

Next Generation Sequencing for Microbial Analysis to Select Prophylactic Antibiotic Selection before Urologic Stone Surgery: A Culture Change

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Abstract

Background: This paper aims to determine if the combination of polymerase chain reaction (PCR) and next-generation sequencing (NGS) could identify bacteria in culture-negative urine that would alter prophylaxis management.

Methods: We sent approximately 5 - 10 mL of a preoperative urine sample to MicrogenDx for PCR/NGS analysis performed after surgery (blind to the surgeon). The physician prescribed standard of care antibiotic prophylaxis. Cases modeling the hospital course of 3 random patients were reviewed by eight urologists after surgery to determine if NGS results would change their prophylaxis regimen. An infectious disease pharmacist reviewed the cases and provided the “ideal” regimen. **Results:** Urine cultures identified bacteria in 11% (2/18) of cases. Culture speciation results were consistent with NGS results. NGS detected a dominant bacteria in 56% (10/18) of negative cultures and targetable bacteria in all samples. There was a 15% (3/20) infection rate. In both cases, NGS results suggest inadequate prophylaxis. In response to the case scenarios, 100%, 88%, and 88% of the urologists reported they would change prophylaxis with NGS results. During a case scenario, physicians would tend to overprescribe antibiotics given PCR/NGS data for prophylaxis selection. **Conclusion:** NGS identifies a targetable bacterium in up to 80% of negative urine cultures before urologic stone surgery. Responses to case scenarios indicate that physicians would change management based on NGS results. Inter-professional (urologic and pharmacy) antibiotic selection with PCR/16S DNA testing may be helpful to improve antibiotic stewardship.

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Keywords

Health-Care Associated Infection, Urinary Tract Infection, Infection Prevention, Surgical Site Infection

1. Introduction

Ureteroscopy for urinary tract urolithiasis is a standard procedure with an estimated 9200 cases in the US per year [1]. Despite antibiotic prophylaxis, the incidence of significant infections after flexible ureteroscopic (URS) lithotripsy is on the rise [2]. To prevent infections, the standard of care is to obtain a negative urine culture before surgery. However, with the next-generation sequencing, we are now able to understand that the urine is not sterile and that pathogenic bacteria may be still present, undetected by urine culture.

One of the challenges when choosing a prophylactic agent is that preoperative urine cultures often show no growth for patients who later develop SIRS [3] [4] [5]. Singh *et al.* found no significant association between pelvic urine cultures or stone cultures and the occurrence of SIRS [6]. We hypothesize the stone or previously placed stent may allow bacteria to form biofilms containing a small number of essential bacteria not detected by standard cultures.

Next-generation sequencing (NGS) poses an alternative to the traditional culture model by using high-throughput sequencing of rapid PCR for resistance genes combined with 16S rRNA (a type of NGS) to detect specific bacterial strains and has been used for the detection of urinary tract infections [7]. With its improved sensitivity, NGS (PCR/16s rRNA) with resistance genes could be used to guide antibiotic therapy. In this study, we pilot this novel approach quantitatively to evaluate the NGS platform's ability to identify bacteria in culture-negative urine that would alter the choice of antibiotic prophylaxis for patients undergoing urologic stone procedures. We also evaluate this approach qualitatively to determine its usefulness to urologic surgeons to inform clinical trial design.

2. Materials and Methods

2.1. Population

After IRB approval (HSC20050234H), subjects were recruited from urology clinics at their preoperative appointment before their planned ureteroscopy (URS) surgery for urinary stone. We did not exclude patients with ureteral stents, including all patients undergoing percutaneous nephrolithotomy (PCNL) or URS within the next two weeks. We informed patients that the results were purely for research purposes, and no analysis performed until after surgery. We also described we would not provide additional information to their physician that could alter antibiotics.

2.2. Next-Generation Sequencing

We collected whole urine (approximately 30 - 50 mL) utilizing special vacutainers supplied by MicrogenDx. We sent around 5 - 10 mL of urine taken for culture to MicroGen Diagnostics, a CAP-accredited and CLIA licensed clinical diagnostic lab, for analysis. MicrogenDx performed rapid PCR for common resistance genes in 21 subjects before URS. Each initial target bacterial or fungi DNA, whose concentration was measured to obtain an initial concentration (ng/uL), was diluted to obtain a six to eight-fold serial dilution series and run on the quantitative PCR (qPCR) panel assay on the Roche LightCycler 480 II instrument. We sequenced the V1-V2 hypervariable bacterial regions with 16S RNA using Ion Torrent (Ion Torrent PGM). Physicians did not obtain results before surgery. The physician proceeded with surgical management and standard of care antibacterial prophylaxis.

