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Urinary Tract Infections with *Aerococcus urinae* in the South of the Netherlands

P. M. H. Schuur^{1*}, M. E. E. v. Kasteren², L. Sabbe³, M. C. Vos¹,
M. M. P. C. Janssens¹, A.G.M. Buiting¹

Aerococcus urinae is an uncommon urinary tract pathogen that causes infections predominantly in elderly persons with local or general predisposing conditions. During a one-year study, the clinical features of *Aerococcus urinae* urinary tract infections ($\geq 10^5$ cfu/ml) were investigated in two large medical microbiology laboratories in the Netherlands. The incidence of *Aerococcus urinae* urinary tract infections ranged between 0.31 and 0.44% for the two laboratories. The median age (range 35–95 years) of patients with this infection was 82.5 years for women and 77.5 for men. Men had significantly ($p < 0.01$) more local predisposing conditions than did women. Underlying systemic diseases such as diabetes mellitus, malignancy, and dementia were found in 67.5% of patients. Most patients (97.5%) had the classic signs of a urinary tract infection, but none of them developed serious symptoms. All isolates tested were susceptible to penicillin, amoxicillin, and nitrofurantoin, 78.3% were susceptible to norfloxacin, and all were resistant to sulfonamides. The majority of patients were treated with amoxicillin, amoxicillin with clavulanic acid, or norfloxacin.

In 1989, Christensen et al. (1) described *Aerococcus*-like organisms (ALO) as previously unreported urinary tract pathogens. Isolates were found at a low but constant rate, showing little phenotypic intervariation. These organisms are catalase-negative, gram-positive cocci that occur most often in clusters and show α -hemolysis on 5% horse or sheep blood agar. Although ALO resemble *Aerococcus viridans*, these organisms can be easily differentiated by their biochemical reactions and antibiotic susceptibility patterns (1, 2). Initially, esculin hydrolysis was found to be negative for all ALO strains, but recently Christensen et al. (3) described an esculin hydrolysis-positive biotype

Because the investigations by Aguirre and Collins (4) in 1992 showed that the ALOs isolated by Christensen have a 94.5% rRNA similarity to *Aerococcus viridans*, they proposed naming these isolates *Aerococcus urinae*. However, the exact taxonomic position of *Aerococcus urinae* remains debatable (3, 5, 6).

Most work on *Aerococcus urinae* has been done by the Danish ALO Study Group; consequently, not much is known about the incidence and clinical presentation of *Aerococcus urinae* infections outside Denmark. The Danish ALO Study Group found that most infections occur in the urinary tract, especially in elderly people with local or general predisposing conditions (1, 2). Severe infections such as urosepticemia and endocarditis have also been reported (7–9).

In order to learn more about the occurrence and phenotypic characteristics of *Aerococcus urinae* and the clinical features of infection with this organism in the Netherlands, we carried out a one-year study. Clinical data were obtained from a questionnaire sent to attending physicians.

*The presentment of Medical Microbiology, St. Elizabeth Hospital, PO Box 74, 5000 AS Tilburg, the Netherlands.

²The presentment of Internal Medicine, St. Elizabeth Hospital, Hilvorchampsweg 40, 5000 LC Tilburg, the Netherlands.

³The general Laboratory for Public Health "Zeeland", PO Box 400, 4600 AA Capelle, the Netherlands.

Materials and Methods

Collection of Strains and Culture. During the study period (1994–1995), two laboratories in the south of the Netherlands, the Laboratory of Medical Microbiology and Immunology, St. Elisabeth Hospital, Tilburg, and the Regional Laboratory for Public Health "Zeeland", Goes, collected strains from urine cultures that had the following characteristics: colonies resembling α -hemolytic streptococci on 5% horse or sheep blood agar; catalase-negative, gram-positive cocci, predominantly in clusters; susceptibility to penicillin and vancomycin and resistance to sulfonamides (1, 2). A Gram stain was made from the urine sediment, and the sediment was cultured on 5% sheep blood agar for 18 h at 35°C in a normal atmosphere. The number of cfu/ml was counted by incubating 1 μ l of urine on C.L.E.D. agar (Oxoid, UK) for 18 h; the presence of $\geq 10^5$ cfu/ml was interpreted as significant bacteriuria.

