ORIGINAL ARTICLE

Gram-positive microorganisms isolated during Chronic Bacterial Prostatitis investigation. A retrospective study

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Abstract

Introduction/Aim: Chronic bacterial prostatitis (CBP) is an inflammatory condition of the prostate that is characterized by pain in the genital or the pelvic area which may accompany urinary disorders and may cause sexual dysfunction. It caused by a variety of uropathogens such as Gram-negative and Gram-positive microorganisms. The pathogenicity of most Gram-positive microorganisms has been questioned, since most leading experts restrict the list of CBP pathogens to the sole *Enterobacteriaceae plus Enterococcus spp.* In order to clarify the role of Gram-positive microorganisms on CBP and investigate the treatment options we reviewed our database of CBP cases from 2008 onwards.

Material: The material of this retrospective study consisted in Gram-positive bacterial isolates from urine and/or prostatic secretions or sperm cultures (total ejaculate) obtained from individuals with reported chronic pelvic discomfort and genital pain, with or without lower urinary tract symptoms and sexual dysfunction, and from patients with febrile relapses of

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CBP, visiting the Urology Department of the Tzaneio Prefecture General Hospital of Piraeus, Greece, from 03/2008 to 11/2018. Demographic, microbiological and clinical history of each assessed patient were reviewed.

Results/Conclusions: In total, 188 out of 314 Gram-positive bacterial isolates were monomicrobial and the remaining 126 polymicrobial. A vast variety of Gram-positive bacteria was found in positive cultures, with coagulase negative *Staphylococci* (CoNS, mainly *S. haemoliticus*, *S. hominis*, *S. epidermidis* and rarely *S. lugdunensis*) being the most frequent pathogens (85 monomicrobial and 43 polymicrobial isolates). As far as the outcomes of follow-up visits are concerned, bacterial eradication was achieved in 213 cases though 135 were completely clinically cured. In the remaining 78 cases bacterial elimination was not accompanied by clinical improvement. Bacterial persistence occurred in 70 cases. 41 out of these were superinfections and the remaining 29 were true persistences. In conclusion, the data from the present study suggest that Gram-positive pathogens can be responsible for prostatic infection. Multidrug resistance for CoNS and *Enterococci* is an emerging medical problem that may cause important threats to public health in the future.

INTRODUCTION

Chronic bacterial prostatitis (CBP) is an inflammatory condition of the prostate that is characterized by pain in the genital or the pelvic area which may accompany urinary disorders and may cause sexual dysfunction. It caused by a variety of uropathogens such as Gram-negative and Gram-positive microorganisms. The pathogenicity of most Gram-positive microorganisms has been questioned, since most leading experts restrict the list of CBP pathogens to the sole *Enterobacteriaceae* plus *Enterococcus spp.*[1]. According to a conservative approach, Gram-positive organisms represent contamination when found in a culture specimen, and patients with these bacteria localized into prostate specimens are currently considered to have CPPS[2]. However, prompt symptom resolution after antibiotic therapy of patients showing *Streptococci* or *Staphylococci* in their prostatic secretions indicates, albeit indirectly, that species other than *E. coli, Proteus* spp. or *Klebsiella spp.* may be involved in the pathogenesis of CBP. In order to clarify the role of Gram-positive microorganisms on CBP and investigate the treatment options we reviewed our database of CBP cases from 2008 onwards.

Key words
prostate, Prostatitis, Chronic Bacterial Prostatitis, Fluoroquinolones, Levofloxacin; Macrolides; Azithromycin, Gram-positive pathogens, *Enterococcus faecalis*, *Coagulase-negative Staphylococci*

METHODS

Material:

The material of this retrospective study consisted in Gram-positive bacterial isolates from urine and/or prostatic secretions or sperm cultures (total ejaculate) obtained from individuals with reported chronic pelvic discomfort and genital pain, with or without lower urinary tract symptoms and sexual dysfunction, and from patients with febrile relapses of CBP, visiting the Urology Department of the Tzaneio Prefecture General Hospital of Piraeus, Greece, from 03/2008 to 11/2018. Demographic, microbiological and clinical history of each assessed patient were reviewed.

Inclusion criteria

The only Inclusion criteria were a diagnosis of category II CBP according to National Institutes of Health (NIH) criteria and a microbiological assessment of causative pathogens.

Exclusion criteria

Patients suffering from conditions that influence bacterial virulence or host response (eg. immunodeficiency, abnormalities of the urogenital system) and patients who received antibiotics or immunosuppressive treatment within 4 weeks of the recorded visits were excluded from the study. Patients diagnosed upon investigation of diseases other than CBP (e.g. category I acute bacterial prostatitis, category III chronic prostatitis/chronic pelvic pain syndrome, overt symptomatic benign prostatic hyperplasia, neoplasia, etc.) as well as patients harboring confounding factors (such as indwelling catheters, cystostomy, ureterostomy, ureteral

stents, previous prostatic surgery or radiotherapy, incomplete compliance to antibacterial therapy assessed by interviewing patients at V1) were also excluded.

Patient assessment

Briefly, in all patients attending the prostatitis clinic a complete clinical history is collected and a copy of NIH Chronic Prostatitis Symptom Index (NIH-CPSI) and International Prostate Symptom Score (IPSS) questionnaires is administered. Urological visit include also digitorectal examination and urine and/or prostatic secretion sample collection, abdominal ultrasound and post-void residual measurement.