2.3. Data Collection

We recorded data from physician notes and medical record review regarding the details of patient history, urologic stone procedure, postoperative course, and infection outcomes. We recorded all speciation results from the standard of care urine cultures. We then compared these results to speciation results in the PCR/NGS (16s rRNA).

2.4. Case Study Creation

We perform a qualitative review in the form of case scenarios to determine if NGS would indeed alter antibiotic prophylaxis. Choosing from the cases enrolled, we randomly selected three cases to be reviewed by eight board-certified Urologists. One infectious disease pharmacists also reviewed the case reports and recommended the “ideal” antibiotic regimen for each case. The cases are highlighted in **Supplemental Figures S1-S3** representing each case scenario to include:

Case 1: Standard culture No Growth, PCR/16s showed primary bacteria with *Enterococcus* (82%) and secondary Staphylococcus (17%) with resistance genes for macrolide and aminoglycoside.

Case 2: Standard culture showed no growth; PCR/16s showed primary bacteria of *Enterococcus* (99%) with no resistance genes.

Case 3: Standard culture showed “normal flora,” PCR/16s showed *Citrobacter* (79%), and *E. coli* (12%) with resistance genes of methicillin, beta-lactam, quinolone, and macrolide.

The questions included:

- 1) Based on PCR/16s results, would you change your antibiotic?
- 2) Choose from your usual antibiotic choice, what antibiotic would you choose?
- 3) Exit survey questions:
 - a) Would this test improve my confidence to prevent infection?
 - b) Would I use this test again?
 - c) Would I recommend this test to other urologists?

- d) How important do you think this test would be in urologic practice?
 e) I have concerns about using this technology for antibiotic prophylaxis selection?

3. Results

3.1. Patient Characteristics

Participants had a median age of 59 (interquartile range: 37 - 70), were predominantly white (80%), and non-Hispanic (60%). They had a median BMI of 30.8 (interquartile range: 24.8 - 34.1). Most patients (14, 70%) were not diabetic. Most patients (12, 60%) did not have any allergies to antibiotics. 5 (25%) patients had indwelling stents when urine was collected, and 5 (25%) patients had percutaneous nephrostomy tubes (Table 1).

3.2. Surgical Methods

A variety of urologic stone procedures were represented in this cohort, with cases of PCNL and URS, laser lithotripsy, and basket retrieval, with and without

Table 1. Patient characteristics.

Characteristics n, (%)	Total patients (n = 20)
Gender	
Men	11 (55%)
Women	9 (45%)
Age years, (IQR)	59 (37 - 70)
Race	
White	16 (80%)
Unknown	3 (15%)
Black	1 (5%)
Ethnicity	
Non-Hispanic	12 (60%)
Hispanic	6 (30%)
Unknown	2 (10%)
Body Mass Index (IQR)	30.8 (24.8 - 34.1)
Diabetes status	
Non-Diabetic	14 (70%)
Diabetic	6 (30%)
History of UTIs	
No	13 (65%)
Yes	7 (35%)
Stent in place on presentation	
No	15 (75%)
Yes	5 (25%)
Catheter in place on presentation	
No	15 (75%)
Yes	5 (25%)

Abbreviations: IQR: interquartile range; URS: ureteroscopy.

nephrostomy tube/ureteral stent placement. There was 1 case of a TURP in addition to URS, and 1 case of an added endopyelotomy (**Table 2**).

3.3. Bacterial Identification and Antibiotic Resistance

Of the 20 cases, only 2 SOC cultures had speciation results and 16s rRNA analysis identified the exact bacteria colonized on both accounts (**Figure 1**). One patient had grown two bacterial species on culture, and NGS identified both species. In the 10 cases where SOC cultures resulted in no growth, NGS was able to identify targetable bacteria. In only 2 of those 10 cases with no growth, the dominant species detected by NGS (*Citrobacter* and *Lactobacillus*) was unlikely to cause postoperative infections. The other dominant species identified in these cases were *E. coli* and *S. epidermidis*, respectively. In nearly 80% of negative cultures, NGS provided dominant speciation data to consider when choosing antimicrobial prophylaxis before urologic stone procedures (**Figure 2**).

