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Next generation sequencing in patients with nephrolithiasis: how does it perform compared with standard urine and stone cultures?

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Abstract

Background: Our aim was to compare microorganism detection between standard culture (Ctx) and next generation sequencing (NGS) in patients undergoing surgery for nephrolithiasis; we prospectively compared both urine and stone culture results using these two techniques.

Methods: We prospectively compared microorganism detection of urine and stone cultures using Ctx *versus* NGS in patients undergoing surgery for nephrolithiasis. We analyzed preoperative voided urine (Voided) using both Ctx and NGS. Intraoperatively, renal stone (Stone) cultures were analyzed with Ctx and NGS. The primary outcome was concordance in microorganism detection between Voided Ctx and Stone NGS, as well as between Stone Ctx and Stone NGS.

Results: We prospectively evaluated 84 patients. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Voided Ctx predicting Stone Ctx were 66.7%, 73.7%, 54.5%, and 82.4%, respectively. Concordance of Voided Ctx microorganisms to Stone microorganisms decreased when NGS was used for the Stone compared with Ctx. The sensitivity, specificity, PPV, and NPV of Voided NGS to predict Stone Ctx microorganisms were 85.2%, 24.6%, 34.8%, and 77.8%, respectively. The concordance of Voided NGS to Stone microorganisms improved when the Stone was analyzed *via* NGS compared with Ctx.

Conclusion: NGS has a higher detection rate of microorganisms than standard culture for both preoperative urine and stone cultures. Voided NGS was the most sensitive in predicting a positive Stone sample, but the specificity and PPV were, overall, low. Further correlation of NGS microorganism detection with patient outcomes will determine which clinical situations may benefit most from NGS *versus* standard culture in patients with urinary-tract stones.

Keywords: bacteriuria, infection, nephrolithiasis, next generation sequencing

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Introduction

Antibiotic stewardship has become the focus of much interest in urology, as omission of antibiotics can lead to sepsis during surgical procedures, and over prescribing can lead to multidrug resistant organisms. In ureteroscopy (URS) and percutaneous nephrolithotripsy Stone surgery (PCNL), the American Urological Association recommends preoperative antibiotic prophylaxis based on level Ib and level IIb evidence, respectively.[1,2] However, voided preoperative urine culture does not have a high concordance to organisms detected in renal aspirate or stone culture.[3] The lack of consistent test results translates to patients being treated with preoperative antibiotics that may not be effective against the organisms inhabiting the urinary tract or within the stone. The discordance between Voided cultures and renal cultures has the potential to lead to a higher incidence of avoidable infectious complications, as well as increased antibiotic resistance.[4–8]

Genetic sequencing in the form of next generation sequencing (NGS) of bacteria provides a novel means of identifying organisms in body fluids, tissues, and on prosthetic devices by detecting microorganism ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA). MicroGen Dx is a company that performs quantitative real-time polymerase chain reaction (PCR) assays and NGS from genomic DNA for multiple specimen types including urine. With respect to urine reporting, MicroGen Dx first detects and quantifies the presence or absence of genomic DNA from the eight most common microorganisms found in urine. Additionally, they test for the presence of antibiotic-resistant genes and return these quantitative real-time PCR results within 24 h in the level 1 panel. Subsequently, MicroGen Dx performs the NGS of genomic DNA with amplicon sequencing of the 16S and ITS regions in bacteria and fungi, respectively. The NGS report is provided within 3–5 days as the level 2 panel, which reports the significant microorganisms detected and their relative proportion.

Data from the orthopedics literature[9,10] shows that NGS is able to pick up organisms even in joints that are aseptic by standard culture techniques (Ctx). Given the aforementioned discordance in bacterial detection between voided urine (Voided), renal aspirate urine, and renal stone (Stone) cultures using standard techniques, a test which can more accurately and concordantly detect microorganisms from any specimen would benefit clinicians. It is unknown how NGS performs relative to Ctx in terms of providing clinically relevant microorganism detection and sample concordance. We planned a prospective study to compare Ctx *versus* NGS for detection of microorganisms in Voided and Stone samples among patients undergoing PCNL and URS for management of nephrolithiasis.

