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Short Communication

Phenotypic differences between coryneform bacteria isolated from seminal fluid of healthy men and men with chronic prostatitis syndrome

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Abstract

We compared the potential phenotypic properties of coryneform bacteria associated with chronic prostatitis syndrome (CPS), such as secretory inhibitor of lysozyme (SIL) and secretory inhibitor of platelet microbicidal protein (SIPMP). A total of 110 clinical isolates of coryneform bacteria isolated from the seminal fluid of healthy men and men with CPS were tested. SIPMP production was tested by inhibiting platelet microbicidal protein (PMP) bioactivity against *Bacillus subtilis*, and was expressed as percentage of inhibition of PMP bactericidal activity. SIL production was tested by inhibiting lysozyme activity against *Micrococcus lysodeikticus* and was expressed in microgram per millilitre of inactivated lysozyme. A significantly higher proportion of CPS strains (58.7% *vs.* 19.2 %) was SIPMP-positive compared with non-CPS strains ($P < 0.01$). Of the CPS strains tested, 77.8% were SIL-positive compared with 34% of the non-CPS isolates ($P < 0.05$). These results suggest that the diagnosis of CPS should not rely solely on classical parameters, for example, the identification and counting of microorganisms, but the functional significance of these parameters must be estimated, possibly by the concentration of different bacterial substrains, detection of opportunistic microorganisms with pathogenic properties, such as pronounced resistance to the cationic antimicrobial peptides, and/or the ability to inhibit the antimicrobial host defence factors.

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Chronic prostatitis syndrome (NIH category III prostatitis) (CPS) is a common urological condition that many clinicians find difficult to diagnose and treat effectively [1, 2]. Recently, coryneform bacteria have been found in segmented specimens (including prostatic secretions) and are postulated to play a role in CPS [1, 3]. On the other hand, coryneform bacteria are part of the normal bacterial flora of seminal fluid [1, 4]. Given that it is difficult to establish precisely the roles of various species of coryneforms in the pathogenesis of CPS, it is imperative to delineate both microbial and host factors that contribute to its development [1, 4].

It has been shown that seminal fluid probably has a highly efficient defence system involving the production of microbicidal agents, probably either peptides or proteins, such as lysozyme, complement and cationic peptides [4–6].

The results of our recent study [7] showed that the production of secretory inhibitor of platelet microbicid-

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al protein (SIPMP) by urethral isolates correlates with the diagnosis of prostatitis. Moreover, we showed that the bacteria's ability to inactivate lysozyme is associated with spermatozoa motility and development of infertility [8]. However, the relationship between production of inhibitors of components of innate immunity by seminal isolates of coryneform bacteria and the clinical source of CPS has not been well studied. Thus, the aim of this study was to assess and compare potential phenotypic properties of coryneforms associated with the source of CPS, such as secretory inhibitor of lysozyme (SIL) and SIPMP.

This study included 127 men ranging from 20 to 35 years old. Patients were recruited from the Orenburg Regional Center for Family Planning and Reproduction. They underwent the following assessment: a physical examination, Meares–Stamey 4-glass localization [9], semen analysis and *trans*-rectal ultrasound. Controls were recruited by poster advertisements and were excluded from the study, if they had a history of symptoms suggestive of CPS. They underwent the same assessment as the patients.

A total of 110 non-duplicate, clinical isolates of coryneform bacteria, isolated from seminal fluid of healthy men and men with CPS, were tested.

The isolates were screened for SIL production according to the method of Bukharin *et al.* [10]. In brief, standard lysozyme solutions (10–100 μg mL^{-1}) were prepared by dissolving the egg-white lysozyme (Sigma Chemical Co., St Louis, MO, USA) in deionized water. To prepare the lysozyme-containing media, 1 mL of each lysozyme dilution was mixed with 9 mL nutrient agar medium (Sifin, Berlin, Germany) and cooled to 48ºC. The agar was solidified in polystyrene culture dishes (Corning Glass Works, Corning, NY, USA). A small (approximately 1-mm) loop of the broth culture of a strain to be tested for production of SIL was stabbed onto the previously poured and dried nutrient agar plates. After incubation for 48 h at 37ºC, the cultures were killed by exposure to chloroform vapours for a period of approximately 1 h, and the chloroform was then allowed to evaporate. After being removed from the chloroform source, each of the plates was overlaid with 3 mL of warm (45ºC) 0.5% nutrient agar containing 0.01 mL of a 24-h broth culture of *Micrococcus lysodeikticus* NCTC 2665. After the overlay of agar, solidified plates were incubated for 24 h at 37ºC and then examined for zones of growth of *M. lysodeikticus* in the areas surrounding the isolate. SIL production of each strain was indicated by growth of *M. lysodeikticus* at maximal lysozyme concentration and was expressed in μg mL^{-1} of inactivated lysozyme. SIPMP production was tested according to the earlier described procedures [11]. The mean values and s.e.m. were calculated. Statistical analyses were carried out using the unpaired *t*-test.

