Original Research Article

The impact of *Corynebacterium glucuronolyticum* on semen parameters: a prospective pre-post treatment study

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Abstract

Background: *Corynebacterium glucuronolyticum* (*C. glurucornolyticum*) is a rare isolate that is only recently being acknowledged as a potential urogenital pathogen. The bibliographic references on this bacterial species are scarce, and its influence on all semen parameters was hitherto unknown.

Methods: A prospective approach to compare semen parameters before and after treatment was used in this study. *C. glucuronolyticum* in semen specimens was identified by using analytical profile index biotyping system (API Coryne) and additionally confirmed by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS), with the determination of antimicrobial susceptibility by Kirby–Bauer method. Semen analysis was done according to the criteria from the World Health Organization (with the use of Tygerberg method of sperm morphology categorization). Very strict inclusion criteria for participants also included detailed medical history and urological evaluation.

Results: From a total of 2169 screened semen specimens, the inclusion rate for participants with *C. glucuronolyticum* that satisfied all the criteria was 1.11%. Antibiogram-guided treatment of the infection with ensuing microbiological clearance has shown that the resolution of the infection correlates with statistically-significant improvement in the vitality of spermatozoa, but also with a lower number of neck and mid-piece defects. Parameters such as sperm count, motility and normal morphology were not affected. In addition, susceptibility testing revealed a trend towards ciprofloxacin resistance.

Conclusions: Albeit it is rarely encountered as a monoisolate in significant quantities, *C. glucuronolyticum* may negatively influence certain sperm parameters; therefore, it has to be taken

into account when assessing urogenital samples.

Introduction

Corynebacterium species are a group of Gram-positive bacilli from the class *Actinobacteria* that are increasingly being recognized as opportunistic pathogens [1,2]. They are pleomorphic rods without spores and without a capsule that often appear as hieroglyphic clusters in Gram-stains [3]. Among them, species that found their niche in the urogenital tract are being discerned in taxonomic studies [4-7]. Moreover, one rare isolate characteristic for male individuals that has been properly acknowledged, quite recently, is *Corynebacterium glucuronolyticum* (*C. glucuronolyticum*).

This entity was initially regarded as two distinct species (and subsequently named *Corynebacterium glucuronolyticum* and *Corynebacterium seminale*) [8,9], which was further backed by certain metabolic dissimilarities in esculin hydrolysis. However, genotypic analyses and the observation of 96–97% DNA homology in strains isolated from patients with prostatitis confirmed that it is actually the same species [4,10]. Therefore, nomenclatural priority should be given to the name *C. glucuronolyticum*, albeit the designation *C. seminale* is still commonly encountered in the literature (even in recent publications) – hence, it can be considered as a synonym.

Coryneform bacteria (also referred to as “diphteroids”) in urogenital tract have been generally regarded as saprophytes, but pathogenic potential of *C. glucuronolyticum* is becoming increasingly evident [11]. Akin to some other studies [11,12], our research group previously confirmed its role in male urethritis syndrome [13]; others have shown its potential of causing monomicrobial paucisymptomatic bacterial prostatitis [14]. This species may even cause

encrusted cystitis without the presence of predisposing factors [15].

Nonetheless, thus far only a dozen studies on this pathogen can be found in the relevant references. One of the reasons is that the identification of coryneform bacteria to the species level is usually cumbersome and seldom pursued. There is only one non-prospective, comparative study from France that evaluated the potential influence of *C. glucuronolyticum* known that a panoply of different bacterial pathogens may negatively influence semen parameters; however, only one study has recently observed the influence of seminal coryneform bacteria, as a group, on semen parameters in infertile men, without appraising a putative role of distinct species [17].

Therefore, by employing prospective, pre-post treatment study design with strict inclusion/exclusion criteria, our primary aim was to assess whether the presence of *C. glurucornolyticum*, as an etiologically relevant monoisolate, may negatively affect semen parameters. Additionally, we wanted to obtain insights into the antimicrobial sensitivity profile of isolated strains.