Methods

Study design

We performed a prospective study comparing microorganism detection using Ctx *versus* NGS with MicroGen Dx (Lubbock, TX, USA) on samples obtained *via* Voided urine, as well as Stones collected from patients undergoing URS and/or PCNL between 1 February 2019 and 15 March 2020. Each patient provided a preoperative urine sample typically *via* spontaneous voiding; however, Voided samples also included specimens from patients with urinary diversions, patients intermittently catheterizing, and patients with indwelling catheters.

The primary outcomes included the percentage of patients with positive Voided urine Ctx *versus* positive Stone NGS (i.e. the sensitivity of Voided Ctx for predicting positive Stone NGS), and the percentage of patients with positive Stone Ctx *versus* positive Stone NGS (i.e. the sensitivity of Stone Ctx for predicting a positive Stone NGS). For our primary outcome measure we defined concordance as cultures that were either both positive, or both negative for any microorganisms. The secondary outcome was the overall percentage of patients with positive Voided NGS.

Inclusion and exclusion criteria

Inclusion criteria:

(1) ⩾18 years of age;
(2) scheduled for PCNL and/or URS for renal calculi (unilateral or bilateral);
(3) patients with no prior urinary-tract infections and patients with known current or prior urinary-tract infections. This includes patients on antibiotics or who have taken antibiotics for a urinary-tract infection.

Exclusion criteria:

(1) inability to provide informed consent;
(2) pregnancy.

Study procedures

Each patient provided a preoperative urine sample *via* voiding (Voided) as described above. This specimen was sent for Ctx, and also for NGS with MicroGen Dx. It should be noted that patients

Table 1. Patient population characteristics ($n = 84$).

Characteristic	*n* (%)
Mean age at surgery, years (SD)	51.6 (15.8)
Female	51 (60.7)
Bilateral surgery	38 (45.2)
PCNL procedure	51 (60.7)
URS procedure	50 (59.5)
Any Voided microorganism detected by Ctx	33 (39.3)
Any Stone microorganism detected by Ctx	27 (32.1)
Any Voided microorganism detected by NGS	66 (78.6)
Any Stone microorganism detected by NGS	40 (47.6)

Ctx, standard culture; NGS, next generation sequencing; PCNL, pecutaneous nephrolithotripsy; SD, standard deviation; Stone, renal stone; URS, ureteroscopy; Voided, voided bladder urine.

with a positive Voided Ctx were treated with culture-directed antibiotics for a urinary-tract infection, as is our routine preoperative practice. The NGS samples were obtained as research specimens only and as such were not contained in the medical record. Therefore, the treating provider did not utilize Voided NGS data to guide medical decisions.

On the day of surgery (PCNL and/or URS), Stone specimens were obtained intraoperatively. In the setting of bilateral URS or bilateral PCNL the Stone specimen was obtained from the first renal unit accessed. For cases with PCNL and contralateral URS the Stone specimen was obtained from the renal unit that underwent PCNL. A portion of each Stone specimen was sent for both Ctx and NGS analysis by grasping the Stone with a forcep (PCNL) or basket (URS), placing the Stone in a culture container *via* a no touch technique, and then crushing the Stone into a saline solution with a sterile curved clamp. Similar to Voided data, the Stone NGS samples were obtained as research specimens only and as such were not contained in the medical record. Therefore, the treating provider did not utilize Stone NGS results to guide medical decisions in the postoperative period, such as whether or not to discharge the patient home with antibiotics.

This study received Institutional Review Board approval from Indiana University School of Medicine (no. 1907218746). We obtained both verbal and written consent from each patient.