Accordingly to our database eligible patients underwent either the Meares-Stamey "4-glass" test (based on cultures of first-void -VB1, midstream/pre-prostatic massage -VB2, expressed prostatic secretions -EPS and post-prostatic massage urine -VB3 specimens) or the "two-glass" test[3], assessing the sole VB2 and VB3 specimens. Few patients rejected digital rectal examination -and the subsequent "2-glass" or "4-glass" test- and were evaluated with total ejaculate cultures (sperm cultures).

Depending on medical history and specific symptoms, urethral smear cultures and total ejaculate cultures were additionally obtained from several patients. Patients presenting with febrile prostatitis were investigated by a midstream urine culture (MUC) only. Appropriate antimicrobials -accordingly to antimicrobial susceptibility test- were administered to confirmed cases of CBP for a period of 4 weeks (a few patients received a 2 week treatment regimen).

Microbiological evaluation

The Meares-Stamey and the two-glass tests were considered positive when: 1) bacteria grew in the culture of expressed prostatic secretion (EPS) and VB3 urine sample and did not in VB1 and VB2 sample; 2) bacterial colonies in VB3 were higher in number compared to VB1 and VB2 samples. Given that no standard cut-off level of the number of bacteria in both urine and prostate secretion samples is defined by consensus for the diagnosis of chronic bacterial prostatitis, we defined no lower acceptable level for either one. Cultures, identification and semi-quantitative assay for *Mycoplasma hominis* and *Ureaplasma urealyticum* were performed using the Mycoplasma IST 2 kit (bioMerieux). *Chlamydia trachomatis* was detected by direct immune-fluorescence (monoclonal antibodies against lipopolysaccharide membrane, Kallestad). Urine samples were cultured undiluted in blood and MacConkey agar plates (Kallestad Lab., TX, USA) and subjected to centrifugation for microscopic examination of the sediment. Evaluation of culture results was performed by two specialist microbiologists, who not informed about patient records. Identification of traditional pathogens was performed by conventional methods and the Vitek-2 Compact (bioMerieux, France) system and susceptibility testing was performed by disc diffusion and/or the Vitek-2 system. Interpretation of susceptibility results was based on Clinical and Laboratory Standards Institute (CLSI) guidelines[4].

Outcome

Follow-up included interview, physical examination and the "2-glass" or "4-glass" test. The microbiological response to antibacterial therapy was defined in a manner similar to that of Naber et al.: (i) eradication: baseline pathogen was eradicated; (ii) persistence: baseline pathogen was not eradicated; (iii) superinfection: baseline pathogen was eradicated with the appearance of a new pathogen[5]. Clinical symptoms were scored with the NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) and the International Prostate Symptom Score (IPSS).

Statistical analysis

Statistical analysis was performed using the Fisher's exact test. The level of significance accepted in this study was 0.05 (P value <0.05 is significant).

The local Ethical Committee approved the research protocol for the present retrospective study.

RESULTS

Demographics

357 Gram-positive bacterial isolates were obtained from eligible patients assessed in 1549 visits recorded during a period of 10 years (2008-2018). In 43 of them, bacterial colonies in VB3 were smaller in number compared to VB1 and VB2 samples and they were excluded from further evaluation. Finally, 314 positive bacterial isolates were considered as the material of this study. 153 out of these patients were evaluated with the two-glass test, 14 were evaluated solely with total ejaculate cultures and the remaining 147 with the Meares-Stamey test. Demographic and microbiological data for the present study are presented in Table 1. There was a wide variety of chronic symptoms and symptom combina-

Table 1 *Patient demographic and microbiological data*

Clinical sample	Number
Number of Patients	314
Average Age	45.1
Patient assessment	
Two Glass Tests	153
Four Glass Tests	147
Mid-stream urine only cultures (febrile cases)	3
Sperm cultures (total ejaculate)	14
Microbiological sample	
Cultures of prostatic secretions	45
Urine samples collected after prostate massage	255
Mid-stream urine only cultures (febrile cases)	3
Sperm cultures (total ejaculate)	14
monomicrobial infection	188
polymicrobial infection	126

Table 2 *Main and coexisting symptoms*

N	Main symptom	Coexisting symptoms, if any
114	Scrotal and/or testicular pain	Pain in the pelvic area, penile pain, attenuation of libido, erectile dysfunction, frequent micturition
58	Pain in the pelvic area	Pain at the lower back, perineal pain, burning on the top of the penis or along the urethra, erectile dysfunction, urinary frequency and urgency, intermittent flow of urine, urethral discharge, hematuria
44	Perineal discomfort	Painful urination, sexual dysfunction, frequency and urgency, disorders of sexual desire
32	Penile burning	Pain localized to the lower back, erectile dysfunction, premature ejaculation, urethral discharge
28	Pain localized to the prostate	Pain or burning sensation during micturition, sexual dysfunction
21	Suprapubic pain	Pain in the pelvic/penile area, painful ejaculation
11	painful ejaculation	Pain in the pelvic/penile area, premature ejaculation, painless epididymal swelling
3	High fever or low-grade fever associated with a history of prostatitis	Intermittent flow of urine, frequency and urgency
3	High fever or low-grade fever associated with a history of prostatitis	Intermittent flow of urine, frequency and urgency

tions reported by the patients with scrotal/testicular discomfort being the most frequent (Table 2). In most cases, symptoms lasted more than three months before the diagnosis.

