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Original Research Article

Corynebacterium species causing urinary tract infections.

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 of the Infant Jesus Teaching Hospital, Warsaw, Poland from January2010 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. 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% (penicillin), 53.4% (ciprofloxacin), 83.3% (gentamycin), 77.8% (tetracycline), with all the evaluated strains susceptible to vancomycin.

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The number of the *Corynebacteria* species isolated from urine samples consecutively raised during analyzed years, from 4 in the year of 2010 up to 87 in the last analyzed year.

Conclusion: The observed increase in the number of *Corynebacterium* species isolated from urine samples over the years suggests a more and more important role of these bacteria in 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 was 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 microflora in females and males may differ, but the microorganisms commonly isolated from both sexes are *Staphylococcus epidermidis, Lactobacillus* spp., *Corynebacterium* spp., *Bacteroides* spp., *Streptococcus* spp. (α-hemolytic strains).

In contrast, the most commonly isolated etiological factors of UTIs 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 be also caused by multiple pathogens.

The bacteria constituting patients' physiological skin and mucosal microflora were, until recently, treated merely as contaminants of analyzed urine specimens. However, these microorganisms are more and more often considered to be among the etiological factors

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behind nosocomial and community-acquired infections. These bacteria include the majority of 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 paucity of data may be the fact that this group of bacteria has highly specific growth requirements (culture media supplemented with blood, lipids). 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 being reserved only for those cases where they are highly probable causative pathogens.

Another problem involves the identification of bacteria of the genus *Corynebacterium*. If the specimen is believed to be contaminated, microbial identification is not even attempted. In those cases where identification is conducted, it is most commonly phenotypic; i.e. based on bacterial morphology; biochemical properties; and, in some species, a synergistic hemolysis test – CAMP (Christie, Atkins, Munch-Petersen).

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 specialized equipment; which is why they are mostly used in 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 challenges associated with culture and identification of bacteria from the genus *Corynebacterium* have resulted in our limited knowledge of the role these microorganisms

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play in infection etiology and on their antimicrobial susceptibility. Widespread, often inappropriate, use of antibiotic therapy in nosocomial and community-acquired infections contributes to the emergence and spread of multi-drug resistant strains, including strains of the genus *Corynebacterium*.

Apart from the toxigenic strains of *C. diphtheriae*, which are strict human pathogens, the following *Corynebacterium* species were reported to be pathogenic in humans: fermenting species: *C. diphtheriae* group (including non-toxigenic strains), *C. xerosis, C. striatum, C. minutissimum, C. amycolatum/striatum, C. glucuronolyticum, C. argentoratense, C. matruchotii*; non-lipophilic, non-fermenting species: *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*. [4]

More recently, a number of other species, isolated from human clinical samples, were identified. Interestingly, *C. renale*, which 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 a 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] with, so far, only one report [7] of the involvement of this bacterial species in UTIs.

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, we decided to include it in our study.

2. MATERIALS AND METHODS

2.1 SAMPLES

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Our study involved a retrospective analysis of isolates identified as representatives of the genus *Corynebacterium* derived from 211 urine specimens collected from the Infant Jesus Teaching Hospital patients of the following clinics: “General, Oncologic, and Functional Urology”, “General and Transplantation Surgery”, “Immunology, Transplantation, and Internal Medicine”, “Transplantation Medicine, Nephrology, and Internal Medicine”, “Internal Medicine and Cardiology”, “Ophthalmology”, “Dermatology”, “Obstetrics and Gynecology”, “Anesthetics and Intensive Care”, “Orthopedic and Accident Surgery of the Musculoskeletal System”, in the period from Jan. 1, 2010, to Dec. 31, 2016.

The study population comprised 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 in the case when urinary tract infection was recognized by the clinician based on 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. In the period from January 2010 to September 2014, bacteria of the genus *Corynebacterium* were identified with API Coryne bacterial identification test kits (bioMérieux France), according to the manufacturer’s instructions; in the period from October 2014 onward, identification was conducted via MALDI-TOF MS with a Vitek MS system (bioMérieux, France).

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

2.3 DETERMINATION OF ANTIMICROBIAL RESISTANCE OF THE CORYNEBACTERIA STRAINS

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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) [8] antibiotic susceptibility was examined by MIC determination by the E-test gradient diffusion method AB Biodisc, Sweeden), 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 methods Mueller Hinton agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) (bioMérieux, France) was used. For disc diffusion antibiotic-impregnated disks (OXOID Limited, UK); benzylpenicillin 1 unit, ciprofloxacin 5 µg, gentamycin 10 µg, vancomycin 5 µg, tetracycyline 30 µg were applied onto the surface of the inoculated plates. After incubation, strains were characterized as susceptible or resistant to antibiotics based on the inhibition zone size or the MIC value according to interpretive criteria of the appropriate guidelines.

3. RESULTS

During the analyzed 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%) could be 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 detailed information on the identified species and their number, stratified by year.

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162 Table 1. Individual *Corynebacterium* strains grown from urine specimens in 2010–2016.

	Number of isolates (%)	Number of isolates from:	
Identification	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

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The number of the *Corynebacteria* species isolated from urine samples consecutively raised during analyzed years, from as few as 4 and 5 in the years of 2010-2011 up to 80 and 87 in the last two analyzed 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 urine cultures from female patients. The *C. jeikeium* species was identified only in the sample collected from males. All other grown species were isolated from both female and male urine cultures.

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, exempt from susceptibility testing.