Of the CPS strains tested, 49/63 (77.8%) were considered to be SIL-positive (Table 1) compared with only 16/47 of the non-CPS isolates (34%, $P < 0.05$). Furthermore, a significantly higher proportion of CPS strains (58.7% *vs.* 19.2%) was SIPMP-positive compared with non-CPS strains ($P < 0.01$).

Among the SIPMP-producing strains, organisms

Table 1. SIL and SIPMP production of seminal Corynebacterium isolates.

Organism	SIL-producing strains/total		SIPMP-producing strains/total	
	Healthy men	CPS	Healthy men	CPS
Corynebacterium equi	0/0	11/16	0/0	10/16
C. genitalium	13/37	18/22	6/37	12/22
C. pseudogenitalium	3/10	5/6	3/10	3/6
C. seminale	0/0	15/19	0/0	12/19

Abbreviations: SIL, secretory inhibitor of lysozyme; SIPMP, secretory inhibitor of platelet microbicidal protein; CPS, chronic prostatitis syndrome

Table 2. SIPMP production of seminal Corynebacterium isolates.

No. of organisms	No. of SIPMP-producing strains (healthy men/CPS) with different levels (%) of SIPMP			
(healthy men/CPS)	0	0.1–10.0	10.1–20.0	> 20
Corynebacterium equi (0/16)	0/6	0/10	0/0	0/0
C. genitalium (37/22)	31/10	6/7	4/4	0/10
C. pseudogenitalium (10/6)	7/1	2/3	1/2	0/0
C. seminale (0/19)	0/4	0/2	0/4	0/9

Abbreviations: SIPMP, secretory inhibitor of platelet microbicidal protein; CPS, chronic prostatitis syndrome

Table 3. SIL production of seminal Corynebacterium isolates.

No. of organisms (healthy men/CPS)	No. of SIL-producing strains (healthy men/CPS) with different levels (μg mL^{-1} of inactivated lysozyme) of SIL			
	0	1	2	≥ 3
Corynebacterium equi (0/16)	0/5	0/3	0/3	0/5
C. genitalium (37/22)	24/4	9/4	4/4	0/10
C. pseudogenitalium (10/6)	7/1	2/3	1/2	0/0
C. seminale (0/19)	0/4	0/2	0/4	0/9

Abbreviations: SIL, secretory inhibitor of lysozyme; CPS, chronic prostatitis syndrome

isolated from patients without CPS had mean SIPMP production levels of 6.5% ± 2.2% (Table 2). The culture supernatants of coryneforms from the CPS group were more active in decreasing the levels of PMP-induced killing of *Bacillus subtilis* (16.5% ± 2.5%, $P < 0.05$).

In contrast to the bacteria isolated from the control group (Table 3), strains isolated from men with CPS showed more intensive inhibition of the bactericidal activity of lysozyme (3.5 ± 0.5 μg mL^{-1} *vs*. 0.8 ± 0.5 μg mL^{-1}, $P < 0.05$).

The results of several studies [1, 4, 7] suggest that most persistent infections of the prostate are because of the bacteria that are usually considered normal constituents of the seminal fluid, and hence routinely disregarded and discarded as 'contaminants' in the laboratory. A role for coryneforms in the causation of prostatitis has been suggested earlier [1, 3, 4, 12–13]. There is an urgent need to understand the virulence properties of bacteria that are associated with chronic infection of the prostate. Identifying such factors would be helpful in devising effective treatment strategies. Pathogenic bacteria counteract the host immune defence by excreting various inhibitors. In this work, we detected extracellular bacterial products with remarkable anti-PMP and anti-lysozyme potential. As these peptides may play an important role in the killing of opportunistic microorganisms and preventing the onset of bacterial infection of the prostate, we believe that SIL and SIPMP might enhance the infectivity of corynebacteria. The strategy underlying this process would be straightforward and effective. For example, Bukharin *et al.* [14] showed that production of the SIL by urethral strains correlates with the diagnosis of urethritis. In addition, a recent study by Deckers *et al.* [15] showed that lysozyme inhibitors promote bacterial survival or growth in specific niches in the host. Collectively, our study and the results of several others [4, 7, 8, 11, 14] suggest that the inactivation of components of innate immunity may be important for bacterial pathogens to induce and perpetuate chronic infections in different locations by surviving or avoiding clearance by microbicidal proteins. We believe that new treatment strategies for CPS should aim at neutralizing these bacterial secretory products or inhibiting other pathogenic properties of microorganisms, thereby improving the prostate function [4].

In conclusion, our study and the results of several others [4, 8, 11] suggest that in the future it will be important to discriminate between different forms of persistent infection of the prostate. The diagnosis should no longer rely only on classical parameters, such as the identification and counting of microorganisms. In addition, the functional significance of these parameters must be determined, possibly by analyzing the concentration of different bacterial substrains, detecting opportunistic microorganisms with pathogenic properties, such as a pronounced resistance to cationic antimicrobial peptides, and/or the ability to inhibit the antimicrobial host defence factors, such as PMP and lysozyme.

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