Statistical analysis

All statistical analysis was performed using IBM SPSS software, version 25 (Armonk, NY, USA). For sample size calculation, we estimated that 50% of patients would have a positive Voided Ctx and Stone Ctx. This was based on prior local institutional data of patients undergoing URS and PCNL. We estimated 95% of patients will have a positive Voided NGS and 70% of patients will have positive Stone NGS.[11] We defined concordance as cultures that were either both positive, or both negative for any microorganisms. Using McNemar's test for paired proportions with 40% discordance, two-sided 5% significance level, and 80% power, we required inclusion of 84 patients to complete this study. This sample size calculation included both of the primary outcome measurements.

Results

We included 84 patients for analysis, all of whom had a Voided Ctx, Voided NGS, Stone Ctx, and Stone NGS data available by completion of the study. A total of 79/84 (94%) patient urine samples were spontaneously voided *per urethra* while the remaining patients provided urine *via* ileal conduit samples (2.4%), clean intermittent catheterization (2.4%), and indwelling catheter techniques (1.2%). All patient population characteristics are outlined in Table 1. For the entire cohort, we observed positive detection of microorganisms in 39.3%, 32.1%, 78.6%, and 46.7% of Voided Ctx, Stone Ctx, Voided NGS and Stone NGS samples, respectively.

The sensitivity, specificity, PPV, and NPV of positive Voided Ctx predicting positive Stone Ctx were 66.7%, 73.7%, 54.5%, and 82.4%, respectively (Table 2). The sensitivity, specificity, PPV, and NPV of positive Voided Ctx predicting positive Stone NGS were 47.5%, 68.2%, 57.6%, and 58.8%, respectively. Voided NGS demonstrated a higher sensitivity but lower specificity than Voided Ctx for predicting positive Stone Ctx with a sensitivity, specificity, PPV, and NPV of 85.2%,

Table 2. Test characteristics of preoperative voided *via* Ctx or NGS technique relative to intraoperative Stone culture (primary outcomes). Concordance defined as cultures are either both positive, or both negative for any microorganism.

	Characteristic	Technique	Intraoperative Stone culture	
			Ctx	NGS
Preoperative Voided	Sensitivity	Ctx	66.7	47.5
	Specificity		73.7	68.2
	PPV		54.5	57.6
	NPV		82.4	58.8
	Sensitivity	NGS	85.2	90.0
	Specificity		24.6	31.8
	PPV		34.8	54.5
	NPV		77.8	77.8

Ctx, standard culture; NGS, next generation sequencing; NPV, negative predictive value; PPV, positive predictive value; Stone, renal stone; Voided, voided bladder urine.

24.6%, 34.8%, and 77.8%, respectively. The sensitivity, specificity, PPV, and NPV of positive Voided NGS predicting positive Stone NGS were 90.0%, 31.8%, 54.5%, and 77.8%, respectively. There was minimal concordance between NGS and Ctx with a sensitivity, specificity, PPV, and NPV of Voided Ctx predicting Voided NGS of 42.4%, 72.2%, 84.8%, and 25.5%, respectively. This concordance between tests somewhat improved with the surgically obtained Stone samples with a sensitivity, specificity, PPV, and NPV of positive Stone Ctx predicting positive Stone NGS of 50.0%, 84.1%, 74.1%, and 64.9%, respectively.

The majority (62.5%) of NGS samples returned both a predominant growth microorganism along with at least one additional microorganism representing ⩾10% of the total microorgansims detected on the level 2 panel. The three most commonly detected microorganisms by Voided NGS were *Escherichia coli* (16.7%), *Enterococcus faecalis* (13.6%), *Proteus mirabilis* (7.6%) compared with Voided Ctx *E. coli* (15.2%), *E. faecalis* (12.1%) and *P. mirabilis* (9.1%). The three most commonly detected microorganisms by Stone NGS were *E. coli* (25.6%), *P. mirabilis* (12.8%), *E. faecalis* (7.7%) *versus* Stone Ctx which detected *E. coli* (22.2%), *P. mirabilis* (11.1%) and *E. faecium* (7.4%). Detection of microorganisms between Ctx and NGS for Voided and Stone cultures when including exactly matching and/or at least one overlapping microorganism is presented in Table 3.

