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Corynebacterium Species Causing Urinary Tract Infections

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Authors' contributions

This work was carried out in collaboration between all authors. Author KR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SGA and MG managed the analyses of the study. Author KR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the prevalence and to assess the antimicrobial susceptibility of individual *Corynebacterium* species isolated from patients with urinary tract infections.
Place and Duration of Study: Department of Medical Microbiology, Infant Jesus Teaching Hospital, Warsaw, Poland from January 2010 to December 2016.
Methodology: This retrospective analysis included *Corynebacterium* strains derived from 211 urine samples. Microbial identification had been performed with the use of biochemical panels and matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS). Antibiotic susceptibility tests were conducted according to the Polish Reference Center for Antimicrobial Susceptibility and the European Committee on Antimicrobial Susceptibility Testing guidelines.
Results: The predominant *Corynebacterium* species isolated from female urine were *C. coyleae, C. aurimucosum*, and *C. amycolatum.* The following species: *Arcanobacterium haemolyticum*, *C.*

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freneyi, *C.* group G1, *C. muciefacines*, *C. propinquum*, *C. pseudodiphtheriticum*, and *C. renale* were identified only in urine cultures from female patients. Antimicrobial susceptibility rates were 41.9% for penicillin, 53.4% for ciprofloxacin, 83.3% for gentamycin, and 77.8% for tetracycline with all the evaluated strains susceptible to vancomycin.
The number of the *Corynebacteria* species isolated from urine samples consecutively raised from 4 in 2010 up to 87 in the last analysed year.
Conclusion: The observed increase in the number of *Corynebacterium* species isolated from urine samples over the years indicates the important role of these bacteria in the urinary tract infection epidemiology, especially in women. Therefore, novel diagnostic procedures should be developed for the specimens from patients with urinary tract infections, especially in the case of transplant recipients.

Keywords: Corynebacterium; urinary infections; antimicrobial susceptibility; MALDI-TOF MS.

1. INTRODUCTION

The presence of bacteria in the urinary tract above the urethral sphincter typically indicates a urinary tract infection (UTI). Customarily, the urinary tract is recognized as a sterile environment, with the exception of the distal end of the urethra. However, conclusions drew from recent studies on the microbial composition of healthy individuals indicate that urinary tract and the bladder are inhabited by several bacterial species forming so-called urinary microbiome [1]. The composition of the normal urethral microorganisms in females and males may differ, but *Staphylococcus epidermidis, Lactobacillus* spp., *Corynebacterium* spp., *Bacteroides* spp., *Streptococcus* spp. (α-hemolytic strains) are common in both sexes.

In contrast, the most commonly isolated etiological factors of UTI include: *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis*, *Proteus mirabilis, Pseudomonas aeruginosa*, and *Streptococcus agalactiae*. The prevalence of the individual species differs in males and females and depends on the patient's age. UTIs can also be caused by multiple pathogens.

Until recently, the bacterial microflora, found in patients' physiological skin and mucus treated merely as contaminants of analysed urine specimens. However, these microorganisms are often considered to be among the etiological factors behind nosocomial and community-acquired infections. Majority of these bacteria includes Gram-positive species of the genus *Corynebacterium*.

Epidemiologic data on the role of Gram-positive bacilli in UTIs are very scarce, which is most likely due to the common belief that these bacteria, as part of the physiological urethral microflora, are only accidental contaminants of the evaluated urine specimens. Another reason for this lack of data may be that this group of bacteria has highly specific growth requirements (culture media supplemented with blood, lipids, etc.). Moreover, these bacteria need a longer incubation time to grow than most Gram-positive as well as Gram-negative species. It is not uncommon for them to show growth only after a 72–96 hour incubation. Thus, Gram-positive bacilli can be often disregarded even in comprehensive microbiological analyses. Their identification is reserved only for the cases where they are highly probable causative pathogens.

The identification of bacteria of the genus *Corynebacterium* is considered as another problem. Generally microbial identification is not done for contaminated specimens. Most commonly phenotypic identification based on the bacterial morphology, biochemical properties and, in some species, a synergistic hemolysis test i.e., CAMP (Christie, Atkins, Munch-Petersen) is done for such contamination.

More advanced species identification techniques for Gram-positive bacilli include total cellular fatty acid profiling, phospholipid-derived fatty acid (PLFA) profiling, cell-wall composition analysis, and DNA or RNA nucleotide sequencing. However, these methods are expensive, time-consuming, and require specialised equipments and therefore, are mostly used in the reference centers for research purposes. Mass spectrometry (MALDI-TOF MS), which is one of the most modern methods of bacterial identification, is becoming more and more common in routine microbial diagnostics [2,3].