Microbiological assessments

Only 45 out of the 147 Meares-Stamey tests provided sufficient amounts of expressed prostatic secretions (EPS). In only 16 out of these 45 cases, findings of EPS were identical to that of the subsequent VB3. In the remaining cases (microbiologically investigated either with the Meares-Stamey "4-glass" test or the "two-glass" test) the microbiological diagnosis was mainly based on VB3 culture findings. Of a total of 51 total ejaculate cultures performed, 33 were obtained complementary to EPS/VB3 cases. In 16 out of 33 cases sperm cultures were similar to EPS/VB3 cultures. The remaining 14 cultures allowed diagnosing bacterial infection cases, while the EPS/VB3 cultures were negative.

In total, 188 out of 314 Gram positive bacterial isolates were monomicrobial and the remaining 126

Table 3a *Monobacterial isolates from EPS samples*

N	Pathogen	cfu/ml	Susceptibility
3	*Enterococcus faecalis*	Not provided	full sensitive
2	*Enterococcus faecalis*	Not provided	res to quinupristin, gentamycin
2	*Enterococcus faecalis*	Not provided	res to erythromycin, tetracyclin, gentamycin
1	*Enterococcus faecalis*	5000	**sens to minocycline**
1	*Enterococcus faecalis*	Not provided	res to te, intermediate to rd
1	*Enterococcus faecalis*	Not provided	res to ery, teicoplanin
1	*Enterococcus faecalis*	Not provided	res to cn, te, erythromycin
1	*Enterococcus faecalis*	Not provided	res to amc, cxm, kf, sam, ampicillin
1	*Enterococcus faecalis*	Not provided	res to lev, ery, gn, teicoplanin
1	*Enterococcus faecalis*	Not provided	res to te, lev, rd, ery, gn
1	*Enterococcus faecalis*	10.000	res to quinolones
2	*CoNS (not identified)*	Not provided	res to penicillin, macrolides, tetracycline
2	*CoNS (not identified)*	Not provided	res to TMP-SMX
1	*CoNS (not identified)*	300	full sensitive
1	*CoNS (not identified)*	Not provided	res to e, da, te, fd, p, fox, intermediate to lev
1	*CoNS (not identified)*	Not provided	res to p
1	*CoNS (not identified)*	Not provided	res to e, fd, sxt, lev, cn, fox, p
1	*CoNS (not identified)*	Not provided	Not provided
1	*CoNS (not identified)*	Not provided	res to Penicillin, Macrolides, Tetracycline
1	*CoNS (not identified)*	Not provided	**sens to ciprofloxacin, gentamycin**
1	*CoNS (not identified)*	Not provided	res to fd
1	*Staphylococcus lugdunensis*	Not provided	res to p
1	*Streptococcus anginosus*	Not provided	full sensitive
1	*Streptococcus agalactiae*	Not provided	full sensitive
1	*Streptococcus agalactiae*	Not provided	res to tetracycline, erythromycin
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polymicrobial. A vast variety of Gram-positive bacteria was found in positive cultures, with coagulase negative *Staphylococci* (CoNS, mainly *S. haemoliticus*, *S. hominis*, *S. epidermidis* and rarely *S. lugdunensis*) being the most frequent pathogens (85 monomicrobial and 43 polymicrobial isolates). In addition, 18 out of the 26 urethral smear cultures revealed coexisting urethral infection. Detailed microbiological data for the present study are presented in Table 3.

Follow-up visits

As far as the outcomes of follow-up visits are concerned, bacterial eradication was achieved in 213 cases though 135 were completely clinically cured. In the remaining 78 cases, bacterial elimination was not accompanied by clinical improvement. Bacterial persistence occurred in 70 cases. 41 out of these were superinfections and the remaining 29 were true persistences. 31 cases were lost to follow up.

Table 3b *Polybacterial isolates from EPS samples*

N	Pathogen	cfu/ml	Susceptibility
1 1	*CoNS (not identified)* *Gemella morbillorum*	10000 11000	res to TMP-SMX full sensitive
1 1	*CoNS (1st)* *CoNS (2nd)*	3000 500	res to meth, pen, tetra, macrolides full sensitive
1 1	*CoNS (not identified)* *Streptococcus mitis oralis*	Not provided Not provided	full sensitive full sensitive
1 1	*Enterococcus Faecalis* *CoNS (not identified)*	Not provided Not provided	**sensitive to vanc, teicopl, linez, levofloxacin** full sensitive
1 1	*Enterococcus* *Streptococcus milieri*	Not provided Not provided	res to quin, ery, tetracycline full sensitive
1 1	*CoNS (1st)* *CoNS (2nd)*	Not provided Not provided	res to pen ,fd ,te, fox ,ery res to pen, ery, fd, te ,sxt ,cn
1 1	*CoNS (1st)* *CoNS (2nd)*	Not provided Not provided	full sensitive full sensitive
1 1	*CoNS (1st)* *CoNS (2nd)*	Not provided Not provided	res to p,fd,c,tob,ery res to ery,c
1 1	*CoNS (not identified)* *Enterococcus faecalis*	Not provided Not provided	res to te ,p, fox, tob e, da, ak, cn res to te, ,intermediate to erythromycin
1 1	*Enterococcus faecalis Escherichia coli*	Not provided Not provided	res to te,e res to ampicillin, ,te
1 1	*Staphylococcus CoN* *Streptococcus agalactiae*	Not provided Not provided	res to da,e,te,fd,p,c,fox,tob res to e
1 1 1	*Enterococcus faecalis* *E coli* *CoNS (not identified)*	Not provided Not provided Not provided	res to ery,te res to amp,amc,sam,kf,fox,sxt res to p,fox,sxt,ery,da,tob,cn,fd
1 1 1	*Enterococcus faealis* *Klebsiella pn* *Proteus*	Not provided Not provided Not provided	full sensitive full sensitive full sensitive
1 1 1	*Enterococcus,* *E Coli,* *Proteus*	Not provided Not provided Not provided	full sensitive full sensitive full sensitive
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DISCUSSION

With the exception of the very low number of febrile prostatitis relapses (3 cases) and the higher average age of patients, no differences in demographic and clinical features and epidemiological characteristics exist between patients with Gram-positive and patients with Gram-negative CBP since they are all largely consistent with that of our previous published or unpublished studies[6].

