

# Quinolone Resistance in *Oligella Urethralis*-Associated Urinary Tract Infection

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## ABSTRACT

The importance of oxidase-positive, nonfermentative, Gram-negative bacilli in a variety of pathological processes is becoming increasingly important. *Oligella urethralis* is an organism which is normally isolated as a commensal from the genitourinary tract. We have described one case of urinary tract infection caused by this organism. This isolated organism is found to be resistant to quinolones, while relatively sensitive to a wide variety of antibiotics. These findings clearly indicate that this organism may be an opportunistic pathogen and that extensive application of quinolones may provide a selective pressure for the emergence of resistance. We believe that this is the first report of isolation and quinolone resistance in *Oligella* in Iran.

**Keywords:** *Oligella urethralis*, Identification, UTI, Quinolone resistance

A 73-year-old female with a three day history of increasing urinary frequency along with urgency and dysuria, was admitted to the clinic in May 2003. She had a history of prior urinary tract infections (UTIs) of uncertain aetiology. However, she was completely asymptomatic for four weeks prior to this admission. The patient had received ciprofloxacin on her previous episodes of UTI.

A clean-catch midstream (CCMS) urine was collected, showing 12-14 white cells, 5-7 red blood cells and many bacteria under high power, which was suggestive of a urinary tract infection. Standard bacteriologic procedures for cultivation of the specimen were followed [1, 2]. The urine culture yielded more than  $10^5$  colony forming units per milliliter (CFU/mL) of a pure growth of bacteria, in both 5% sheep blood agar (SBA) and MacConkey agar (MAC) plates after overnight incubation at 35°C. A confirmatory second specimen which demonstrated the same result was obtained 24 hours later.

Presumptive identification was made on the basis of schemes described by Schreckenberger and Graevenitz. In order to obtain the most reliable microscopic morphology, Gram staining was performed with a sample from enrichment broth culture, incubated for 2h at 35°C. Motility was determined by preparing a wet mount preparation of a young colony from a SBA plate. This strain failed to acidify the butt of triple sugar iron (TSI)

agar and also was inactive in oxidation/fermentation of D-glucose. An oxidase test was performed using N, N, N, N-tetramethyl-p-phenylenediamine dihydrochloride (1%) [3]. According to the above mentioned tests, the isolated strain was characterized as a small Gram-negative, non-motile, nonfermentative, oxidase-positive bacillus which indicated a *Moraxella*-like organism.

The preliminary identification was followed by traditional and more specific biochemical reactions as described by Moss [4] and Gilardi [5]. This strain was found to have the following key characteristics: (i) growth on MacConkey's agar and forming colorless colonies, (ii) alkalization of Christensen's citrate, (iii) production of catalase, (iv) growth at 25, 37 and 42°C, (v) reduction of nitrite, (vi) production of phenylalanine deaminase (PAD) and lecithinase, (vii) sensitivity to Penicillin 10-U.

Other characteristics of this isolated strain are as follows: It was unable to reduce nitrate and hydrolyze gelatin, urea and aesculin. It could not produce any H<sub>2</sub>S, DNase and indole. It was non-pigmented and did not produce any haemolysis on Tryptic Soy agar (TSA) and SBA respectively. Methods used to grow and identify this microorganism were those that used for *Pseudomonas* spp. Based upon all the above mentioned characteristics, and on the basis of retrospective inspection of bacteriologic reports, the strain isolated in this reported case was finally confirmed as being *Oligella urethralis* [3-5, 6, 7, 10].

Even though there is no validated testing method [1, 3] and little documentation of the results of the sensitivity testing of *O. urethralis* in literature [7], we performed the antibiogram by the comparative Kirby-Baure disk diffusion method. A standardized inoculum of bacteria ( $1.5 \times 10^8$  CFU/mL) was swabbed onto the surface of a Mueller-Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents were placed on the agar. After 16-18 hours of incubation at 35°C in ambient air, the antimicrobial susceptibility pattern was interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [8]. The antimicrobial susceptibility pattern showed that this strain was resistant to nalidixic acid (30 µg), ciprofloxacin (1 µg), norfloxacin (5 µg), ofloxacin (5 µg) and nitrofurantoin (200 µg). However, it was sensitive to cefazolin (30 µg), cephradine (30 µg), cefotaxime (30 µg), ceftizoxime (30 µg), ceftazidime (30 µg) and cefixime (5 µg). (Oxoid Ltd., London, United Kingdom)

The patient was started on oral cefixime 400 mg once daily for five days. All her urinary symptoms settled two days after starting the treatment. A further urine culture was obtained three days after the completing treatment, which proved to be negative.

