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Chen Z[1], Bates L J[1], Ognenovska K[1], McIver C[2], Phan M[3], Peters K M[3], Moore K H[1], Schembri M[3]
1. *University of New South Wales,* ***2.*** *St George Hospital,* ***3.*** *University of Queensland*

ANTIBIOTIC RESISTANCE/16S RRNA ANALYSIS OF BACTERIAL ISOLATES IN WOMEN WITH RECURRENT URINARY TRACT INFECTIONS AND DETRUSOR OVERACTIVITY

Hypothesis / aims of study

In the last 5 years recurrent urinary tract infection (UTI) has become an increasing problem in women with refractory detrusor overactivity (DO), affecting at least 40% of such women. Furthermore, the high rate of infections caused by antibiotic resistant bacteria impacts our ability to successfully treat UTIs. This is especially true for uropathogenic *E. coli*, which is responsible for over 80% of all UTIs and is increasingly becoming multi-resistant. Our aim was to investigate women with refractory DO and co-existent recurrent UTI over an extended time period. This was carried out using a combination of routine microbial culture to determine the primary causative agent during episodes of symptomatic UTI, as well as periodic analysis of urine using culture-independent methods (rRNA analysis) to determine the composition of bacteria present in the urine during the same time period.

Study design, materials and methods

Recurrent UTI was defined as ≥2 infections in 6 months or ≥3 infections in 1 year (1). Multiple mid-stream urine specimens with careful labial toilet were collected from women who had a prior history of recurrent UTI. Of these, women with proven recurrent UTI were chosen for further analysis (all of whom had refractory DO). The number of confirmed UTIs over the proceeding 24 months were noted. Patients with symptomatic UTI were treated with appropriate antibiotics. Voiding dysfunction was defined as maximum flow rate <10 centile, residual >100mL. Half of the urine sample was sent to the Microbiology Unit, cultured routinely at a threshold of $>10^6$ CFU/mL, to identify the major causative organism and antibiotic resistance. The results of routine culture, resistance patterns and isolates obtained from the agar plate were recorded. The remaining samples were stored in frozen aliquots (-20°C), from which total DNA was extracted. Genus-level characterization of the bacteria present in the urine samples was determined by employing 16S rRNA gene amplification and amplicon pyrosequencing(2).

ID	*UTI in 24 mo*	*No/Dates of urine samples*	*Organisms reported on routine microscopy*	*Antibiotic Resistance*	*Bacteria isolated (stored in glycerol)*	*No of Genera (16S rRNA)*
1	15	6 / Nov '14 – Jul '15	*E. coli; 2x nil growth; E. faecalis; nil; perineal flora*	*E. coli* – amoxicillin	N/a; nil x2; *E. faecalis; nil, mixed – nil coliforms*	31
2	17	6 April – July '15	*C. freundii; K. penumoniae; perineal/bowel; E. coli* x2*;* nil growth	C.f: amoxicillin, Augmentin, ceph, triprim K.p: nitrofurantoin; 1st *E.coli*: amoxicillin, tripim 2nd *E.coli* amoxicillin	*Citrobacter*; *Klebsiella* and scant coliform; mixed flora; *E. coli* x2; nil	25
3	5	4 June-July '15	Perineal & Bowel flora x2; perineal flora only x 2;	Nil	*Morganella* sp and E.*coli*; *Klebsiella*; single coliform; *Staph & enterococcus*; mixed *E. coli / staph*	42
4	3 (in 6 mo)	7 June – August '15	Perineal & Bowel flora x4; streptococcus, Perineal & Bowel flora; *E. coli*	Strep: Nitrofuratoin *E. coli*: amoxicillin	mixed *E. coli* and *staph*; mixed coliform and staph; mixed flora; *E. coli*	32
5	15	8 Jun '15 – July '15	Perineal & Bowel flora; *E. faecalis; E. coli* x3; Perineal & Bowel flora x2; *E. coli*	All 4 *E. coli*: amoxicillin, triprim;	Mixed samples grew coliform, enterococci and staph	40
6	14	3 Aug '15	*E. coli*, Strep, perineal flora	Nil	n/a; Strep; mixed staph and enterococci	19
7	3 (in 7 mo)	2	*E. coli*; perineal floral	*E. coli* ; amoxicillin/ Augmentin	n/a; mixed coliforms	24
8	8	*3* July '14 – Aug '15	*E. coli,* ESBL *E. coli; K. pneumoniae*	*E. coli* - Amoxicillin, triprim; ESBL E.coli - Amoxicillin, Augmentin, ceph, triprim, K.p amoxicillin	*E. coli; E. coli* and *Klebsiella*; *Klebsiella*	5
9	5 (in 12 mo)	3	Nil; *E. coli;* perineal flora	*E. coli* – triprim	N/a; *E. coli;* mixed staph + enterococci	23

Results

39 women gave urine samples over a 2-year period, of these nine women had proven recurrent UTI and refractory DO. Often the only UTI symptom was worsening urgency, frequency and urge leak. Median age was 75 years (range 57-81yo). In the previous

6 - 24 months there were 3-18 confirmed UTI (average 8/woman). Six of the 9 women have a history either past or present voiding issues, treated by double emptying, not requiring CISC.
Of the 42 urine samples collected from 9 patients (median 5 samples per patient; range 2-10), traditional microbiology culture results showed only 4 samples had no growth. 18 samples had an organism reported: 13 samples grew *E. coli,* 2 grew *Enterococcus faecalis,* 2 grew *Klebsiella pneumonia*, 1 *Citrobacter freundii.* 17 samples were reported as mixed perineal +/- Bowel flora (common isolated organisms *Morganella*, *Klebsiella*, *Staphylococci*, and *Enterococcus*). 3 patients had documented changes in the bacterial flora on routine microbiology culture results over time. Antibiotic resistance occurred in all but two of these women. Of the18 samples with confirmed bacteria on routine microbiology, only 4 were not resistant to multiple antibiotics. Culture-independent 16S rRNA sequencing has revealed that a diverse array of organisms are present in the urine samples from individual patients (see table). Further assessment of these populations will determine how the bacterial populations vary in each patient over time. Each patient yielded an average of 26.7 different genera (SD 11.2, Median 25, IQR 21, 36)

Interpretation of results
Most laboratories do not routinely report all organisms grown, preferring to report only the dominant organism, especially when there is mixed growth. However, if multiple bacteria are actually colonising the bladder, then treatment with antibiotics (specific for the predominant organism), may encourage unreported organisms to proliferate and become resistant. For example Patient 2 (below table) is a particularly striking example; She had 17 confirmed UTI over 24 months. Her samples clearly show how both the organisms and antibiotic resistance has changed over a relatively short period of time.

Concluding message
Results to date show that the organisms isolated from women with recurrent UTI and a history of refractory DO alter over time, as does antibiotic resistance. In these patients, reporting all identified bacteria may help guide treatment. Culture independent 16S rRNA sequencing data will enable us to profile all of the organisms present in the urine over an extended time period, and enable us to link changes in the bacterial population to episodes of symptomatic UTI.
Overall, these findings may enable us to understand why a proportion of women with refractory DO do not respond to anticholinergic medicines. Such pharmacotherapy is unlikely to be efficacious in the presence of constantly evolving UTI.

References
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