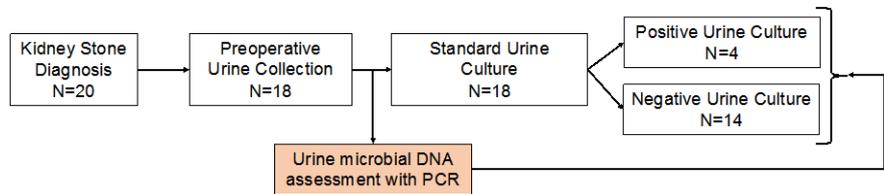
3.4. Cases of Infection

Of the 18 patients who underwent their procedure, 2 cases (11%) developed infections postoperatively. In the first infection case, the patient did not supply a urine culture before URS. The surgeon chose cefazolin for antibiotic prophylaxis. NGS detected a high bacterial load (>10⁷): 53% *Morganella morganii* and 45% *E. coli*. NGS was also able to identify resistance genes against beta-lactams and fluoroquinolones. Based on this data, we would recommend trimethoprim-sulfamethoxazole or a 3rd generation cephalosporin rather than a 1st generation cephalosporin. In the second case of infection, three or more organisms present, each higher than 10,000 cu/mL, per SOC UC results. The surgeon chose cefazolin and piperacillin/tazobactam for prophylaxis. NGS detected *Citrobacter* (64%), *Veillonella atypica* (27%), and resistance genes to methicillin, beta-lactams, macrolides, and aminoglycosides. NGS also detected *Candida albicans* in its fungal

Table 2. Surgical methods.

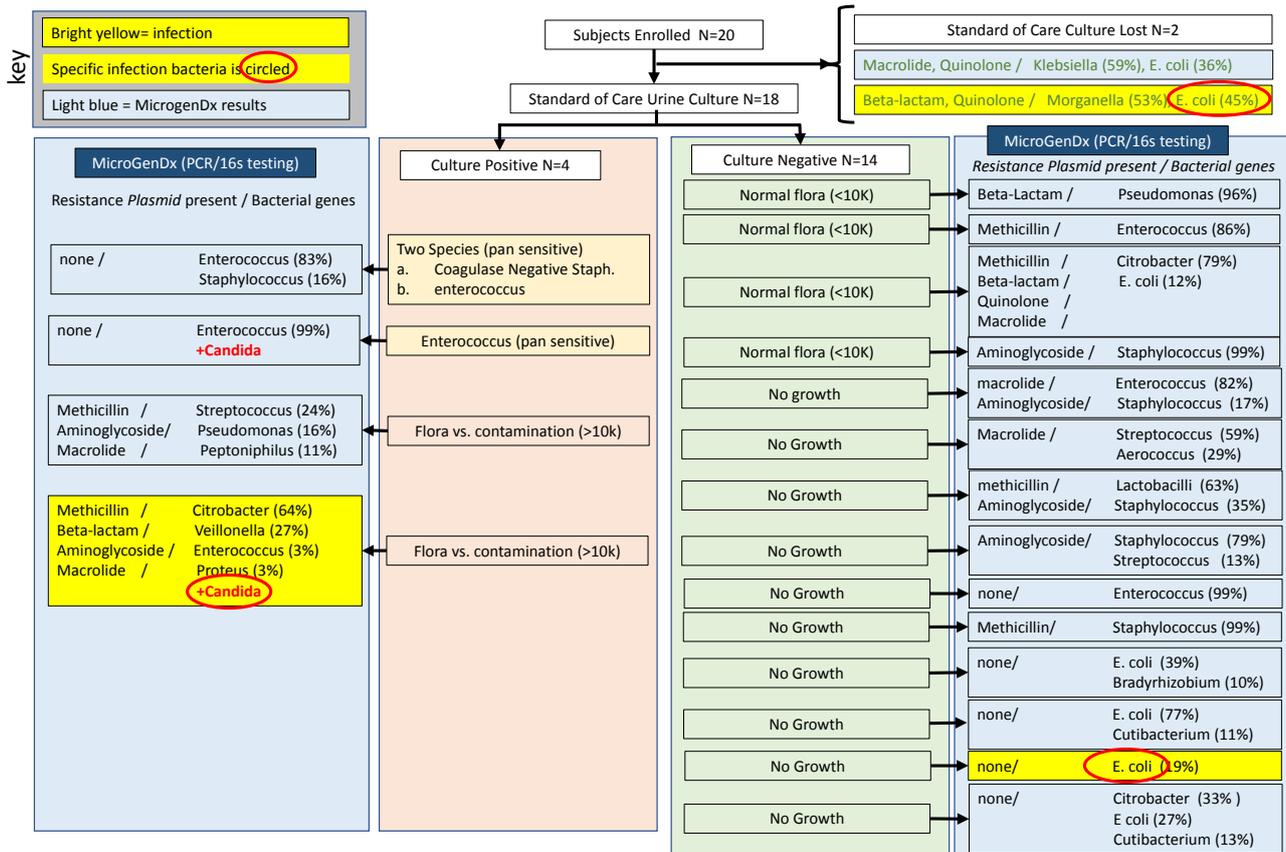
Surgical procedure	Total Patients (n = 20)
U PCNL with nephrostomy placement	3 (15%)
B PCNL with nephrostomy placement	1 (5%)
U PCNL	1 (5%)
U URS, LL with stent placement	5 (25%)
B URS, LL with stent placement	4 (20%)
B URS, LL with stent placement	1 (5%)
B URS, LL and unilateral Basket retrieval, bilat stent placement	1 (5%)
U URS, Basket retrieval, TURP	1 (5%)
U URS, endopyelotomy with stent placement	1 (5%)
Surgery cancelled	2 (10%)