Materials and Methods

Subjects

A total of 2169 male individuals (aged between 18 and 68 years), who visited an outpatient clinic for sexually transmitted diseases during a 4-year period (between 2013 and 2017), gave their semen specimens for microbiological analysis. Following the initial screening and examination of all specimens, only those that exhibited pure-culture growth of *C. glucuronolyticum*, with a large number of colony forming units or CFUs (by employing specific microbiological techniques and analyses described below), were submitted for further diagnostic workup. All patients gave their consent to proceed with the investigation for the purposes of this study.

A first step was to ascertain the presence or absence of any symptoms of the genitourinary tract. After that, each patient's medical history was used to pinpoint any behavioural patterns and/or risk factors that may have a substantial impact on their semen parameters. Such history included questions pertaining to childhood diseases, developmental patterns, prior surgeries, allergies, systemic medical conditions (like diabetes mellitus), family reproductive history, the usage of prescription and non-prescription medications, as well as a review of systems and lifestyle exposures.

Microbiological workup

The postulates for the sterile collection of semen specimens were given to all individuals as instructions prior to sampling. This was done in order to avoid any microbiological contamination from potential non-semen sources (such as commensal organisms from the skin). The cultivation procedure for all semen specimens entailed the usage of Blood Agar Base No. 2 (Oxoid, UK) with 7% defibrinated sheep blood and chocolate agar at 36.7 °C in an aerobic atmosphere supplemented with CO_2, as well as by using MacConkey and Sabouraud agar (Oxoid, UK). White to yellow, non-haemolytic colonies that grew on blood and chocolate agar plates were subjected to Gram-staining, revealing Gram-positive, non-spore-forming bacilli in a distinguishing 'Chinese letters' arrangement. A positive CAMP reaction with staphylococcal β-haemolysin was seen after incubation at 36.7 °C for 24 hours. Subsequently, the final identification of the microorganism as *C. glucuronolyticum* was done by using analytical profile index biotyping strip system – API Coryne (bioMérieux, France).

Only those individuals whose specimens yielded *C. glucuronolyticum* in pure-culture (without the presence of any other isolates) and with colony counts larger than 10^4 CFU/mL were submitted for further diagnostic workup. These isolates (originally confirmed by API Coryne) were additionally confirmed by using matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) (Microflex™ MALDI Biotyper MS, Bruker-Daltonik, Fremont, CA), and these two techniques showed complete correlation. The presence of other aerobic urogenital pathogens that may influence semen parameters (such as *Neisseria gonorrhoeae*, *Escherichia coli*, *Enterococcus faecalis*, etc.), as well as the presence of sexually-transmitted pathogens (such as *Chlamydia trachomatis*, *Ureaplasma* spp., *Mycoplasma hominis*, *Trichomonas vaginalis*), was excluded by employing appropriate

microbiological diagnostic procedures.

Antimicrobial susceptibility was performed by using agar disk diffusion or Kirby–Bauer method [18], according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines and breakpoint tables [19]. Approximately 4–5 colonies of *C. glucuronolyticum* were inoculated in 5 ml of nutrient broth and subsequently incubated for up to 8 hours, until the suspension matched McFarland 0.5 turbidity standard. Those suspensions were then spread over Müller-Hinton agar plates (Oxoid, UK), followed by the placement of applicable antimicrobial discs and incubation in an aerobic environment at 36.7 °C for 24 hos. The inhibition zones were then measured in millimetres and compared to a standard interpretation chart in order to categorize the isolates as susceptible, intermediate susceptible, or resistant.

Semen analysis

In further diagnostic workup, every participant was given clearly written and verbal instructions regarding the proper collection of the semen sample and was provided with a clean, wide-mouthed container made of plastic that is non-toxic for spermatozoa. Semen samples were collected by masturbation after 3-5 days of abstinence from sexual activity, with the initial analysis ensuing soon after liquefaction (30 minutes at 36.7 °C). The concentration of inflammatory cells (leukocytes) was calculated relative to spermatozoa by appraising fixed and stained smears made from undiluted semen.