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

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

		Benzylpenicillin n=179	**%**	**Ciprofloxacin n=103**	**%**	**Gentamicin n=54**	**%**	**Tetracycline n=54**	**%**	**Vancomycin n=179**	**%**
Identification	**Number of**	**S**		**S**		**S**		**S**		**S**	

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	isolates										
A. haemolyticum	1	1	100,0	* nt	nt	nt	nt	nt	nt	1	100
C. amycolatum	25	11	44,0	5	35,7	5	100,0	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	100,0	11	78,6	32	100
C. glucuronolyticum	3	0	0,0	1	33,3	nt	nt	0	0,0	3	100
C. group G 1	1	1	100,0	1	100,0	nt	nt	nt	nt	1	100
C.	3	1	3	0	0,	0	0,	1	1	3	1

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jeikeium			3,3		0		0		00,0		00
C. minutissimum	3	2	66,7		nt	nt	nt	nt	nt	3	100
C. mucifaciens	2	0	0,0	2	100,0	nt	nt	nt	nt	2	100
C. propinquum	1	1	100,0	nt	nt	nt	nt	nt	nt	1	100
C. pseudodiphtheriticum	1	1	100,0	nt	nt	nt	nt	nt	nt	1	100
C. renale	1	1	100,0	nt	nt	nt	nt	nt	nt	1	100
C. simulans	4	2	50,0	1	50,0	2	100,0	2	100,0	4	100

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C. striatum	5	3	60,0	nt	nt	nt	nt	nt	nt	5	100
C. tuberculostearicum	15	5	33,3	2	22,2	3	60,0	4	57,1	15	100
C. urealyticum	5	2	40,0	nt	nt	nt	nt	nt	nt	5	100
C. xerosis	14	1	7,1	4	33,3	6	66,7	9	90,0	14	100
C. species	37	21	56,8	11	55,0	7	87,5	6	75,0	37	100
Sum witout *C. species*	**142**	**54**	**38,0**	**44**	**53,0**	**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

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.

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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 1 *C. amycolatum* strain and 1 *C. species* strain intermediately susceptible to penicillin and 1 *C. species* strain intermediately susceptible to ciprofloxacin. These strains constituted 1.1% and 1% of the analyzed 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 analyzed 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

For a long time, only obtaining a single species of primary (eg. *Enterobacteriaceae*) or secondary uropathogens (eg. *Enterococcus* spp.), with a significant bacterial count in a urine culture from patients with bacterial UTIs was believed to warrant further microbiological testing, which involved identifying the strain and determining its susceptibility. Currently, microbiological analyses are continued, especially in case of immunocompromised or transplantation patients, even when the urine culture yields two or even three bacterial species. [12].

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Classifying bacteria from the genus *Corynebacterium* as uropathogenic species is controversial and is mostly a result of the established belief that their growth in urine culture implies that the urine specimen had been contaminated with skin microflora.

Our analysis showed that 33% of the analyzed specimens yielded pure cultures of *Corynebacterium* spp. These findings are fairly consistent with those by Reddy et al. [10], 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 analyzed urine specimens yielded pure *Corynebacterium* cultures. The slight discrepancy may be due to the different numbers of evaluated specimens.

Discounting bacteria of the genus *Corynebacterium* as possible etiological factors of UTIs may result in incorrectly interpreting the clinical presentation and omitting to introduce antibiotic therapy, which 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 reports, *C. urealyticum* is an established pathogen of the urinary tract. Reddy et al. [10] reported to culture *C. urealyticum* only from samples where this species constituted 6.1% of all pathogens isolated. Prospective studies by López-Medrano et al. [11] 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 a population at a high risk of infections caused by this pathogen. They suggested that this group of patients should receive antibiotic therapy when suffering from a UTI, even with microbiologically-confirmed non-significant bacteriuria.

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Our analysis, which encompassed a 7-year period, found only six strains of *C. urealyticum*. However, the specimens we analyzed 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 already after 24 hours. [12] Moreover, this species grows 5 times less often on standard urine-culture media than on selective media for *Corynebacterium* 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 our study, may be similarly underestimated. Like in the case of *C. urealyticum*, the main problem with isolating this species is its slow growth (colonies are visible after a minimum of 24-hour incubation). Bacteria of this species have been reported to cause urogenital infections in men, in whom they may be part of physiological flora. The prevalence of these bacteria in women is unknown. [13]

Surprisingly, the bacterial species most commonly isolated in our study was *C. coyleae*. This is a relatively new species. It was first described in 1997 [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 such specimens 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 our study, the first instance of *C. coyleae* identification was in 2014 (1 strain). In subsequent years, this species was identified in 14 (2015) and 17 (2016) cultures. At this stage, we should emphasize that bacterial identification with mass spectrometry (MALDI-TOF) was first

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introduced at our laboratory in October 2014; earlier bacterial identification had been based on biochemical tests alone, which may explain the significant increase in *C. coyleae* identification in 2015–2016. What is noteworthy is the fact that, in our study, *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 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 been also reported to be part of genitourinary tract microflora in healthy women. [16, 17]

To 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 species most commonly isolated in our study was *C. amycolatum*, which constituted 11.8% of urine culture isolates, 72% of which were collected from women. Like in the case of other species, there is no available literature reporting 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* the predominant species in this group (constituting 37.1%). [20].

In summary, our findings indicate that an increase in the number of strains of the genus *Corynebacterium* isolated from urine specimens from one year to the next, with culture

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methods remaining the same, may indicate the growing role of this genus in UTI epidemiology, especially in women. These findings suggest that 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.

4. CONCLUSION

In summary, our findings indicate that an increase in the number of strains of the genus *Corynebacterium* isolated from urine specimens from one year to the next, with culture methods remaining the same, may indicate the growing role of this genus in the UTI epidemiology, especially in women. These findings suggest that 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.

ETHICAL APPROVAL

Not required

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