Abbreviations: B: bilateral, U: unilateral, LL: laser lithotripsy, URS: ureteroscopy, TURP: transurethral resection of prostate.



We display our enrollment of 20 subjects through the study. The urine was split between culture and next-generation sequencing with PCR of plasmid genes. We then compared the results of the PCR to the culture specimen as ground truth.

Figure 1. Consort diagram.



We display a diagram of urine culture and corresponding PCR results from subjects On the left are the positive urine cultures noting alignment between genetic and phenotypic results. Of note, Candida is not routinely cultured before urologic stone procedures. On the right are all of the negative urine culture results and their associated NGS/PCR results. Bright yellow represents those that had postoperative clinical infection with a red circle to indicate the causative organism. The orange squares were those subjects selected for the case reports in the supplemental figures.

Figure 2. Results diagram.

screen as a standard part of the test. The patient developed funguria postoperatively with *Candida albicans*, indicating that NGS may be specific enough to recommend antifungal prophylaxis appropriately. These findings would be helpful in PCNL patient's that have had multiple antibiotics with high level of suspicion for candida

3.5. Case Scenario Data

Eight urologists responded to the three case scenarios, and in each of the 3 cases, 100%, 88%, and 88% of the physicians would have changed their prophylaxis

management given NGS results (**Supplemental Figures S1-S3**). The ideal antibiotic of choice based on the ID Pharmacist recommendations was oral Bactrim for all cases, and only 50%, 0%, and 0% selected this option. In contrast, 38%, 25%, and 63% chose to escalate the antibiotic to vancomycin, Zosyn, or Amikacin in conjunction with a second antibiotic. Overall, on exit survey, urologists reported that it is moderately important (n = 2, 25%), important (n = 4, 50%), or very important (n = 2, 25%) to test this technology in this context, while 38% have concerns about using this technology for antibiotic prophylaxis related primarily to implementation.

4. Discussion

We report several findings from our pilot study that include: NGS may provide actionable information above standard urine culture, urologists find the information useful, and concern for antibiotic escalation may benefit from interprofessional collaboration with pharmacists to select preoperative antibiotics in the context of PCR/NGS testing. Testing NGS is a practical extension to standard urinary culture in that the specimen can be split and sent for culture and NGS. Results of NGS are available in 48 hours to allow for time for preoperative antibiotic selection well before surgery.