The limited knowledge about the infection etiology and the antimicrobial susceptibility of

these microorganisms are due to the challenges associated with culture and identification of bacteria from the genus *Corynebacterium*. Widespread, often inappropriate use of antibiotic therapy in nosocomial and community-acquired infections contribute to the emergence and spread of multidrug resistant strains, including strains of the genus *Corynebacterium*.

Apart from the toxigenic strains of *C. diphtheriae*, an exclusive human pathogen, other *Corynebacterium* species were reported to be pathogenic in humans. Among these *C. diphtheriae* group (including non-toxigenic strains), *C. xerosis, C. striatum, C. minutissimum, C. amycolatum/striatum, C. glucuronolyticum, C. argentoratense, C. matruchotii* are the fermenting species. On the other hand *C. afermentans, C. auris, C. pseudodiphtheriticum, C. propinquum;* and lipophilic species: *C. jeikeium, C. urealyticum, C. lipophilum, C. accolens, C. macginleyi,* CDC groups F-1 and G, and *C. bovis* are the non-lipophilic, non-fermenting species [4].

More recently, a number of other species, isolated from human clinical samples, were identified. Interestingly, *C. renale*, which was used to be considered pathogenic in animals, has been isolated from human clinical samples [5].

Moreover, bacterial taxonomic studies have led to some species being reclassified into other taxa. One example of this is the reclassification of *C. haemolyticum* as *Arcanobacterium haemolyticum*. This species has been reported as an etiologic factor of pharyngeal, skin and soft-tissue infections, as well as sepsis [6]. So far, only one report Ciraj et al. [7] of the involvement of this bacterial species in UTIs has been recorded.

Since, for many years, this species was classified as part of the genus *Corynebacterium*, as well as due to the growing interest in this species as an important human pathogen, it was included in the present study. The aim was to determine the prevalence and to assess the antimicrobial susceptibility of individual *Corynebacterium* species isolated from the patients suffering with urinary tract infections.

2. MATERIALS AND METHODS

2.1 Samples

The study involved a retrospective analysis of isolates identified as representatives of the genus *Corynebacterium* derived from 211 urine specimens. These were collected from the patients of the following clinics of the Infant Jesus Teaching Hospital: “General, Oncologic, and Functional Urology”, n=84; “General and Transplantation Surgery”, n=16; “Immunology, Transplantation, and Internal Medicine”, n=27; “Transplantation Medicine, Nephrology, and Internal Medicine”, n=69; “Internal Medicine and Cardiology”, n=8; “Ophthalmology”, n=1; “Dermatology”, n=1; “Obstetrics and Gynecology”, n=2; “Anesthetics and Intensive Care”, n=2; “Orthopedic and Accident Surgery of the Musculoskeletal System”, n=1; from January 1, 2010, to December 31, 2016.

The study population comprised of 164 females and 47 males. The patient's age ranged from 20 to 92 years, (median 57 years, mean 56 years).

All the cultures were performed when urinary tract infection was recognised by the clinician based on the laboratory and/or clinical findings.

2.2 Isolation and Identification of Bacteria from Urine Sample

Midstream urine specimens were cultured according to a standard protocol (1-µL samples plated onto the chromogenic CHROMID® CPS 3 and the MacConkey media (both manufactured by bioMérieux, France). The cultures were incubated at 37°C for 24 hours in aerobic conditions. , The bacterial microflora of the genus *Corynebacterium* were identified by Analytical Profile Index API® Coryne bacterial identification test kits (bioMérieux France), according to the manufacturer’s instructions for the period of January 2010 to September 2014. From October 2014 onward, the identification was performed using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) with the Vitek MS system (bioMérieux, France).

In some cases, bacterial identification was conducted only to the genus level. Isolates were analysed only if the culture yielded significant bacterial counts i.e., 10^5 cfu/mL.

2.3 Determination of Susceptibility to Antibiotics of the *Corynebacteria* Strains

Antibiotic susceptibility tests to penicillin, ciprofloxacin, gentamycin, tetracycline, and vancomycin were performed.