The analysis was done by employing methods recommended by the World Health

Organization (WHO) [20]. Semen volume was measured by transferring the sample directly into a commercial graduated glass measuring cylinder with a wide mouth. The pH was measured by using pH paper (Merck KgaG, Germany) that was checked against known standards. Total sperm number was calculated by using the Makler counting chamber (Sefi Medical Instruments Ltd., Israel) with 5 μL of well-mixed semen. In order to measure total motility, the parameters of progressive motility, non-progressive motility and immotile spermatozoids were measured at room temperature.

Sperm vitality was determined with the exclusion of vital dye (*i.e.* eosin) from spermatozoid head membranes by using a one-step staining technique with eosin–nigrosin; these results were confirmed with the help of the hypo-osmotic swelling (HOS) test. Papanicolaou staining procedure was used to prepare slides for sperm morphology assessment, and in the analysis a strict (or Tygerberg) method of categorization was used. Alongside measuring the percentage of normal forms, the percentage of head, neck, mid-piece and tail defects, as well as excess residual cytoplasm (ERC), were also noted.

In order to distinguish normal semen specimens from abnormal ones, the WHO's lower reference limits were used for threshold values. Normal specimens were characterized by normozoospermia. This meant that the values of three pivotal sperm parameters (number, motility and morphology) were above the threshold as described by the WHO. Abnormal specimens showed deviations from the references for one or more of the aforementioned parameters – resulting in oligozoospermia when number was affected, asthenozoospermia when motility was affected, and teratozoospermia when morphology was affected (or a combination of these semen quality issues).

Urological examination

A urological examination was conducted on each subject, with a special focus on the genitalia, which included examination of the penis and the urethral meatus, palpation and measurement of the testes, and the eventual presence (and consistency) of both the epididymis and vasa. Also included in the exam was the identification of palpable varicoceles, as well as the appraisal of secondary sex characteristics (such as body habitus and hair distribution). In addition to the extensive physical examination, scrotal colour Doppler ultrasonography was performed on all patients to exclude the possible presence of varicocele or testicular tumours. Furthermore, for approximate measurement of prostate dimension and volume, suprapubic ultrasonography was pursued. Philips ClearVue 650 ultrasound machine with Active Array technology was used for all ultra-sonographic examinations (Philips, Netherlands).

Patients participating in treat/re-test study protocol

From the 35 individuals with *C. glucuronolyticum* > 10^4 CFU/mL, nine of them were excluded from the further study protocol due to the presence of co-infecting agents. Two additional individuals were excluded due to the presence of varicocele and previous radiotherapy. Each of the remaining 24 study participants with *C. glucuronolyticum* > 10^4 CFU/mL and in pure culture was treated according to the antibiogram results of the isolate in question, after providing a semen sample for semen analysis. Microbiological clearance after treatment was tested one week, one month and two months after treatment by using culturing techniques (as described previously). A control semen specimen for semen analysis and final

microbiological clearance confirmation was tested three months after the treatment. Two participants did not return for a control semen analysis; thus, they were not included in the final analysis. Each semen specimen was blinded from the biomedical engineer doing the sperm analysis in order to prevent researcher bias, and the same person did all of the analyses in order to avoid inter-rater reliability issues.

Statistical analysis

The obtained data was double entered into Excel sheets and exported to Statistical Package for Social Sciences version 17 for subsequent analysis (SPSS Inc., USA). Descriptive statistics, including means, medians and standard deviations, were calculated for variables as appropriate. A paired t-test (dependent sample t-test) and analysis of partial correlations were employed in the analysis (their use is further justified in the “Results” section for the sake of clarity). A p-value of less than 0.05 was considered statistically significant.

Results

From a total of 2169 semen specimens screened, 498 of them (22.96%) revealed growth of coryneform bacteria (with a majority of them displaying colony counts smaller than 10^4 CFU/mL). *C. glucuronolyticum* in pure culture and with colony counts larger than 10^4 CFU/mL was established in 35 of semen specimens (1.61%). After applying all the above-mentioned rigorous inclusion and exclusion criteria, as well as ruling out co-infections, a total of 24 individuals (age range from 21 to 52 years with mean age of 35 and median age 34) constituted our cohort group in this study (1.11%). MALDI-TOF MS for final confirmation of these isolates has shown 100% concurrence with API Coryne system. Semen analysis (in line with the WHO criteria) was pursued in study participants before they were subjected to antimicrobial therapy according to the respective antibiogram results.