In our first question, we address the usefulness of a PCR/NGS based urine profile before URS in this pilot study. We found that NGS detects targetable bacteria and fungi in culture-negative urine and propose utilizing this data in a prospective trial to use NGS to determine preoperative antibiotic prophylaxis. NGS may be more effective and specific than those predicated upon SOC preoperative urine cultures mostly negative before surgery. For example, we identified several culture-negative patients with dominant bacterial types that may influence a physician to prescribe a 1st generation cephalosporin compared to a gram-negative dominant group that may need a fluoroquinolone or 3rd generation cephalosporin. Importantly, urologists do not typically send a urine specimen specifically for fungi (*candida* sp.). The PCR/NGS test readily makes this information available, and in one patient that was positive did have sepsis caused by *Candida*, which was later cultured from the patient's blood. Many studies have reported the low sensitivity of preoperative urine cultures for predicting infectious complications of urologic stone procedures [3] [4] [5] [8]. Eswara *et al.* (2013) compared sensitivities among preoperative and perioperative pelvic urine and stone cultures for pathogen detection, reporting that of the patients who develop urosepsis, 0% had positive midstream preoperative urine cultures, while 73% had positive stone cultures. [9] However, they report a 64% concordance rate in urosepsis patients between stone cultures and readmission cultures, indicating that stone cultures did not always appropriately guide antibiotic selection in up to 36% of cases. Moreover, an antibiotic selection from stone culture rarely is provided in a timely fashion to alter postoperative antibiotics, and many of our patients go home the same day of the procedure. Our study, while small,

found that NGS was able to detect pathogens and provide alternate antimicrobial prophylaxis, especially in those who did have an infection.

Our findings are consistent with those of Long *et al.* (2016) and Grumaz *et al.* (2016), who reported that NGS is more sensitive than blood cultures for detecting pathogens in ICU patients. Notably, their studies observe the clinical utility of NGS to guide therapy in a high-risk population of ICU patients [10] [11]. Many studies have reported the increased sensitivity of NGS when compared to cultures, but further research is needed to establish cases where the improved sensitivity delivers cost-effective, clinically applicable data. The results of this study suggest that NGS may improve the standard of care in patients undergoing invasive urologic stone procedures.

Infectious complications are the most common cause of death following urologic stone procedures and present a sizeable economic drain on the American healthcare system [1] [12]. Other studies have found that up to 17% of urosepsis cases follow urologic interventions [13] [14] [15] [16] [17]. Koras *et al.* (2014) observed signs of SIRS in up to 27% of patients who underwent PCNL, 7.6% of which were diagnosed with sepsis [13]. Given the mortality rates and economic costs of urosepsis, SOC practices must be optimized to reduce the risk of infectious complications following urologic procedures.

Our qualitative questions address the usefulness of PCR/NGS testing to urologists before URS. We identified that 88% - 100% of urologists reported that they would have changed their prophylactic regimen based on NGS results in the post case survey. However, during the cases, most urologists did not choose the ideal regimen recommended by infectious disease pharmacists and in many escalated antibiotic use to either more antibiotics or broader spectrum. The findings of this study suggest that interdisciplinary collaboration between physicians and pharmacists may prevent excessive use of aggressive therapy when utilizing highly sensitive pathogen detection modalities, such as NGS.

We have several limitations to our study. The sample size limits the power of this study; however, this is a pilot study to inform a larger clinical trial and is informative for planning. We decided to publish these results due to the novelty of the research and its potential to change the preoperative antibiotic selection for a large number of patients. The increased sensitivity of NGS compared to SOC urine cultures reported herein was observed in a cohort of 20 patients. However, 88% - 100% of urologists surveyed stated that NGS with PCR for resistance genes would change their management in the 3 case scenarios we prepared, suggesting that physicians would use NGS in cases where it detected bacteria not found on preoperative cultures. More extensive studies are required to conclude that NGS can detect targetable microbes in culture-negative urine. Another limitation of this study is the lack of direct comparisons to perioperative cultures. However, as these perioperative cultures cannot guide antimicrobial prophylaxis, a direct comparison between preoperative studies was used. Lastly, this was a repository study using data from chart review. We did not call patients to follow

up to confirm the postoperative course. We did not perform a full cost analysis and the current cost of the specific PCR method in this study through Micro-GenDx is \$199.00 US. Urine testing is variable depending on if an analysis was sent first and if the culture is positive then species detection and resistance profiling is performed (average \$30 - \$80).