A very interesting finding of this study is the variety of Gram-positive pathogens detected, as well as the variety of their combinations in polymicrobial isolates from EPS and VB3 samples.

Some clinicians and microbiologist debate the role of Gram-positive organisms other than *Enterococci*[7] and for this reason colony forming unit (cfu) data for several bacteria (of the isolates from EPS samples are missing from our database.

Arguments against Gram-positive organisms' pathogenicity are mainly based on three facts. First, the low incidence of Gram-positive organisms other than *Enterococci* in isolates from expressed prostatic secretions (EPS) and post-prostatic massage urine (VB3) specimens of patients with CBP, second the rarity of concomitant leucocytic reaction in EPS (that always occurs in the pres-

Table 3c *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1	*Enterococcus faecalis*	400	**sens to: vanco, levofloxacin**
16	*Enterococcus faecalis*	200-100000	full sensitive
6	*Enterococcus faecalis*	200-6000	res to: ery, tetracycline
1	*Enterococcus faecalis*	400	res to: levo, macrolides
1	*Enterococcus faecalis*	200	**sens to: amoxicilin**
6	*Enterococcus faecalis*	400-13000	res to: tetra, erythromycin
3	*Enterococcus faecalis*	800-2000	res to: ery, tetra, quinupristin
1	*Enterococcus faecalis*	1400	res to: macrolides, sxt
20	*Enterococcus faecalis*	600-1000	res to: erythromycin
1	*Enterococcus faecalis*	400	res to: tetra, levo, gn, erythromycin
1	*Enterococcus faecalis*	2000	**sens to: vanco, linez, dalfo, teicoplanin**
1	*Enterococcus faecalis*	60000	**sens to: amp, line, teicoplanin**
2	*Enterococcus faecalis*	1500-10000	res to: quinolones
3	*Enterococcus faecalis*	500-10000	res to: ery, genta, dalfopristin
1	*Enterococcus faecalis*	600	res to: tetra, interm to erythromycin
1	*Enterococcus faecalis*	2000	res to: tetra, vanco, tigecyline
2	*Enterococcus faecalis*	200	res to: tetra, inter to rd
2	*Enterococcus faecalis*	5000-40000	res to: ery, cipro, levofloxacin
1	*Enterococcus faecalis*	5000	res to: dalfo, tetracycline
1	*Enterococcus faecalis*	1500	res to: ampicillin
1	*Enterococcus faecalis*	9000	res to: ampicilin, sxt
3	*Enterococcus faecalis*	3000-10000	res to: ery, genta, tetra, dalfo, clindamycin
1	*Enterococcus faecalis*	2500	res to: cn, te, e, rd
2	*Strept mitis-oralis*	300-2200	full sensitive
2	*Staph aureus MRSA*	>100000	res to pen,fox,e,da,lev,tob
2	*Stahp haemoliticus*	8000	Not provided
1	*Staph hominis*	5000	Not provided
1	*Staphylococcus aureus*	2000	res to penicillin, tobramycin
4	*Streptococcus agalactiae*	100-12000	full sensitive
1	*Streptococcus agalactiae*	200	res to ery, dalfopristin
1	*Strept parasanguinis*	3000	Not provided
1	CoNS (not identified)	1000	res to p, fox, c, lev, fd, sxt, te, e, da
1	CoNS (not identified)	100000	res to: tetracyclines
1	CoNS (not identified)	800	res to ery, pen, methicillin,fusidic acid
6	CoNS (not identified)	200-1400	res to: fd, ery
1	CoNS (not identified)	400	res to pen, fd, c, tob, erythromycin
1	CoNS (not identified)	900	res to: pen, fox, ak, ery, sxt, tob, lev, cn
5	CoNS (not identified)	1200-8000	res to: erythromycin
21	CoNS (not identified)	400-100000	full sensitive
1	CoNS (not identified)	2000	**sens to cefoxitin, clindamycin, penicillin**

Table 3c *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1	CoNS (not identified)	Not provided	res to: sxt, tetracyclin
2	CoNS (not identified)	500-10000	res to: pen, fox, ery, da, fd, sxt, lev
1	CoNS (not identified)	500	res to: pen, fox, e, fd, tetracycline
5	CoNS (not identified)	400-3500	Not provided
1	CoNS (not identified)	100	res to: fd, cn, ery, da, pen, tetracycline
2	CoNS (not identified)	1000-30000	**sens to: tetra, linez, rifampicin**
1	CoNS (not identified)	1000	res to: meth, pen, clind, ery, gentamycin
2	CoNS (not identified)	200-400	res to: pen, fd
4	CoNS (not identified)	3000-10000	res to: ampicillin
1	CoNS (not identified)	500	**sens to: ciprofloxacin, gentamycin**
3	CoNS (not identified)	100-6000	res to: fd, erythromycin
1	CoNS (not identified)	>100000	res to: pen, fox
2	CoNS (not identified)	300-700	res to pen, fd, ery, fox, tetracycline
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ence of Gram-negative in the EPS)[8] and third the lack of documentation of recurrent urinary tract infections[9].