## DISCUSSION

*Oligella urethralis* (formerly *Moraxella urethralis* and CDC group M-4) was first described as a *Moraxella*-like organism by Lautrop et al in 1970[9]. The genus *Oligella* was named because of the small size of the bacilli on the Gram stain [10]. This genus belongs to a group of taxonomically diverse nonfermentative bacilli, and it could be distinguished from these similar organisms by a variety of traditional biochemical tests [1, 3-6].

In this case report, the isolated strain was primarily identified as a *Moraxella*-like organism based upon preliminary tests. *Oligella urethralis* is phenotypically similar to the *Moraxella* species, in that its isolates are coccobacillary, oxidase-positive, nonmotile, indole-negative and asaccharolytic Gram-negative bacteria. This strain of *O. urethralis* was ultimately differentiated from the similar *Moraxella* spp. based on more specific biochemical reactions. Some important features that help to differentiate *O. urethralis* from human isolates of *Moraxella* spp. and related organisms are as follows: *M. catarrhalis*, *M. lacunata* and *M. nonliquefaciens* all fail to grow on MacConkey agar, however they are able to reduce nitrate. *M. osloensis* and *M. atlantae*, are both PAD negative and unable to reduce nitrite. *M. canis* produces H<sub>2</sub>S (lead acetate paper) and reduces nitrate, whereas *M. lincolnii* neither grows on MacConkey agar nor reduces nitrite. Finally *Psychrobacter phenylpyruvicus* is a urease positive bacterium [3, 4, 11, 12].

Cellular fatty acid analysis can also be used to differentiate these microorganisms. Moss et al demonstrated that *O. urethralis* differed from the *Moraxella* species by the presence of large amounts (49%) of *cis*-vaccenic acid (18:1ω7c), small amounts (1%) of 3-

hydroxyhexadecanoate (3-OH-16:0), and the absence of 10:0 and 3-hydroxydodecanoate (3-OH-12:0) [4].

*Oligella urethralis* is a commensal of the genitourinary tract, and most clinical isolates are from urine and the female genital tract [1, 3, 7, 10, 12]. Although symptomatic infections are rare, bacteremia, septic arthritis (mimicking gonococcal arthritis) [13] and peritonitis in two patients who were receiving chronic ambulatory peritoneal dialysis have been described [7]. *O. urethralis* is occasionally identified as a source of urinary tract infections [12].

Although validated antimicrobial susceptibility testing methods for *Oligella* and other similar nonfermentative bacteria have not been established [1, 3], in vitro susceptibility studies have been published and antimicrobial agents that had potential activity against isolated organisms, have been noted [7, 14].

The strain described here was resistant to ciprofloxacin, norfloxacin, ofloxacin, nalidixic acid and nitrofurantoin, but sensitive to other antimicrobial agents, including the first and the third generation of cephalosporins, penicillins, chloramphenicol and aminoglycosides.

In our health system, quinolones have been widely used to treat uncomplicated upper and lower urinary tract infections, due to their high efficacy against the Gram-negative organisms, and also their oral administration. Our patient had received ciprofloxacin on her previous episodes of UTI and consequently, it seems that extensive application of quinolones may be responsible for emergence of resistance.

Despite being contemporary, there is little documentation of the results of sensitivity testing of *O. urethralis*. Riley and colleagues demonstrated that this organism is not intrinsically resistant to quinolones, because the five previously isolated cases, including the type strain NCTC 11999, were found to be sensitive to ciprofloxacin [7]. It is possible that quinolone resistance is readily acquired by this organism, as occurs with *Pseudomonas aeruginosa* [15, 16] and *Staphylococcus aureus* [17].

The two major mechanisms of quinolone resistance include alteration of the target sites, which are the organism's DNA gyrase (encoded by *gyrA* and *gyrB*) and topoisomerase IV (encoded by *parC* and *parE*), as well as active efflux of the drug out of the cell, which limits access of the drug to the target site. Resistance is usually associated with point mutations in the *gyr* or *par* loci [18].

Strains of *O. urethralis* are usually susceptible to β-lactam antibiotics; in one case, however, a β-lactamase producing strain has been reported by Pugliese et al [19]. Mammeri and colleagues demonstrated that chromosomal integration of a cephalosporinase gene from *Acinetobacter baumannii* into *Oligella urethralis*, could be the source of acquired β-lactamas resistance among two different species [20].