Briefly, according to the of the Polish Reference Center for Antimicrobial Susceptibility 2010 (KORLD) Łętowska and Olender [8] antibiotic susceptibility was examined by MIC determination by the E-test gradient diffusion method AB Biodisc, Sweden), and since 2014, according to the European Committee on Antimicrobial Susceptibility Testing 2014 (EUCAST) guidelines [9] by the Kirby-Bauer disk diffusion method. For both the methods, Mueller Hinton agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) (bioMérieux, France) were used. For disc diffusion antibiotic-impregnated disks (OXOID Limited, UK); benzylpenicillin 1 unit, ciprofloxacin 5 µg, gentamycin 10 µg, vancomycin 5 µg, tetracycline 30 µg were applied onto the surface of the inoculated plates. After incubation, strains were characterised as susceptible or resistant to antibiotics based on the inhibition zone size or the MIC value according to the interpretive criteria of the appropriate guidelines.

3. RESULTS

During the analysis period, urine cultures yielded significant bacterial counts from a total of 211 strains of the genus *Corynebacterium.* A total of 144 (68.2%) out of the 211 grown strains were identified to the species level, whereas the remaining 67 strains (31.8%) were identified only to the genus level (*Corynebacterium*). Predominant species included: *C. coyleae*, 32 strains (15.2%); *C. aurimucosum*, 26 strains (12.3%); *C. amycolatum*, 25 strains (11.8%); *C. tuberculostearicum*, 15 strains (7.1%); and *C. xerosis*, 15 strains (7.1%). Other species constituted less than 3% each. Table 1 presents the detailed information on the identified species and their number, stratified by year.

The number of the *Corynebacteria* species isolated from the urine samples consecutively raised during the study period, from as few as 4 and 5 during 2010-2011 and up to 80 and 87 in the last two experimental years.

Corynebacterium strains were isolated from 164 (77.7%) female urine specimens and from 47 (22.3%) male urine specimens. The species isolated from women were predominantly: *C. coyleae* (n=31), *C. aurimucosum* (n=24), and *C. amycolatum* (n=19). The following species: *A. haemolyticum*, *C. freneyi*, *C.* group G1, *C. muciefaciens*, *C. propinquum*, *C. pseudodiphtheriticum*, and *C. renale* were identified only in the female urine cultures. The *C. jeikeium* species was identified only in the male samples. All other grown species were isolated from both female and male urine cultures.

Table 1. Individual *Corynebacterium* strains grown from the urine specimens during 2010–2016

Identification	Number of isolates (%)	Number of isolates	
	Total (%)	Females	Males
A. haemolyticum	1 (0,5)	1	0
C. amycolatum	25 (11,8)	19	6
C. aurimucosum	26 (12,3)	24	2
C. coyleae	32 (15,2)	31	1
C. freneyi	1 (0,5)	1	0
C. glucuronolyticum	3 (1,4)	1	2
C. group G 1	1 (0,5)	1	0
C. jeikeium	3 (1,4)	0	3
C. minutissimum	3 (1,4)	1	2
C. mucifaciens	2 (0,9)	2	0
C. propinquum	1 (0,5)	1	0
C. pseudodiphtheriticum	1 (0,5)	1	0
C. renale	1 (0,5)	1	0
C. simulans	4 (1,9)	3	1
C. striatum	5 (2,4)	3	2
C. tuberculostearicum	15 (7,1)	8	7
C. urealyticum	6 (2,8)	4	2
C. xerosis	15 (7,1)	10	5
C. species	66 (31,3)	52	14
Sum	211 (100)	164	47

Antimicrobial susceptibility tests were conducted for 179 out of all (n=211) grown strains. The remaining cultures, despite having the number of colonies forming units/mL greater than 10^5, were, perhaps incorrectly assumed to have been contaminated with urethral microflora and were, thus, exempted from susceptibility testing.

Table 2 presents antimicrobial sensitivity data for the individual species and jointly for all *Corynebacterium* species.

The number of strains tested for susceptibility to each individual antibiotic was considered to constitute 100%. Susceptibility to penicillin and vancomycin was tested in all 179 strains, ciprofloxacin susceptibility was tested in 103 strains, and gentamycin and tetracycline susceptibility were tested in 54 strains.

The proportion of penicillin-susceptible strains (out of all strains tested (n=179), including *Corynebacterium* spp. (n=37)) was 41.9%.

The proportion of ciprofloxacin-susceptible strains (out of all strains tested (n=103), including *Corynebacterium* spp. (n=20)) was 53.4%.

Very few strains exhibited intermediate antimicrobial susceptibility, with one *C. amycolatum* strain and one *C. species* strain intermediately susceptible to penicillin and one *C. species* strain intermediately susceptible to ciprofloxacin. These strains constituted 1.1% and 1% of the analysed isolates, respectively.