Biochemical Tests. Further identification of *Aerococcus urinae* consisted of acid production from carbohydrates as tested using Bacto nutrient broth (Difco, USA) containing 0.5% carbohydrates (Merck, Germany). For this organism, acid production should be positive for glucose, sucrose, mannitol, and sorbitol, and negative for maltose, lactose, trehalose, raffinose, salicin, arabinose, and inulin (1, 2). There should be growth in 6.5% NaCl and no growth on 5% sheep blood agar at 45°C or after 30 min at 60°C; the β -glucuronidase reaction should be positive (Rosco Diagnostic Tablets, Denmark). We used the API 20 Strep (bioMérieux, France) for testing hippurate hydrolysis (positive), leucine aminopeptidase (positive), pyrrolidonyl aminopeptidase activity (negative), esculin hydrolysis (variable), and Voges-Proskauer reaction (negative). Isolates typical morphologically and microscopically were identified as *Aerococcus urinae* when additional tests showed no more than two discrepancies.

Susceptibility Testing. Antibiotic susceptibility was tested by the direct agar diffusion method using Rosco Neo-Sensitabs (Rosco) on 5% sheep blood agar for penicillin and vancomycin, and Mueller-Hinton agar (Oxoid) for sulfamethoxazole. Strains were reported as susceptible (S) when zone diameters were ≥ 26 mm for penicillin, ≥ 23 mm for vancomycin, and ≥ 26 mm for sulfamethoxazole. Microdilution susceptibility testing was performed in Mueller-Hinton broth (Oxoid). The following break points indicated susceptibility: nitrofurantoin, ≤ 16 μ g/ml; amoxicillin, ≤ 2 μ g/ml; cefamandole, ≤ 4 μ g/ml; norfloxacin, ≤ 1 μ g/ml; trimethoprim, ≤ 1 μ g/ml; and cotrimoxazole, ≤ 1 μ g/ml.

Clinical Data. The following clinical and demographic information data were obtained: sex, age, symptoms of disease, local conditions predisposing to urinary tract infection (e.g., kidney stones, hyperplasia of the prostate), systemic predisposing conditions (e.g., diabetes mellitus, leukopenia, malignancy), laboratory results (e.g., leukocytosis, pyuria), clinical condition of the patient (good when mobile, moderate when partly mobile, poor when bedridden), and use of antibiotics.

Statistical Analysis. Differences in group proportions were assessed using the chi-square test.

Results

Both laboratories received urine specimens from patients in hospitals and nursing homes and from

Table 1: Phenotypic discrepancies among 54 *Aerococcus urinae* isolates.

Test result	No. (%)
Voges-Proskauer (API) positive	36 (66.7)
Esculin hydrolysis (API) positive	14 (25.9)
Ribose (API) negative	1 (1.8)
Sorbitol (API and acid production in Bacto nutrient broth) negative	1 (1.8)
Leucine-aminopeptidase (API) negative	2 (3.7)
β -glucuronidase (API and Rosco Diagnostic Tablets) negative	13 (24.1)
Sucrose (Bacto nutrient broth) negative	5 (9.3)
Maltose (Bacto nutrient broth) positive	1 (1.8)
Salicin (Bacto nutrient broth) positive	18 (33.3)

patients of general practitioners. Annually, approximately 18,000 positive urine cultures (i.e., $\geq 10^5$ cfu/ml) are found by these laboratories together. The estimated incidence of *Aerococcus urinae* is 0.31% for one laboratory and 0.44% for the other.

During the period of the study, 86 urinary cultures were collected that contained colonies suspected to be *Aerococcus urinae*. Further testing showed that only 54 isolates could be identified as *Aerococcus urinae*, since they showed no more than two discrepancies. All discrepancies encountered among these 54 isolates are presented in Table 1. The majority of the 32 isolates we discarded were identified as either *Streptococcus* spp. or *Enterococcus* spp.