5. Conclusion

Infectious complications are the most common cause of death following urologic stone procedures. This study found that NGS can identify a targetable bacterium in up to 80% of negative urine cultures before urologic stone surgery. Responses to case scenarios indicate that physicians would change management based on NGS results. Using this data we have initiated a clinical trial using NGS to augment antibiotic selection in urine culture negative patients prior to ureteroscopy for stone surgery (NCT04404855).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplemental Figures

Case Scenario 1

A man in his late 50's presents with nephrolithiasis and blood in the urine. He is allergic to penicillins. The patient is planned to undergo bilateral ureteroscopy. SOC urine culture result showed no growth.

What would you select as your antibiotic for surgery prophylaxis? _____

Based on Microgen results:

1. Would you change your antibiotic? _____

2. What would you select? _____



Case Scenario 1

Next Generation Sequencing Results

MicroGen Diagnostics' comprehensive testing (patent pending) is a relative quantitative universal test for bacteria/fungi. DNA sequencing methods are used to identify the microorganisms' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Rapid Screening (PCR Results)		Comprehensive Identification (Sequencing Results)	
Bacterial Load (Medium)	10 ⁵ -10 ⁷	Detected Bacteria:	
Enterococcus faecalis	2.57 x 10 ⁵	Enterococcus faecalis	82%
Klebsiella pneumoniae	Not Detected	Staphylococcus epidermidis	11%
Streptococcus agalactiae	Not Detected	Staphylococcus lugdunensis	6%
Pseudomonas aeruginosa	Not Detected	NO FUNGAL SPECIES DETECTED	
Staphylococcus aureus	Not Detected		
Proteus mirabilis	Not Detected		
Escherichia coli	Not Detected		
Mobiluncus curtisii	Not Detected		
Mobiluncus mulleris	Not Detected		
Gardnerella vaginalis	Not Detected		
Ureaplasma urealyticum	Not Detected		
Ureaplasma parvum	Not Detected		
Staphylococcus saprophyticus	Not Detected		
Prevotella bivia	Not Detected		
Mycoplasma hominis	Not Detected		
Lactobacillus gasseri	Not Detected		
Lactobacillus crispatus/acidophilus	Not Detected		
Resistance Genes Detected			
Macrolide			
Aminoglycoside			
Resistance Genes Not Detected			
Vancomycin			
Methicillin			
Beta-lactam			
Carbapenem			
Tetracycline			
Quinolone			

Complete Antibiotic Analysis [Next Page(s)]

ANTIBIOTIC DISCLAIMER: Southwest Regional PCR assumes no liability to patients with respect to the actions of physicians, health care facilities and other users, and is not responsible for any injury, death or damage resulting from the use, misuse or interpretation of information obtained through this antibiotic report. Therapeutic options listed by the program are based upon national antibiotic susceptibility data and antibiograms. Therapy should not be undertaken without a thorough assessment of the indications, contraindications and side effects of any prospective drug or intervention. Furthermore, the database is curated and derived from incidence and prevalence statistics whose accuracy will vary widely for individual diseases and regions of the country. Changes in endemicity, incidence, and drugs of choice may occur. The list of drugs, infectious diseases and even country names will vary with time. Although we endeavor to include such new information on a timely basis, a delay cannot be avoided. For more information please contact us at 855-208-0019

Case Scenario 1

					82	11	6	
					Enterococcus faecalis	Staphylococcus epidermidis	Staphylococcus lugdunensis	Enterococcus faecalis
					Gram +	Gram +	Gram +	Gram +
Class	Generic	Topical	PO	IV	Resp	FAn	FAn	FAn
Aminoglycoside	amikacin*	✓		✓		✓		✓
Extended spectrum penicillin/beta-lactamase inhibitor	amoxicillin/clavulanate (Augmentin)		✓			✓	✓	✓
fluoroquinolone	levofloxacin		✓	✓		✓	✓	✓
	ciprofloxacin (Cipro)	✓	✓	✓			✓	
	moxifloxacin		✓	✓				✓
Glycopeptide	vancomycin	✓		✓		✓	✓	✓
	nitrofurantoin		✓			✓		✓
	tmp/smx (Bactrim)	✓	✓	✓		✓	✓	✓
Oxazolidine	linezolid	✓	✓	✓		✓	✓	✓
penicillins	penicillin v		✓			✓		✓
anti-psuedomonal penicillins	piperacillin/tazobactam	✓		✓			✓	
Carbapenem	doripenem			✓			✓	
Cephalosporin 1st generation	cephalexin (Keflex)		✓				✓	
	cefazolin			✓				✓
RNA sythetase Inhibitor	Mupirocin (bactroban)	✓					✓	✓

