bacteria seem to be poorly predictable.

Fluoroquinolones are suggested as cost-effective and safe drugs. At the same time, *C. amycolatum* and *C. striatum* can serve as examples of species against which the activity of fluoroquinolones has rapidly decreased [14]. In our study, *C. jeikeium*, *C. striatum* and majority of the *C. seminale* strains were susceptible to norfloxacin. Although bimodal spread of susceptibility (MIC either <0.5 or >4 µg/mL) to ciprofloxacin has been described [15], similar pattern did not appear in our study.

Resistance to beta-lactam antimicrobials among the coryneforms varies, *C. seminale, T. otitidis* and *Arthrobacter* sp. have been shown to be more susceptible to penicillin G than *C. jeikeium*, *C. striatum*, and *D. hominis* [7, 12, 16, 17]. Our strains were highly susceptible, except *Cellulomonas/Microbacterium* group.

Our data corresponds to several studies that have shown high resistance of coryneforms to macrolides and lincosamines [6, 7, 15, 17, 18] and there is a distinct resistance pattern, MLSb (a co-occurring resistance to Macrolide, Lincosamine and Streptogramin B) [8]. In our study, 16 strains (13 *C. seminale*, 2 *Cellulomonas/Microbacterium* sp. and 1 *Corynebacterium* group F1) showed concurrent resistance to erythromycin and clindamycin.

Unlike earlier studies [6,8], in our study, *C. jeikeium* and *C. seminale* did not have low MICs of nitrofurantoin. Another discord with earlier reports was revealed in case of TMP/SMX that was highly active on the majority of the strains studied by us while weak or missing activity of it against *C. striatum*, *C. jeikeium*, *D. hominis* and *T. otitidis* has been described [18-20]. Our results justify the role of TMP/SMX as the drug of choice for prostatitis in Canada [21].

Resistance to tetracyclines among corynebacteria is controversial – generally multiresistant species like *C. jeikeium* and *C. amycolatum* are relatively susceptible, while *C. seminale* and *C. striatum* quite resistant [7, 18]. *D. hominis, T. otididis* [20] and *Cellulomonas* sp. [7] have been susceptible. With that, our data generally agreed, except that our *C. striatum* strains were susceptible to doxycycline.

In conclusion, ampicillin-sulbactam, penicillin G and TMP/SMX revealed the highest activity against coryneforms isolated from semen that were frequently resistant or intermediate to several other antimicrobials. Norfloxacin revealed only moderate activity against prostatitis-associated *Corynebacterium* group G.

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