Aerococcus urinae was found as a monoculture in 82.5% of patients. In seven female patients, *Aerococcus urinae* was found in a mixture with *Escherichia coli* ($n = 5$), *Klebsiella pneumoniae* ($n = 1$), or *Enterococcus faecalis* (1). The results of susceptibility testing are summarized in Table 2.

Questionnaires regarding 40 of the 54 isolates were available for clinical data analysis. Those who returned the questionnaires were mostly urologists

Table 2: Susceptibility of 54 *Aerococcus urinae* isolates.

Antimicrobial agent	No. of isolates	No. (%) susceptible	No. (%) resistant
Penicillin	54	54 (100)	0
Amoxicillin	53	53 (100)	0
Vancomycin	54	54 (100)	0
Nitrofurantoin	42	42 (100)	0
Cefamandol	44	43 (97.7)	1 (2.3)
Norfloxacin	46	36 (78.3)	10 (21.7)
Trimethoprim	40	18 (45.0)	22 (55.0)
Cotrimoxazole	35	20 (57.1)	15 (42.9)
Sulfamethoxazole	54	0	54 (100)

Table 3: Conditions predisposing to urinary tract infection among patients with *Aerococcus urinae* urinary tract infection.

Condition	No. (%) of patients (n = 40)
Local	
Kidney stones	4 (10)
Bladder stones	2 (5)
Hyperplasia of the prostate ^a	11 (55)
Urethral stricture	5 (12.5)
Vesicoureteral reflux	1 (2.5)
Transurethral resection of the prostate ^a	5 (25)
Cystocele	3 (7.5)
Neurogenic bladder	2 (5)
Indwelling catheter	4 (10)
Other	5 (12.5)
No local conditions	14 (35)
Systemic	
Diabetes mellitus	8 (20)
Malignancy ^b	8 (20)
Immunosuppressive therapy ^c	3 (7.5)
Dementia	8 (20)
Cerebrovascular accident	2 (5)
Kidney failure; creatinine >150 µmol/l	2 (5)
Other	8 (20)
No systemic conditions	11 (27.5)

^a Applies only to males (n = 20).

^b Cervical carcinoma, 2; colon carcinoma, 1; gastric carcinoma, 1; prostate carcinoma, 3; carcinoma of unknown origin, 1.

^c All 3 patients used oral corticosteroids for chronic obstructive pulmonary disease.

(47.5%), nursing home physicians (22.5%), and general practitioners (12.5%). The age of the patients ranged from 35 to 95 years, with a median of 82.5 years for women (n = 20) and 77.5 for men (n = 20); the majority of patients (88.6%) were older than 65 years. Almost all patients (97.5%) had one or more symptoms of urinary tract infection: dysuria (50%); pollakisuria (60%); abdominal pain (32.5%); flank tenderness (10%); fever (temperature > 38.5°C) (25%); or general discomfort (42.5%). Forty-five percent of patients, most of whom were women (12/20) (0.05 < p < 0.1), complained of urinary incontinence. Most patients had local (65%) or systemic (67.5%) conditions predisposing to urinary tract infections. Table 3 details these conditions. Men had significantly more local problems (p < 0.01) such as hyperplasia of the prostate or urethral stricture, whereas women had more systemic problems, such as dementia or cerebrovascular accidents (p < 0.05). The physical condition of the pa-

tients was good in 50%, moderate in 45%, and poor in 5%. Men were usually in better physical condition than women (p < 0.01).

Laboratory results were available for 77.5% of the cases. Many patients had pyuria (67.7%) and erythrocyturia (41.9%). Leukocytosis (> 11 x 10⁹ leukocytes/l) was found in 9.7%, and 51.6% had an elevated erythrocyte sedimentation rate (> 7 mm/h for males, > 12 mm/h for females). Most patients (80%) received antibiotics: amoxicillin, 25%; norfloxacin, 25%; amoxicillin plus clavulanic acid, 15.6%; either trimethoprim, cotrimoxazole, nitrofurantoin, ciprofloxacin, cefuroxime, or an unknown antibiotic, 34.4%.