Gram Stain
 +: Positive, -: Negative, I:Indeterminate, N: not applicable U: Unknown
 Respiration
 Ae: Aerobic, An: Anaerobic, FAn: Facultative anaerobic, Unk: Unknown
 * Resistance genes found. Consultation with a pharmacist on an appropriate course of treatment with recommendations made at the discretion of the physician based on known interaction and concentrations is recommended.

Supplemental Figure S1. Case scenario 1 with microgen.

Case Scenario 2

A man in his mid 30's presents with nephrolithiasis and a right ureteral calculus. He has no known allergies to antibiotics. The patient is planned to undergo bilateral ureteroscopy. SOC urine culture result showed no growth.

What would you select as your antibiotic for surgery prophylaxis? _____

Based on Microgen results:

1. Would you change your antibiotic? _____
2. What would you select? _____



Case Scenario 2

Next Generation Sequencing Results

MicroGen Diagnostics' comprehensive testing (patent pending) is a relative quantitative universal test for bacteria/fungi. DNA sequencing methods are used to identify the microorganisms' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Rapid Screening (PCR Results)	Amount per mL	Comprehensive Identification (Sequencing Results)
Bacterial Load (Medium)	10 ⁵ -10 ⁷	
Enterococcus faecalis	3.04 x 10 ⁵	Detected Bacteria: Enterococcus faecalis 99%
Klebsiella pneumoniae	Not Detected	NO FUNGAL SPECIES DETECTED
Streptococcus agalactiae	Not Detected	
Pseudomonas aeruginosa	Not Detected	
Staphylococcus aureus	Not Detected	
Proteus mirabilis	Not Detected	
Escherichia coli	Not Detected	
Mobiluncus curtisii	Not Detected	
Mobiluncus mulieris	Not Detected	
Gardnerella vaginalis	Not Detected	
Ureaplasma urealyticum	Not Detected	
Ureaplasma parvum	Not Detected	
Staphylococcus saprophyticus	Not Detected	
Prevotella bivia	Not Detected	
Mycoplasma hominis	Not Detected	
Lactobacillus gasseri	Not Detected	
Lactobacillus crispatus/acidophilus	Not Detected	
Resistance Genes Detected		
None		
Resistance Genes Not Detected		
Vancomycin		
Methicillin		
Beta-lactam		
Carbapenem		
Macrolide		
Aminoglycoside		
Tetracycline		
Quinolone		

Complete Antibiotic Analysis [Next Page(s)]

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Case scenario 2

Class	Generic	Topical	PO	IV	Resp	Gram	Enterococcus faecalis	Enterococcus faecalis
						+	+	+
Aminoglycoside	amikacin	✓		✓			✓	✓
Extended spectrum penicillin/beta-lactamase inhibitor	amoxicillin/clavulanate (Augmentin)		✓				✓	✓
fluoroquinolone	levofloxacin		✓	✓			✓	✓
Glycopeptide	vancomycin	✓		✓			✓	✓
	nitrofurantoin		✓				✓	✓
	tmp/smx (Bactrim)	✓	✓	✓			✓	✓
Oxazolidine	linezolid	✓	✓	✓			✓	✓
penicillins	penicillin v		✓				✓	✓

Gram Stain
 +: Positive, -: Negative, I: Indeterminate, N: not applicable U: Unknown
 Respiration
 Ae: Aerobic, An: Anaerobic, FAn: Facultative anaerobic, Unk: Unknown
 * Resistance genes found. Consultation with a pharmacist on an appropriate course of treatment with recommendations made at the discretion of the physician based on known interaction and concentrations is recommended.

Supplemental Figure S2. Case scenario 2 with microgen.

Case Scenario 3

A woman in her early 60's presents with a right ureteral stone and nephrolithiasis. She has no known allergies to antibiotics. Approximately 2 months prior, she developed a fever and chills and was diagnosed with a UTI. She was treated with antibiotics and her symptoms improved. The patient is now planned to undergo bilateral ureteroscopy. SOC urine culture result showed 'Multiple organisms present, each less than 10,000 CFU/mL. These organisms, commonly found on external and internal genitalia, are considered to be colonizers. No further testing performed.'