Generally, isolated strains of *C. glucuronolyticum* showed excellent sensitivity to rifampicin and vancomycin (100% of strains susceptible), very good sensitivity to penicillin G and gentamicin (97.14% and 91.43% of strains susceptible, respectively), modest sensitivity to ciprofloxacin (68.57% of strains susceptible) and low susceptibility to tetracycline and clindamycin (45.71% and 40% of strains susceptible, respectively) (Figure 1). Post-treatment semen analysis was done three months after a successful course of therapy (with microbiological confirmation of bacteriological clearance). Before treatment, five individuals presented with frank symptoms of urethritis and/or prostatitis, whereas after treatment the symptoms persisted in only one of them.

As a part of prospective study approach, comparisons were then made between the semen parameters of individuals before and after the treatment. In the pre-treatment group there were

15 normal semen specimens (*i.e.* normozoospermia) and 9 abnormal ones (*i.e.* those with oligozoospermia, asthenozoospermia, teratozoospermia or a combination), according to the WHO criteria. After treatment just one of the abnormal specimens reverted to the normal group (*i.e.* all parameters were now within normal ranges), while the opposite was not observed. Leukocytospermia was present in 7 specimens before treatment, whereas after treatment it was present in 3 specimens. However, in order to reveal the true influence of treatment (and consequently of *C. glucuronolyticum*) on specific seminal parameters, precise statistical analyses were performed on both normal and abnormal group of semen specimens.

A summary of descriptive data for pre-treatment and post-treatment sperm parameters is presented in Table 1, while detailed breakdown of their sperm morphology is presented in Table 2. Regarding the morphology subcategories, by applying t-test for paired samples, there was a statistically significant difference in the number of spermatozoa with neck and mid-piece aberrations in the group of normal semen specimens ($p < 0.001$); more specifically, although the parameters in this group were generally within reference values, there were significantly less neck and mid-piece defects after treating the infection than before the treatment. Conversely, there were no statistically significant differences in the number of normal forms of spermatozoa, those with aberrant heads or tails, or those with ERC for either of the two groups of semen specimens ($p > 0.05$).

Data summary in Table 1 may point to the conclusion that practically all parameters marginally improved after treatment. In order to properly ascertain whether treating the infection with *C. glucuronolyticum* actually had any statistically significant influence on semen parameters, a stringent partial correlation analysis was pursued (Table 3, Table 4). Consequently, control variables, such as participant's age, days of abstinence, seminal pH

value and leukocytospermia, were not included in the regression analysis due to the low ratio of participant number and predictors (df=18). The results have shown that in the pre-treatment group there was a marginal difference between normal and abnormal semen specimens regarding the total sperm concentration ($r = 0.46$, $p = 0.044$), whilst more significant differences have been observed in total motility ($r = -0.80$, $p < 0.001$) and vitality ($r = -0.57$, $p = 0.009$). On the other hand, in the post-treatment group there was again a marginal difference between normal and abnormal semen specimens regarding the total sperm concentration ($r = -0.48$, $p = 0.044$), and significant difference in total motility only ($r = -0.56$, $p = 0.015$), whereas the correlation with vitality was not significant anymore.

In other words, the latter analysis revealed that, when participant's age, days of abstinence, seminal pH value and leukocytospermia are taken into account, a statistically significant increase of spermatozoa vitality can be observed after the treatment of *C. glucuronolyticum*. Other analysed parameters (more precisely sperm count, motility and normal morphology) were not influenced by the treatment and ensuing microbiological clearance.