In conclusion, this case has emphasized the potential role of *Oligella urethralis* as an opportunistic pathogen. Furthermore, it has demonstrated that quinolone resis-

tance may arise in patients for whom quinolones are used extensively.

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#### REFERENCES

- Forbes BA, Sahn DF, Weissfeld AS. *Bailey & Scott's Diagnostic Microbiology*. 10<sup>th</sup> ed. Missouri: Mosby; 1998:350-62.
- Isenberg HD. *Essential Procedures for Clinical Microbiology*. 1<sup>st</sup> ed. Washington: ASM Press; 1998:95-101.
- Schreckenberger PC, Graevenitz AV. *Acinetobacter, Achromobacter, Alcaligenes, Moraxella, Methylobacterium*, and Other Nonfermentative Gram-Negative Rods. In: Murray PR, Baron EJ, Pfaller MA, et al. eds. *Manual of Clinical Microbiology*. 7<sup>th</sup> ed. Washington: ASM Press; 1999:539-60.
- Moss CW, Wallace PL, Hollis DG, et al. Cultural and chemical characterization of CDC groups EO-2, and M-6, *Moraxella* (*Moraxella*) species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. *J Clin Microbiol*. 1988;26:484-92.
- Gilardi GL. 1990. Identification of Glucose-Nonfermenting Gram-Negative Rods. North General Hospital, New York, N.Y.
- Hott JG, Krieg NR, Sneath PA, et al. *Bergey's Manual of Determinative Bacteriology*. 9<sup>th</sup> ed. Maryland: Williams & Wilkins; 1994: 92.
- Riley UBG, Bignardi G, Goldberg L, et al. Quinolone resistance in *Oligella urethralis*-associated Chronic Ambulatory Peritoneal Dialysis Peritonitis. *J Infect*. 1996; 32:155-6.
- National Committee for Clinical Laboratory Standards. 2000. Methods for disk diffusion antimicrobial susceptibility tests for bacteria that grow aerobically, 5<sup>th</sup> ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Lautrop H, Brovre K, Frederiksen W. A *Moraxella*-like microorganism isolated from the genito-urinary tract of man. *Acta Pathol Microbiol Scand*. 1970;78:255-6.
- Steinberg JP, Rio CD. Other Gram-Negative Bacilli. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 5<sup>th</sup> ed. Philadelphia: Churchill Livingstone, 2000:2468.
- Rossau R, Kersters K, Falsen E, et al. *Oligella*, a new genus including *Oligella urethralis* comb. Nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. Nov. (formerly CDC group IVe ): relationship to *Taylorella equigentalis* and related taxa. *Int J Syst Bacteriol*. 1987;37:198-210.
- Graham DR, Band JD, Thornsberry C, et al. Infections caused by *Moraxella urethralis*, *Moraxella*-like groups M-5, and *Kingella kingae* in the United States, 1953-1980. *Rev Infect Dis*. 1990;12:423-30.
- Mesnard R, Sire JM, Donnio PY, et al. Septic arthritis due to *Oligella urethralis*. *Eur J Clin Microbiol Infect Dis*. 1992;11:195-6.
- Bizet C, Tekaia F, Philipon A. In vitro susceptibility of *Alcaligenes faecalis* compared with those of other *Alcaligenes* spp. to antimicrobial agents including seven  $\beta$ -lactams. *J Antimicrobial Chemother*. 1993;32:907.
- Crock SM, Selkon JB, McLanly, et al. Clinical resistance to long-term oral ciprofloxacin. *Lancet*. 1985;2:275.
- Chapman ST, Speller DCE, Reeves DS. Resistance to ciprofloxacin. *Lancet*. 1985;2:39.
- Humphreys H, Mulvihill E. Ciprofloxacin resistant *Staphylococcus aureus*. *Lancet*. 1985;2:383.
- Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev*. 1997;61:377-92.
- Pugliese A, Pacris B, Schoch PE, et al. *Oligella urethralis* urosepsis. *Clin Infect Dis*. 1993;17:1069-70.
- Mammeri H, Poirel L, Mangeney N, et al. Chromosomal integration of a cephalosporinase gene from *Acinetobacter baumannii* into *Oligella urethralis* as a source of acquired resistance to  $\beta$ -lactamase. *Antimicrob Agents Chemother*. 2003;47:1536-42.

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