On the other hand, the literature strongly suggests that urologic diseases involving Gram-positive bacteria may be easily overlooked due to limited culture-based assays typically utilized for urine in hospital microbiology laboratories[10]. Moreover, "negative" cultures may be often reported despite the presence of Gram-positive bacteria due to high bacterial count cut-offs established by laboratories (e.g., 50 000 CFU)[11]. Actually, low-count bacterial infection is possible, given the nature of CBP, the local conditions of the prostate gland and the peculiarities of EPS and urinary specimens after prostatic massage.

Still, current evidence suggests that the finding of high leukocyte counts in EPS has not been shown to give meaningful information regarding chronic prostate inflammation. In confirmation to the above, a recent study demonstrated no significant differences in white blood cell (WBC) counts in expressed prostatic secretion (EPS), between culture-positive and negative groups in patients with new bacterial prostatic infection after transrectal biopsy[12].

Finally, category II chronic bacterial prostatitis (CBP) was traditionally defined as recurrent symptomatic UTIs caused by the same organism detected in prostatic secretions, occurring between asymptomatic periods[13]. Nonetheless, current evidence suggests that, regardless of causative pathogens, CBP patients are mainly presenting with symptoms comprising pain accompanied or not by urinary, sexual and/or ejaculatory disturbances[14]. In fact, the majority of our study population showed a complex clinical presentation combining pain with genitourinary symptoms. Testicular/scrotal pain was highlighted as the patients' main clinical manifestation (36.3%). This finding is in accordance with that of other studies (showing even greater incidence of testicular pain -44.3%[15]). The reason explaining the high prevalence of this specific symptom is unknown however it is possibly caused by spasm of ejaculatory dycts.

In the present article, we have focused on Gram-positive microorganisms isolated during CBP investigation. In order to explore possible geographical and time trends in CBP pathogen prevalence, we have extracted synchronous (years 2009-2015) data from an Italian database from a secondary referral prostatitis clinic. The database contained data from 151 consecutively assessed patients, diagnosed with cat. II CBP matching the inclusion/exclusion criteria for the present study. Besides the high frequency of *E. faecalis* isolates, the most remarkable similarity between Greek and Italian databases was the wide array of different Gram-positive species isolated from CBP patients (Tables 5a,5b).

Currently, Gram-positive bacteria tend to be the

Table 3d *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1 1	CoNS (1st) CoNS (2nd)	100 1000	res to: meth, pen, tetra, macrolides
1 1	*Enterococcus faecalis* CoNS (not identified)	1000 700	**sens to: vanco, teico, linez, levo** full sensitive
4 4	*Streptococcus agalactiae* CoNS (not identified)	1000-2600 400-3100	full sensitive full sensitive
2 2	*Enterococcus faecalis* *E Coli*	1500-1800 1500-5500	res to sxt res to ampicillin
1 1	CoNS (not identified) *E Coli*	5000 10000	**sens to clindamycin, linesolid** res to sxt,ciprofloxacin
1 1	*Enterococcus faecalis* *Citrobacter freundii*	30000 5000	res to dalfopristin, tetracycline res to cefoxitin, piperacillin
5 5	*Enterococcus faecalis* CoNS (not identified)	4000-15000 500-3000	res to dalfopristin, tetracycline full sensitive
2 2	Enterococcus faecalis CoNS (not identified)	100-10000 1000-4000	full sensitive res to tetracycline, erythromycin
1 1	CoNS (not identified) *Staphylococcus aureus*	80000 10000	res to penicillin res to penicillin, erythromycin
1 1	*Enterococcus faecalis* CoNS (not identified)	2000 800	res to tetra, dalfo, clindamycin res to ampicillin
1 1	*E coli* *Staphylococcus aureus*	400 200	full sensitive full sensitive
1 1	*Enterococcus faecalis* *Staph epidermidis*	1200 1100	res to: sxt res to: fusidic acid
1 1	*Enterococcus faecalis* CoNS	>100000 not provided	res to: tetra, ery, quinupristin not provided
1 1	CoNS (1st) CoNS (2nd)	2600 300	res to: p, fox, ak, e, sxt, tob, lev, cn res to: p, fox, fd
1 1	CoNS (1st) CoNS (2nd)	1400 1000	res to: p, fd res to: cn, ery, da,fd,te intermediate to tob
1 1 1	CoNS (1st) CoNS (2nd) CoNS (3rd)	600 400 300	res to fd, ery, da full sensitive res to p, cn, te, fox
2 2	*E Coli* CoNS (not identified)	300-1500 800-1500	full sensitive full sensitive
1 1	*Enterococus faecalis* *Klebsiella oxytoca*	200 100	res to: ery, gn, rif res to: amp, sxt, te
1 1	CoNS (1st) CoNS (2nd)	Not provided Not provided	**sens to: macrolides, aminoglycosides** **sens to: macrolides, aminoglycosides**
2			
2 2	CoNS (1st) CoNS (2nd)	1000 3000	res to: ery, sxt, fusidic acid not provided
1 1	E Coli CoNS (not identified)	5000 >100	full sensitive res to: fusidic acid, erythromycin
1 1	*Staph haemolyticus* *Staph hominis*	100.000 100.000	not provided not provided