In the entire cohort, three patients had a complication related to infection, of which all three were urinary-tract infections, managed with antibiotics as outpatients. There were no Clavien–Dindo grade 3 or higher complications in our patient population. In two of the three patients that experienced complications, Voided Ctx, Voided NGS, Stone Ctx, and Stone NGS were all positive. In the third patient, only the Voided NGS and Stone NGS were positive. Three additional patients had emergency room visits for pain unrelated to infection.

Discussion

We performed the first comparison of microorganism detection using standard Ctx to NGS in both Voided and Stone samples among patients undergoing surgical management of nephrolithiasis. We observed generally low rates of concordance between samples with both Ctx and NGS. While standard Ctx is the current gold standard for microorganism detection, NGS detected microorganisms more often, frequently identifying multiple organisms within a single positive

Table 3. Microorganism detection in voided and stone cultures using Ctx and NGS techniques including exactly matching and/or at least one overlapping microorganism.

Comparison	*n* (%)
Exactly matching Voided Ctx and Voided NGS	16 (19.0)
Exactly matching or at least one overlapping organism between Voided Ctx and Voided NGS	32 (38.1)
Exactly matching Voided Ctx and Stone Ctx	53 (63.1)
Exactly matching or at least one overlapping organism between Voided Ctx and Stone Ctx	56 (66.7)
Exactly matching* Voided Ctx and Stone NGS	35 (41.7)
Exactly matching or at least one overlapping organism between Voided Ctx and Stone NGS	42 (50.0)
Exactly matching Voided NGS and Stone Ctx	13 (15.5)
Exactly matching or at least one overlapping organism between Voided NGS and Stone Ctx	26 (31.0)
Exactly matching* Stone NGS and Stone Ctx	44 (52.4)
Exactly matching or at least one overlapping organism between Stone NGS and Stone Ctx	55 (65.5)
Exactly matching Voided NGS and Stone NGS	12 (14.3)
Exactly matching or at least one overlapping organism between Voided NGS and Stone NGS	35 (41.7)

*Exactly matching includes samples that showed no microorganism growth in addition to samples that showed the exact same microorganism growth.
Ctx, standard culture; NGS, next generation sequencing; Stone, renal stone; Voided, voided bladder urine.

sample. We found that overall, Voided NGS was the most sensitive in predicting a positive Stone sample, but the specificity and PPV were overall low, meaning a positive result was not always clinically relevant. However, we were less likely to miss a microorganism that may be present in the Stone when NGS testing was utilized on Voided samples. While this more sensitive test may allow treatment and prevention of unwanted infectious complications, it could also result in over treatment of many asymptomatic individuals. Overall, we did note that either test, Ctx or NGS, performed best when compared with themselves; meaning, a Voided Ctx had a better sensitivity and specificity when predicting a Stone Ctx and a Voided NGS was best when predicting a Stone NGS.

These data raise the question of what the true gold standard should be for microorganism detection in order to guide optimal preoperative antibiotic prophylaxis for Stone surgery. Overall, the concordance of the current gold standard Voided preoperative urine culture with the Stone culture using the Ctx technique is low in our cohort at 66%. Although not observed in the current study, some patients will have a negative preoperative urine culture, but still proceed to postoperative systemic inflammatory response syndrome (SIRS) response or urosepsis, suggesting that there may be undetected clinically significant bacteriuria or additional factors contributing (i.e. intraoperative urinary-tract pressurization or funguria that is not being detected or mitigated by the Voided Ctx). There is no literature on how preoperative treatment of positive urine cultures prior to Stone surgery affects the intraoperative Stone NGS results and we did not specifically aim to assess that within our study. Further study regarding the clinical significance of positive results for both Ctx and NGS is warranted along with the effect of recent antibiotic use on intraoperative Stone NGS results.