What would you select as your antibiotic for surgery prophylaxis? _____

Based on Microgen results:

1. Would you change your antibiotic? _____

2. What would you select? _____



Case Scenario 3

Next Generation Sequencing Results

MicroGen Diagnostics' comprehensive testing (patent pending) is a relative quantitative universal test for bacteria/fungi. DNA sequencing methods are used to identify the microorganisms' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Rapid Screening (PCR Results)	Amount per mL	Comprehensive Identification (Sequencing Results)	
Bacterial Load (High)	> 10 ⁷	Detected Bacteria:	
Escherichia coli	8.25 x 10 ⁵	Citrobacter freundii	79%
Enterococcus faecalis	1.49 x 10 ⁵	Escherichia coli	12%
Klebsiella pneumoniae	3.19 x 10 ⁴	Streptococcus anginosus	6%
Streptococcus agalactiae	Not Detected	NO FUNGAL SPECIES DETECTED	
Pseudomonas aeruginosa	Not Detected		
Staphylococcus aureus	Not Detected		
Proteus mirabilis	Not Detected		
Mobiluncus curtisii	Not Detected		
Mobiluncus mulieris	Not Detected		
Gardnerella vaginalis	Not Detected		
Ureaplasma urealyticum	Not Detected		
Ureaplasma parvum	Not Detected		
Staphylococcus saprophyticus	Not Detected		
Prevotella bivia	Not Detected		
Mycoplasma hominis	Not Detected		
Lactobacillus gasseri	Not Detected		
Lactobacillus crispatus/acidophilus	Not Detected		
Resistance Genes Detected			
Methicillin			
Beta-lactam			
Macrolide			
Quinolone			
Resistance Genes Not Detected			
Vancomycin			
Carbapenem			
Aminoglycoside			
Tetracycline			

Complete Antibiotic Analysis [Next Page(s)]

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Case scenario 3

Class	Generic	Topical	PO	IV	Microgen							
					Gram -		Gram +		Gram +			
					Resp	FAn	FAn	FAn	FAn	FAn	FAn	
Aminoglycoside	amikacin	✓		✓	✓	✓		✓				
	gentamicin	✓		✓					✓			
Cephalosporin 3rd/4th generation	ceftriaxone*			✓	✓							
	cefepime*			✓	✓							
Fluoroquinolone	ciprofloxacin (Cipro)*	✓	✓	✓	✓	✓	✓	✓				
	levofloxacin*		✓	✓		✓		✓	✓	✓		
Glycopeptide	nitrofurantoin			✓	✓	✓		✓			✓	
	tmp/smx (Bactrim)	✓	✓	✓		✓	✓	✓			✓	
	vancomycin	✓		✓						✓	✓	
Polymyxin antibiotic	colistimethate (colistin)	✓		✓	✓	✓		✓				
Carbapenem	doripenem			✓		✓		✓				
	ertapenem			✓						✓		
Cephalosporin 1st generation	cephalexin (Keflex)*			✓				✓				
Cephalosporin 2nd generation	cefuroxime*			✓		✓		✓				
	cefprozil*			✓			✓					
Extended spectrum penicillin/beta-lactamase inhibitor	amoxicillin/clavulanate (Augmentin)*			✓		✓		✓			✓	
Tetracycline	doxycycline	✓	✓	✓		✓		✓				
	tetracycline			✓			✓					
anti-pseudomonal penicillins	piperacillin/tazobactam*	✓		✓			✓					
Oxazolidinone	linezolid	✓	✓	✓						✓	✓	
penicillins	penicillin g			✓						✓		
	penicillin v			✓							✓	

Gram Stain
 +: Positive, -: Negative, I: Indeterminate, N: not applicable U: Unknown
 Respiration
 Ae: Aerobic, An: Anaerobic, FAn: Facultative anaerobic, Unk: Unknown
 * Resistance genes found. Consultation with a pharmacist on an appropriate course of treatment with recommendations made at the discretion of the physician based on known interaction and concentrations is recommended.

Supplemental Figure S3. Case scenario 3 with microgen.