Table 3d *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1 1	CoNS (not identified) *E Coli*	3000 1000	not provided res to: cipro, nor, cefuro, sxf, amp, cefotax
1 1	CoNS (not identified) *Enterococcus faecalis*	200 100	res to: p,fox,tob,ery,da,ak,cn, tetracycline res to: tetracycline, interm to erythromycin
1 1	CoNS (1st) CoNS (2nd)	2300 300	res to lev,tob,e,da,sxt,fd res to p,fox,e,fd
1 1	CoNS (not identified) *Streptococcus spp* (n.id)	8000 1800	res to ampicillin not provided
1 1	*Acinetobscter* CoNS (not identified)	200 1 500	full sensitive **sens to: sxt, amikacin, tetracycline**
1 1	*Enterococcus faecalis* *Streptococcus agalactiae*	2000 2500	full sensitive not provided
1 1	*Staph haemoliticus* *Staph epidermidis*	5000 800	full sensitive res to erythromycin, clindamycin
1 1	*E. coli* *Enterococcus faecalis*	8000 20000	res to sxt, tetracycline res to ery, sxt, tetracycline
1 1	*Klebsiella* *Enterococcus faecalis*	200 3000	res to: ampicillin res to: tetracycline, erythromycin
2 2	CoNS (not identified) *Streptococcus agalactiae*	1000-2500 100-500	not provided not provided
1 1	CoNS (1st) CoNS (2nd)	100 200	res to fd,c,e,cn,fox,sxt, penicillin res to penicillin
1 1	CoNS (1st) CoNS (2nd)	1500 2000	res to ery,lev,p,da,fox,fd res to ery,fd,te
4 4 4	*E Coli* *Enterococcus faecalis* CoNS (not identified)	1000-2500 500-1000 200-1300	full sensitive full sensitive res to tetracycline
1 1	*Oligella Urethralis* *Enterococcus faecalis*	300 2500	res to: ciprofloxacin res to: tetracycline, interm to erythromycin
1 1	CoNS (not identified) *Enterococcus faecalis*	1000 2000	res to sxt res to ampicillin
3 3	CoNS (not identified) *Enterococcus faecalis*	500-1300 600-2000	res to cipro, levo, tetra, sxt, erythromycin res to tetracycline
1 1	CoNS (not identified) *Candida*	2500 not provided	full sensitive not provided
1 1	*Proteus mirabilis* *Enterococcus faecalis*	1400 1000	full sensitive full sensitive
2 2	CoNS (1st) CoNS (2nd)	1200 400	res to fd,e res to fd
1 1	*Klembsiella* *Stahp haemolyticus*	800 2000	full sensitive not provided
1 1 1	CoNS (1st) *Enterococcus faecalis* CoNS (2nd)	800 800 1500	full sensitive res to ery, te res to p, fox, e, da, cn, ak, tob, fd
3 3	*E coli* *Enterococcus faecalis*	2500-11000 200-3000	full sensitive full sensitive

Table 3d *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1 1	CoNS (1st) CoNS (2nd)	3900 1000	res to fd, interm to da res to tob,fd,lev,p,cn,sxt,e, interm to ak,da
4 4 4	CoNS (1st) *Enterococcus faecalis* CoNS (2nd)	800-4500 800-7000 1500-11000	full sensitive res to e,te res to p, fox ,ery, da, cn, ak, tob, fd
3 3 3	CoNS (1st) CoNS (2nd) *E coli*	200 100 5000	res to p,fox,ery,da,c,te,fd,lev res to p.fd.ery res to quinolones
1 1	CoNS (1st) CoNS (2nd)	100 300	res to p, fox, fd intermed to lev, gn res to tob
1 1 1	CoNS (not identified) *Enterococcus faecalis E coli*	100 600 2000	res to fd, fox, penicillin res to ery, tetracycline res to quinolones
1 1	CoNS (not identified) *Enterococcus faecal* *E coli*	100 300 1000	res to cipro, levo, tetra, sxt, erythromycin res to tetracycline res to quinolones
1 1	CoNS (not identified) *Brevundimonas dim/vesic* *Streptococcus salivarius*	300 1500 500	res to: pen, fox, levo, fd, ery, sxt, te res to: ct full sensitive
1 1	*Enterococcus faecalis* CoNS (not identified)	2000 800	res to tetra, dalfo, clindamycin res to ampicillin
1 1	CoNS (not identified) *Candida non albicans*	300 1000	res to fd not provided
1 1	*Enterococcus faecalis* *E coli*	30000 80000	full sensitive res to sxt, tetracyclines
10 10	CoNS (1st) CoNS (2nd)	400-30000 100-20000	full sensitive full sensitive
1 1 1	CoNS (1st) CoNS (2nd) *Pseudom oryzihabitans*	100 200 100	res to fd,p res to ery multisensitive
1 1	*Streptococcus agalactiae* CoNS (not identified)	2000 100	res to e, da res to p, fd, e
1 1 1	CoNS (not identified) *Enterococcus faecalis* *Proteus mirabilis*	100 100 200	res to e,da,fd,p res to cn,te,e full sensitive
3 3	CoNS (1st) CoNS (2nd)	900-2000 300-500	res to p, fd, da res to e, da
4 4	*E coli* CoNS (not identified)	1800-10000 400-15000	res to: quinolones, stx, tetracycline res to macrolides
1 1	*E coli* *Enterococcus faecalis*	2000 2000	res to cip, lev, te, kf, ak, sam, sxt, amp, amc, ctx res to ery, lev, gn, te
1 1 1	*E. coli* *Haemoph parainfluenzae* CoNS (not identified)	700 2000 1000	multisensitive full sensitive res to p,fd,e,te
1 1	CoNS (1st) CoNS (2nd)	600 >100000	not provided res to lev,te,fd,sxt,e,cn