Figure 1. Antimicrobial susceptibility of *C. glucuronolyticum* from semen specimens

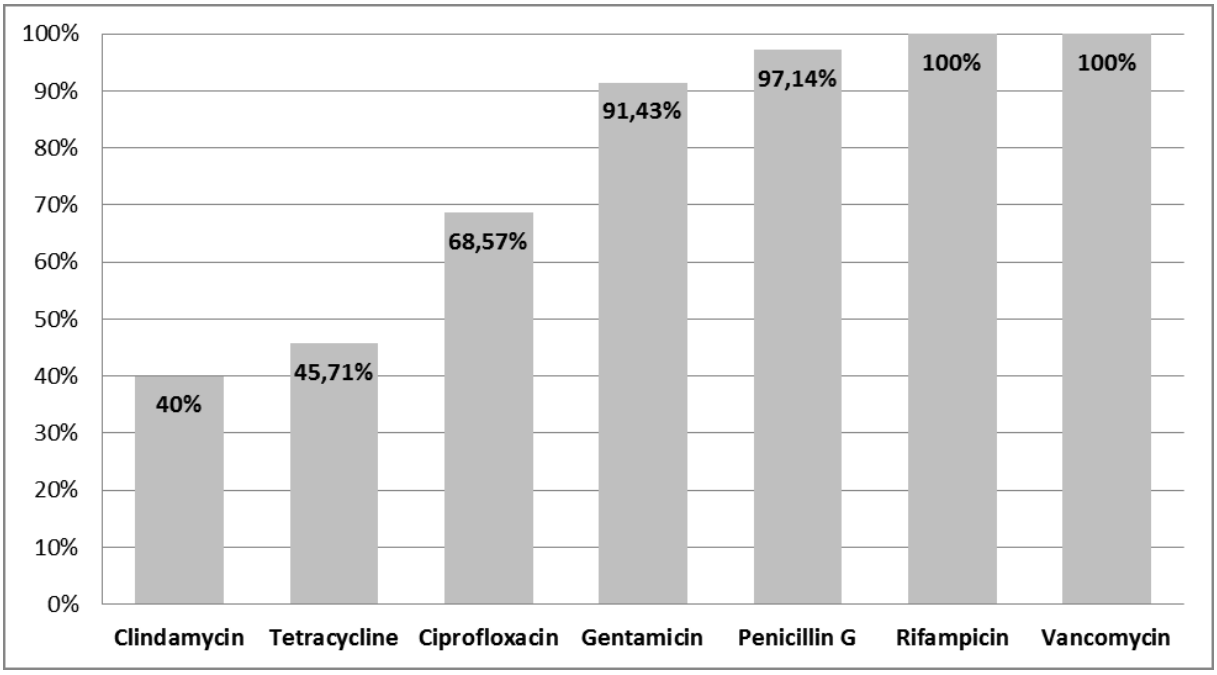

Table 1. Grouped descriptive statistics of the semen parameters before and after the treatment and microbiological clearance of *C. glucuronolyticum* (Note: *SD stands for standard deviation)

		Mean	*Median*	*SD*		*Mean*	*Median*	*SD*
Abstinence (Days)		3.58	3.00	0.72		3.55	3.50	0.60
pH		7.46	7.50	0.30		7.47	7.50	0.25
Sperm Count	**Before Treatment**	52.03	48.50	37.64	**After Treatment**	55.45	47.00	34.79
Total Motility		50.79	52.50	14.76		53.00	56.50	13.65
Vitality		70.79	71.50	8.55		72.86	73.00	6.50
Normal Morphology		16.92	17.00	6.65		18.09	17.00	5.69

Table 2. Grouped descriptive statistics of the spermatozoa morphology before and after the treatment and microbiological clearance of *C. glucuronolyticum* (Note: *SD stands for standard deviation; SEM stands for standard error of the mean; ERC stands for excess residual cytoplasm)

		Mean	*SD*	*SEM*		*Mean*	*SD*	*SEM*
Normal morphology		16.92	6.65	1.36		18.09	5.69	1.21
Head defects		70.42	5.29	1.08		71.05	4.34	0.92
Neck defects	**Before Treatment**	22.75	4.69	0.96	**After Treatment**	18.86	3.88	0.83
Tail defects		11.29	2.35	0.48		11.59	2.32	0.50
ERC		2.25	0.79	0.16		2.41	0.73	0.16