Discussion

The present study shows that *Aerococcus urinae* urinary tract infections are relatively uncommon in the Netherlands and are usually found in patients over 65, who often have local or general predisposing conditions.

When comparing the results obtained in our laboratories to the work of Christensen et al. (1), we found the same low incidence of *Aerococcus urinae* isolates. Nevertheless, we think our figures are probably an underestimation, because the laboratory technicians must learn to recognize suspected colonies and not to mistake them for viridans streptococci or, on microscopy, for *Staphylococcus* spp.

Most of our isolates showed one or two discrepancies in the biochemical tests. These results contrast with those of Christensen et al. (1) who found ALO to have uniform phenotypical characteristics. A possible explanation may be the differences in the methods and materials used for testing reactions. We found several esculin hydrolysis-positive isolates that may resemble the esculin hydrolysis-positive biotype recently described (3). Most of these isolates also produce acid from salicin, which has not been described previously. The β-glucuronidase-negative isolates we found may represent another biotype of *Aerococcus urinae*. Of special interest is the fact that the majority of our isolates show a positive Voges-Proskauer reaction in the API 20 Strep. Further testing will be necessary to explain this result, which contrasts with the results of Christensen et al. (1, 2).

Of potential value in the identification of *Aerococcus urinae* isolates may be the polymerase chain

reaction developed by Aguirre and Collins (10), or the use of antibiograms (5).

The exact taxonomic position of *Aerococcus urinae* is still unclear. The results of Aguirre and Collins (4), based on partial 16S rRNA sequence analysis, could not be confirmed by Facklam and Elliott (6), who performed DNA-DNA hybridization studies and found a relatedness of less than 10% with *Aerococcus viridans*. Unweighted pair-group method with arithmetic averages (UPGMA) clustering of antibiotic susceptibility patterns also showed little similarity between *Aerococcus viridans* and *Aerococcus urinae* (5). Recently, Christensen et al. (3) performed DNA reassociation studies and 16S rRNA sequence analysis with esculin-negative and esculin-positive strains. The esculin-negative strains comprised a homogeneous group and were closely related to, but slightly genotypically different from, the esculin-positive strains. They concluded that, genotypically, *Aerococcus urinae* is somewhat heterogeneous and may contain two or more subspecies. Further investigations are necessary to establish the true taxonomic position of *Aerococcus urinae* and the esculin hydrolysis-positive biotype.

Urinary tract infections are a major health problem in elderly patients (11-14). Most uropathogens encountered in this population are *Enterobacteriaceae*, in both women and men. *Escherichia coli*, although still the most common isolate, is seen less often in elderly patients (12, 13). Unusual pathogens such as gram-positive cocci are encountered more often in the elderly, and one investigator even found more gram-positive isolates than *Enterobacteriaceae* in elderly men (12, 14, 15). Our study shows no male predominance for *Aerococcus urinae* infections. Most patients with *Aerococcus urinae* urinary tract infections had the same symptoms and predisposing conditions as patients with other urinary tract infections.

The virulence of *Aerococcus urinae* does not seem particularly high, but we advise caution because of reports of cases of urosepticemia and fatal endocarditis caused by *Aerococcus urinae* (7-9). None of our patients developed serious clinical symptoms.

Antibiotic therapy for urinary tract infections by *Aerococcus urinae* can consist of penicillin, amoxicillin, or nitrofurantoin, since all isolates tested were susceptible to these drugs, which is in accordance with the investigations done by Christensen et al. (1, 2). Sulfonamides cannot be used, since all isolates were resistant (1, 2). The susceptibility

to trimethoprim and cotrimoxazole is variable; therefore, these antibiotics should only be used after susceptibility is confirmed.

We conclude that *Aerococcus urinae* plays a minor but consistent role in urinary tract infections in the Netherlands, similar to the results reported in Denmark by Christensen et al. (1, 2). Since most patients who are affected are over 65, age is probably an important predisposing factor for *Aerococcus urinae* urinary tract infections.

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