Table 3d *Monobacterial isolates from VB3 samples*

N	Pathogen	cfu/ml	Susceptibility status
1 1	CoNS (not identified) *E coli*	1300 700	**sens to: tetra, linez, rifam, chloramph** res to: cipro, amp, tetracycline.
3 3	CoNS (1st) CoNS (2nd)	900-3200 500-4000	res to pen, fd, da res to ery, da
3 3 3	CoNS (1st) CoNS (2nd) *Haemoph parainfluenzae*	100-1200 600-800 100-800	res to p,fox,c,lev,fd,sxt,te,e,da res to p.te.e,da,fd,lev res to quinolones
1 1 1	*Enterococcus faecalis* CoNS (1st) CoNS (2nd)	400 2500 700	full sensitive res to te,fd,ery,da,p,fox res to p,fox,ery,da,cn,lev,rd,sxt,tob,fd
5	3 different species Gram (+) cocci	not provided	not provided
7	CoNS (1st) CoNS (2nd) *Enterococcus faecalis*	1000 800 500	res to te,e,da,fd res to p,fd res to e,te
5 5	CoNS (1st) CoNS (2nd)	2000-18000 300-14500	res to p,fd,da res to e,da

Table 4 *Clinical and microbiological outcome*

cured	236
Bacterial persistence - Symptom persistence	70
Bacterial eradication - Symptom persistence	78
Unknown outcome	31
Bacterial persistence / superinfections	41
Bacterial persistence / persistence	29

Table 5a *Monomicrobial isolates in an Italian cohort of 151 consecutively assessed patients*

Pathogens	Isolated from EPS/VB3 only	Isolated from total ejaculate only	Isolated from both specimens	TOTAL
Enterococcus faecalis	11	6	3	20
Staphylococcus aureus	3	/	/	3
Staphylococcus coagulase-negative	1	5	1	7
Streptococcus beta-haemolyticus gr. B	/	/	1	1
Streptococcus agalactiae	1	/	/	1
Steptococcus anginosus	/	1	/	1
Kocuria kristinae	/	/	1	1
TOTAL	16	12	6	**34**

Table 5b *Polymicrobial isolates in an Italian cohort of 151 consecutively assessed patients*

Pathogens	Isolated from EPS/VB3 only	Isolated from total ejaculate only	Isolated from both specimens	TOTAL
E.coli + *Enterococcus faecalis*	1	1	2	4
E.coli + *Streptococcus beta-haemolyticus gr. B*	1	/	/	1
E.coli + *Peptostreptococcus spp.*	/	/	1	1
E. faecalis + *Klebsiella spp.*	/	2	/	2
E. faecalis + *Citrobacter spp.*	/	/	1	1
E. faecalis + *Ureaplasma urealyticum*	/	/	1	1
E. faecalis + *Staphylococcus coagulase negative*	1	/	/	1
P. aeruginosa + *Staphylococcus coagulase negative*	/	1	/	1
Streptococcus mitis + *Staphylococcus coagulase negative*	/	/	1	1
E. coli + *E. faecalis* + *Staphylococcus coagulase negative*	/	/	1	1
TOTAL	3	4	7	14

most frequent isolates among EPS and VB3 specimens of patients with CBP. An Italian study of 6221 bacterial isolates from CBP patients showed a 73.9% prevalence of Gram-positive bacterial strains[16]. In a large Chinese cohort of CBP patients, coagulase-negative staphylococcal species were found to be the most prevalent isolates (*S. haemolyticus*, 30%; *S. epidermidis*, 12%)[17]. Three smaller studies from Russia, Spain and Israel also indicated CoNS (mainly epidermidis, hemolyticus and saprophyticus) as the most common causative agent in monomicrobial prostatitis. Other Gram-positive bacteria found among more common isolates in routine culture are other *Streptococcus spp.* and *Staphylococcus aureus*[18, 19, 20].

As a matter of fact, the prostate is prone to infections and any bacteria that reach the urethra, including anaerobes, can cause infection to occur. Although the underlying mechanism remains unknown, urethral dysbacteriosis may be a primary cause of CBP[21]. Other host-related and/or bacteria-related factors may also facilitate the colonization of the prostate gland. Thus, Gram-positive microflora exhibiting pathogenic properties may trigger and maintain chronic inflammation in the prostate. Ivanov et al. supported the above hypothesis by showing phenotypic differences between CoNS isolated from seminal fluid of healthy men and from men suffering from CBP[22]. Similarly, a study on the microbial spectrum of urethra and prostate secretions in patients with CBP showed that the most frequently Gram-positive microorganisms isolated from EPS and urethra had secreted pathogenicity factors and were resistance to multiple antibiotics that could promote their persistence in prostate tissues[23].

The abovementioned facts may explain the boosted resistance patterns of Gram-positive pathogens found in both monomicrobial and polymicrobial isolates of this study. These trends are emerging, given that several Gram-positive microorganisms are tolerant and also develop biofilms on abiotic surfaces such as prostatic calcifications, rendering their eradication difficult[24].

Treating chronic bacterial prostatitis requires prolonged therapy. Resistance patterns and microenvironmental factors should be considered when choosing antibacterial therapy. Traditionally, Gram-positive bacteria were treated with macrolides and tetracyclines. Both agents penetrate the prostate and achieve high concentrations therein. The macrolides are bacteriostatic antibiotics with a broad spectrum of activity against many Gram-positive bacteria. Of them clarithromycin and azithromycin are more active than erythromycin, are effective anti-biofilm agents, exhibit several antinflammatory properties and display antiproliferative and autophagic effects on smooth muscle cells when are

used in long-term treatment.[27] Tetracyclines exhibit activity against a wide range of microorganisms other than Gram-positive, such as Gram-negative bacteria, chlamydiae and mycoplasmas. The introduction of ciprofloxacin in the middle 80s' was a major advancement in CBP treatment since ciprofloxacin demonstrated activity against most uropathogens (*Enterococcus faecalis* included) and displayed good distribution to the prostatic sites of infection, with a convenient pharmacokinetic profile. Numerous modifications have been made to the fluoroquinolone structure in order to further improve the pharmacokinetic profile and antibacterial spectrum resulting in increased activity against Gram-positive bacteria and several atypical microorganisms. In this study, tetracyclines and macrolides were successfully demonstrated to be an alternative to quinolones.