Table 3. Partial correlation analysis of sperm parameters before the treatment (Note: *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001)

	Sperm count	Motility	Vitality	Morphology
Category	-0.46*	-0.80***	-0.57**	-0.17
Sperm count		0.34	0.10	0.04
Motility			0.36	0.15
Vitality				0.02

Table 4. Partial correlation analysis of sperm parameters after the treatment (Note: *p-value < 0.05; **p-value < 0.01)

	Sperm count	Motility	Vitality	Morphology
Category	-0.48*	-0.56*	-0.29	-0.08
Sperm count		0.31	0.38	0.05
Motility			0.65**	0.13
Vitality				0.06

Discussion

This is the first study in the medical literature that aimed to assess whether the presence of specific corynebacterial species (in this case *C. glurucornolyticum*) in monoculture with established etiological relevance may negatively affect all important semen parameters, and the results have shown a negative influence on the vitality and neck morphology of spermatozoa. However, sperm parameters that are considered more important (such as number and motility) were not adversely affected, *i.e.* there were no statistically significant changes following the treatment and microbiological clearance. Albeit our results could consequently point to a minor influence of this species on semen parameters, vitality can be a pivotal parameter when the motility of spermatozoids in semen sample is low [20].

The strengths of our study are a prospective study design, strict inclusion/exclusion criteria for study participants, detailed microbiological workup to ensure clinical significance and conclusive confirmation of the isolates, individually tailored treatment regimens according to the respective antibiograms, methodological sturdiness in semen evaluation, a three-month interval between pre-treatment and post-treatment semen analysis, as well as stringent statistical analysis. Conversely, the main weakness of our study would be the number of included study participants; however, to our knowledge this is the largest collection of *C. glucuronolyticum* isolates from semen specimens with colony counts > 10^4 CFU/mL (and purported etiological relevance) in a single study.

In our study, corynebacteria as a group were found in 22.96% of all specimens. Still, it must be noted that in a majority of those samples their colony count was less than 10^4 CFU/mL and they grew alongside other saprophytes of the distal urethra. On the other hand, after pursuing

species-level identification in specimens with more than 10^4 CFU/mL, *C. glucuronolyticum* in significant numbers was found in only 1.61% of all specimens. Antimicrobial susceptibility testing was done in all those isolates (Figure 1), but only those without any co-infection and those who satisfied strict inclusion criteria were included in a further prospective study protocol (1.11%). Therefore, coryneform bacteria may be commonly encountered in semen specimens (with a quite variable span from 12% up to 86.6% in studies conducted by different research groups) [17,21,22], but probably only a small fraction of them are to be considered clinically relevant.

When it comes to the sensitivity of the isolates, this study revealed considerable resistance to clindamycin and tetracycline, as well as a trend toward ciprofloxacin resistance. The latter finding is particularly worrisome, as fluoroquinolones are often a treatment cornerstone and a sort of wild card in urology practice, as well as the most habitually administered drug for urinary tract infections in men from Croatia [23]. A similar susceptibility pattern has been demonstrated by Mashaly and his co-authors on the isolates from Egypt (albeit with less quinolone resistance) [17], whereas Funke *et al.* have shown that MIC_{50} values were highest for chloramphenicol, clindamycin and tetracycline [24].

The exact instances when the usual commensals (like corynebacteria) of the male genital tract may act as pathogens represent a good question without a good answer, as elegantly stated by Türk *et al.* [25]. We recently described a ciprofloxacin-resistant strain that caused male urethritis syndrome [13], which is a clinical presentation described by other research groups as well [11,12]. Moreover, this entity may be responsible for the encrustation of the bladder mucosa with subsequent chronic inflammation (also known as encrusted cystitis), as described by Curry and her colleagues [15].

Contrarily, association of this species with prostatitis syndrome is not so straightforward. Novo-Veleiro *et al.* highlighted the potential significance of *C. glucuronolyticum* in three patients with monomicrobial paucisymptomatic infectious prostatitis with a fever of unknown origin [14]. However, although coryneform species were more abundant in prostatitis patients when compared to controls in the recent paper by Türk *et al.*, from five molecularly-confirmed *C. glucuronolyticum* species they have described, none have shown the propensity to form biofilms in the prostate gland [25].