The proportion of gentamycin-susceptible strains (out of 54 strains tested, including *Corynebacterium* spp. (n=8)) was 83.3%.

The proportion of tetracycline-susceptible strains (out of 54 strains tested, including *Corynebacterium* spp. (n=8)) was 77.8%.

All the analysed strains identified only to the genus level (n=37), as well as those identified to the species level (n=142), exhibited susceptibility to vancomycin.

The individual *Corynebacterium* species tested, showed no significant differences in their antimicrobial susceptibility. However, in some cases, the number of isolates was insufficient to observe such differences.

4. DISCUSSION

Until recently, obtaining a single species of primary (eg. *Enterobacteriaceae*) or secondary uropathogens (eg. *Enterococcus* spp.), with a significant bacterial count in patients urine culture with bacterial UTIs was believed to warrant further microbiological testing. by identifying the strain and determining its susceptibility. Currently, microbiological analyses are carried out, especially in the case of immunocompromised or transplantation patients, even when the urine culture yields two or even three bacterial species [10].

Classifying bacteria from the genus *Corynebacterium* as uropathogenic species is controversial due to the established belief that their growth in urine culture resulted from skin microflora contamination.

The present analysis showed that 33% of the analysed specimens yielded pure cultures of *Corynebacterium* spp. These findings are fairly consistent with those by Reddy et al. [11], whose study on the prevalence of *Corynebacterium* species in various clinical specimens yielded pure cultures only in the case of non-urine specimens. Reddy also reported that only 11 (25.6%) out of 43 analysed urine specimens yielded pure *Corynebacterium* cultures. The slight discrepancy may be due to the different numbers of evaluated specimens.

Leaving out bacteria of the genus *Corynebacterium* as possible etiological factors of UTIs may result in incorrect clinical interpretation and omission of antibiotic therapy. This may lead to dangerous consequences for the patient, especially in the case of multidrug-resistant strains.

This way of thinking may lead to therapeutic failure, especially in renal transplant recipients.

According to literature, *C. urealyticum* is an established pathogen of the urinary tract. Reddy et al. [11] reported to culture *C. urealyticum* only from samples where this species constituted 6.1% of all isolated pathogens. Prospective studies by López-Medrano et al. [12] on the occurrence of UTIs caused by *C. urealyticum* in renal transplant recipients showed this species to be present (regardless of the bacterial count) in 9.8% of the evaluated patients. Even when only the cultures with a significant bacterial count of 10^5 cfu/mL were considered, *C. urealyticum* was still reported in 3% of the evaluated patients. This is a much higher proportion than that in other patient populations. Therefore, the cited authors concluded that renal transplant recipients should be considered as a high

Table 2. Antimicrobial susceptibility of *Corynebacterium* bacteria derived from urine specimens and grown in numbers exceeding the significant bacterial count

Identification	Number of isolates	Number and percentage of susceptible strains									
		Benzylpenicillin	%	Ciprofloxacin	%	Gentamicin	%	Tetracycline	%	Vancomycin	%
A. haemolyticum	1	1	100	* nt	nt	nt	nt	nt	nt	1	100
C. amycolatum	25	11	44	5	35,7	5	100	2	66,7	25	100
C. aurimucosum	26	10	38,5	13	72,2	9	81,8	7	87,5	26	100
C. coyleae	32	12	37,5	15	71,4	13	100	11	78,6	32	100
C. glucuronolyticum	3	0	0	1	33,3	nt	nt	0	0	3	100
C. group G 1	1	1	100	1	100	nt	nt	nt	nt	1	100
C. jeikeium	3	1	33,3	0	0	0	0	1	100	3	100
C. minutissimum	3	2	66,7		nt	nt	nt	nt	nt	3	100
C. mucifaciens	2	0	0	2	100	nt	nt	nt	nt	2	100
C. propinquum	1	1	100	nt	nt	nt	nt	nt	nt	1	100
C. pseudodiphtheriticum	1	1	100	nt	nt	nt	nt	nt	nt	1	100
C. renale	1	1	100	nt	nt	nt	nt	nt	nt	1	100
C. simulans	4	2	50	1	50	2	100	2	100	4	100
C. striatum	5	3	60	nt	nt	nt	nt	nt	nt	5	100
C. tuberculostearicum	15	5	33,3	2	22,2	3	60	4	57,1	15	100
C. urealyticum	5	2	40	nt	nt	nt	nt	nt	nt	5	100
C. xerosis	14	1	7,1	4	33,3	6	66,7	9	90	14	100
C. species	37	21	56,8	11	55	7	87,5	6	75	37	100
Sum witout *C. species*	**142**	**54**	**38**	**44**	**53**	**38**	**82,6**	**36**	**78,3**	**142**	100
Sum of all isolates	**179**	**75**	**41,9**	**55**	**53,4**	**45**	**83,3**	**42**	**77,8**	**179**	100

** not tested*

infection risk group caused by this pathogen. They suggested that this group of patients should receive antibiotic therapy when suffering from UTI, even with microbiologically-confirmed non-significant bacteriuria.