The pathogens most commonly associated with both clinical relapses and superinfections were Enterococcus faecalis, and CoNS. To our knowledge, Gram-positive cocci like Enterococcus faecalis are at the same time the most common uropathogens and the bacteria carrying the most powerful resistance determinants[24]. Emerging molecular data and special culture results suggest that CoNS species cause bacterial prostatitis relapses while both Enterococcus faecalis and CoNS are biofilm formators[25,26].

In conclusion, the data from the present study suggest that Gram-positive bacteria do colonize the urethra and/or prostatic ducts, and can be responsible for prostatic infection. Multidrug resistance in CoNS and *Enterococci* is an emerging medical problem that may cause important threats to public health in the future.

Περίληψη

Εισαγωγή/Σκοπός: Η χρόνια βακτηριακή προστατίτιδα (ΧΒΠ) είναι μια φλεγμονώδης κατάσταση του προστάτη που χαρακτηρίζεται από πόνο στην περιοχή των γεννητικών οργάνων ή της πυέλου μπορεί να συνοδεύεται απο διαταραχές του ουροποιητικού συστήματος και μπορεί να προκαλέσει σεξουαλική δυσλειτουργία. Προκαλείται από μια ποικιλία gram-αρνητικών και gram-θετικών ουροπαθογόνων. Για τα περισσότερα από τα τελευταία έχει αμφισβητηθεί η παθογέννετική τους ιδιότητα, αφού οι περισσότεροι κορυφαίοι εμπειρογνώμονες περιορίζουν τον κατάλογο των παθογόνων μονο στα Enterobacteriaceae και τα Enterococcus spp. Προκειμένου να αποσαφηνιστεί ο ρόλος των θετικών κατά gram μικροοργανισμών στη ΧΒΠ και να διερευνηθούν οι επιλογές θεραπείας, εξετάσαμε τη βάση δεδομένων μας από το 2008 και μετά.

Υλικό: Το υλικό αυτής της αναδρομικής μελέτης συνίστατο σε θετικές κατά Gram βακτηριακές απομονώσεις από ούρα ή/και προστατικές εκκρίσεις ή καλλιέργειες σπέρματος που ελήφθησαν από άτομα με αναφερθεν χρόνιο πυελικό άλγος και άλγος γεννητικών οργάνων με ή χωρίς συμπτώματα από την κατώτερη ουροφόρο οδο, με ή χωρίς σεξουαλική δυσλειτουργία/ς καθώς και από ασθενείς με εμπύρετες υποτροπές της ΧΒΠ που επισκέφθηκαν το Τμήμα Ουρολογίας του Γενικού Νοσοκομείου Πειραιά από 03/2008 έως 11/2018. Προσδιορίστηκε το δημογραφικό, μικροβιολογικό και κλινικό ιστορικό κάθε ασθενούς.

Αποτελέσματα: Συνολικά, 188 από τις 314 gram θετικές βακτηριακες απομονώσεις ήταν μονομικροβιακές και οι υπόλοιπες 126 πολυμικροβιακές. Μια μεγάλη ποικιλία θετικών κατά Gram βακτηρίων βρέθηκε στις θετικές καλλιέργειες, με τους αρνητικούς στην κοαγκουλάση σταφυλόκοκκους (κυρίως haemoliticus, hominis, epidermidis και σπάνια lugdunensis) να είναι τα πιο συχνά παθογόνα (85 μονομικροβιακές και 43 πολυμικροβιακές απομονώσεις). Όσον αφορά την έκβαση εξάλειψη των βακτηρίων επιτεύχθηκε σε 213 περιπτώσεις, αν και μόνο 135 είχαν θεραπευθεί πλήρως. Στις υπόλοιπες 78 περιπτώσεις η εκρίρωση των βακτηρίων δεν συνοδεύτηκε από κλινική βελτίωση. Βακτηριακή εμμονή παρατηρήθηκε σε 70 περιπτώσεις. 41 από αυτές ήταν επιμολύνσεις και οι υπόλοιπες 29 ήταν αληθινή εμμονές).

Συμπέρασμα: Τα δεδομένα από την παρούσα μελέτη υποδηλώνουν ότι τα Gram-θετικά μικρόβια μπορεί να είναι υπεύθυνα για την χρόνια βακτηριακή προστατίτιδα. Η ανθεκτικότητα σε πολλά φάρμακα τους αρνητικούς στην κοαγκουλάση σταφυλόκοκκους και τους Enterococci είναι ένα αναδυόμενο ιατρικό πρόβλημα που μπορεί να προκαλέσει σημαντικές απειλές για τη δημόσια υγεία στο μέλλον.

Λέξεις ευρετηριασμού

προστάτης, προστατίτιδα, χρόνια βακτηριακή προστατίτιδα. φθοροκινολόνες, λεβοφλοξακίνη, Μακρολίδια, αζιθρομυκίνη, Gram-θετικά παθογόνα, *Enterococcus faecalis*, Σταφυλόκοκκοι αρνητικοί στην κοαγκουλάση

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