The current estimations are that 15% of infertility in men is linked to genital tract infection [26]. For example, some studies suggest that exposure to *Chlamydia trachomatis* may affect sperm function and induce premature sperm death [27,28]. Furthermore, a recent systematic review and meta-analysis conducted by Huang *et al.* showed that *Ureaplasma urealyticum* and *Mycoplasma hominis* are significantly associated with male infertility [29], and various research groups have shown that these two pathogens are associated lower sperm concentrations motility, vitality, density, as well as with higher semen viscosity [30]. A plethora of other bacterial species (not necessarily sexually-transmitted) may also potentially alter sperm quality [31].

However, sporadic detection of this microorganism in clinically relevant numbers (partly due to infrequent species-level identification of coryneform bacteria) is probably a reason why this species has not been thoroughly studied in similar clinical-microbiological research endeavours. Mashaly *et al.* examined the influence of seminal coryneforms as a group on semen parameters in infertile men, and showed that sperm motility was lower in those with the presence of any coryneforms [17]. But from 12 semen cultures that harboured

Corynebacterium species they identified four different genus representatives, which hinders adequate conclusions; moreover, they did not look at sperm vitality or more detailed morphology parameters.

In the study by Riegel and Lepargneur [16] there were 2.7% of *C. glucuronolyticum* isolates in the sample of 1902 patients with colony counts greater than 10^3 CFU/mL. These authors compared the values of only two semen parameters (*i.e.* total motility and pH) in samples with aforementioned numbers of *C. glucuronolyticum* with the values in semen samples harbouring identical numbers of other corynebacteria. The results have shown that normal spermatozoid motility was found in 25.4% of samples with high *C. glucuronolyticum* counts, when compared to 45% of specimens containing similar counts of other corynebacteria. However, there is a myriad of methodological issues with this study, as the authors did not account for the eventual presence of other potential co-infections, there were no inclusion/exclusion criteria, no other examinations conducted, no prospective follow-up, and colony count cut-off value was not high enough to discriminate between colonization and infection.

An inherent problem with a large number of studies in this field is that culture-positive and culture-negative groups are compared without any type of therapeutic intervention or prospective follow-up, which is why we opted for a different, pretest-posttest approach. Nonetheless, there is still an issue of intra-individual variation in semen composition, which is also something that should always be taken *'cum grano salis'* when interpreting all studies exploring how particular pathogens influence semen parameters [20]. An overly short time interval before the re-evaluation may also be an issue, thus we used a three-month time frame to fully account for the entire process of spermatogenesis and transport on ductal system [32]. Also, antibiotic therapy may not always prevent permanent abnormalities of sperm parameters,

likely due to the initiation of persistent immuno-pathological cascades in the genital tract [33].

But by employing rigorous methodology, certain insights can be gained by these types of correlational studies, which is why they are increasingly being conducted. The question then remains what are the underlying pathophysiological mechanisms that result in reduced semen parameters and (in the worst-case scenario) give rise to infertility. Some authors concentrate only on the induction of necrosis and apoptosis [34], while others propose the putative role of antigenic mimicry that may exists between certain components of spermatozoa and bacterial proteins [31]. Although thus far such cross-reaction has been proved only for the flagella of spermatozoa by Moretti *et al.* [31], our study hints that other parts of spermatozoa may be affected as well.

In conclusion, as infertility is becoming a global public health issue with an increasing need for assisted reproduction, even mild issues with semen quality can be paramount when deciding how to approach a sub-fertile or infertile couple. A meticulous andrological workup is warranted, and the presence of corynebacteria in a semen sample should always raise the suspicion of *C. glucuronolyticum*, as this species may exhibit adverse effects on spermatozoa. Future studies should address this topic even further to provide new revelations on the potential pathophysiological mechanisms. In any case, re-evaluating sperm characteristics after treatment is pivotal for reliable assessment of male fertility and confirmation of this microorganism's role in generating defective spermatozoa.

Conflict of interest: None declared.

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