The present analysis, which encompassed a 7-year long period, found only six strains of *C. urealyticum*. However, the specimens were derived from various patients, not only transplant recipients, and the urine culture conditions were not targeted towards this particular species. The resulting underestimation of the rates of UTIs caused by *C. urealyticum* may be due to the slow growth of this species (48–72 hours), whereas the growth of other, more common UTI-causing pathogens is typically observed after 24 hours [12]. Moreover, this species grows 5 times less often on standard urine-culture media than on selective media. This is because, besides the longer time these bacteria need to grow (up to 72 and even 96 hours), they also require supplementary growth factors and prefer a 5% carbon dioxide atmosphere [11].

The rate of infections by *C. glucuronolyticum*, which was detected only 3 times during this study, may be similarly underestimated. Like in the case of *C. urealyticum*, the main problem with isolating this species is its slow growth (colonies were visible after a minimum of 24-hour incubation). Bacteria of this species have been reported to cause urogenital infections in men, may be being part of physiological flora. The prevalence of these bacteria in women is unknown [13].

Surprisingly, the bacterial species most commonly isolated in this study was *C. coyleae*. This is a relatively new species. It was first described in Funke et al. [14] and has since been only rarely reported as a causative agent of infections. There have been only a handful of reports on *C. coyleae* isolation from specimens such as blood, urine, abscess aspirate, prostatic secretion, or pleural fluid [15]. *C. coyleae* may have occurred earlier, however, it had either not been identified to the species level or had been identified incorrectly [14].

Fernandez-Natal et al. [15] reported a strain, which was genetically identified as *C. coyleae* but had been incorrectly identified as *C. jeikeium* based on its biochemical characteristics. In this study, the first instance of *C. coyleae* identification was recorded in 2014 (1 strain). In subsequent years, this species was identified in 14 (2015) and 17 (2016) cultures. At this stage, this could be emphasized that bacterial identification with mass spectrometry (MALDI-TOF) was first introduced at the study laboratory in October 2014. The earlier bacterial identification had been based on biochemical tests alone, which may explain the significant increase in *C. coyleae* identification in 2015–2016. It is noteworthy that, *C. coyleae* was grown from urine specimens collected almost exclusively from females (97%).

The second most commonly isolated species was *C. aurimucosum*. This species was isolated 26 times over the period of 7 years. According to the literature reports, these bacteria have been isolated from patients with acute and chronic arthritis and osteitis, from soft tissues of the foot in patients with diabetic foot, and from biopsy samples (synovial membrane) collected from a patient with arthritis. This bacterial species has also been reported to be part of genitourinary tract microflora in healthy women [16,17].

Till date, there has been only one reported case of UTI caused by *C. aurimucosum,* in a patient who underwent urethroplasty due to urethral stricture. The isolated strain was resistant to penicillin and trimethoprim/sulfamethoxazole and susceptible to vancomycin, tetracycline, ciprofloxacin, and imipenem. The patient received a 10-day treatment with imipenem, which was therapeutically successful [18].

The third most commonly isolated species in the present study was *C. amycolatum*, which constituted 11.8% of urine culture isolates, 72% of which were collected from women. As similar with other species, there is no available literature on the involvement of this species in UTIs. These bacteria have been reported to occur in wound infections, where they constituted 29.2% of the isolated Gram-positive bacilli, [19] and in local surgical site infections. In general, bacteria of the genus *Corynebacterium* were responsible for 24.7% of infections, with *C. amycolatum* being *identified as* the predominant species in this group (constituting 37.1%) [20].

5. CONCLUSION

The present study revealed an increase in the number of strains of the genus *Corynebacterium* isolated from urine specimens from one year to the next, with the same culture methods. This result indicated the growing role of this genus in the UTI epidemiology, especially in women. These findings also suggest that the novel diagnostic algorithms should be developed for

dealing with clinical specimens collected from patients with UTIs, especially transplant recipients. Correct identification of the isolated strains is necessary for accurate UTI diagnoses.

CONSENT AND ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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