One of the more notable trends in this study is the high rate of discordant findings between Ctx and NGS among the same patient samples (Voided and Stone). Voided NGS was positive in twice as many patients as Voided Ctx, and Stone NGS was positive in an extra 15.5% of patients compared to Stone Ctx. Exact matches, meaning both samples were negative or both samples had the exact same microorganisms growing, were variable, both for the same samples and between urine and Stone samples. A prior phase II study by McDonald *et al.*[11] compared standard urine Ctx with NGS of urine samples in both asymptomatic controls ($n=22$) and in patients with acute cystitis ($n=44$). Standard urine Ctx detected positive urine Ctx in 13/44 (30%) symptomatic patients and 5/22 (23%) asymptomatic controls, whereas NGS detected positive urine Ctx in 44/44 (100%) symptomatic patients and 21/22 (95%) of controls. In another study of orthopedic patients, Tarabichi *et al.*[9] evaluated concordance between standard Ctx and NGS in synovial fluid samples obtained from clinically diagnosed periprosthetic joint infections. The authors found that standard Ctx was positive in 35% of cases (30/86 patients), whereas NGS was positive in 42% (36/86) of patients. Only 25 patients (30%) had a concordance between positive samples (i.e. positive standard Ctx and positive NGS). These studies, along with our results, show a high rate of discordance between Ctx and NGS for different types of clinical samples. With a high sensitivity, NGS does have the potential to minimize postoperative infectious complications with more targeted preoperative treatment or prophylaxis *versus* Ctx; however, to determine the clinical impact of such a test would require a very large treatment cohort, since serious infectious complications following Stone surgeries are infrequent (0.3% and 0.9–4.7% sepsis rates post-URS and PCNL, respectively).[12–14] Accordingly, our cohort infectious complications were both uncommon and low grade when using urine Ctx results to guide clinical management. Further studies would be needed to identify the optimal balance between striving for no infectious complications with the use of NGS without over treating individuals and further leading to rising antibiotic resistance.[15] In patients who proceed to have a postoperative urinary tract infection or urosepsis event, the quick turnaround (<24 h) of an intraoperative level 1 NGS panel may help target initial antibiotic selection based on a local antibiogram, as microorganism detection would be available faster than that of a standard Ctx.

The current study does have limitations. One important limitation is the lack of a strong guideline-based algorithm for management of preoperative positive urine cultures prior to surgery for nephrolithiasis. It has been our practice to treat positive urine cultures with at least 48 h, and in most cases, 7 days of culture-specific antibiotics prior to surgical intervention. A related challenge is the difficult nature of determining what organisms are contaminants as opposed to pathogenic and potentially virulent when detected on Ctx or NGS. To this end, we elected to include a real-world generalizable cohort of patients in our analysis, including five patients who used indwelling catheters, clean intermittent catheterization (CIC) or had an ileal conduit, as there is no established guideline for preoperative culture management specific to these patients prior to Stone surgery. Similarly, as a relatively new technique, the clinical significance of detecting multiple microorganisms *via* NGS requires further evaluation. There is a paucity of evidence to guide what microorganisms detected within an NGS analysis are clinically significant and may require antibiotic coverage (i.e. if six microorganisms are detected by NGS, how do you determine whether to cover only the predominant microorganism *versus* the top three most prevalent *versus* all six?). Additionally, different institutions will have access to different testing kits that introduces further variability in the reproducibility of both Ctx and NGS. It is important to note that as with many of the NGS testing kits available, MicroGen Dx does not test susceptibilities to antibiotics for the pathogens. Detection of eight common resistance genes are screened by MicroGen Dx, but it is the responsibility of the treating provider to correlate the microorganisms detected to local antibiograms. Although detection of common antibiotic-resistance genes may be helpful it does not ensure that corresponding phenotype in all microorganisms. Finally, it is important to acknowledge that urinary-tract calculi can fully obstruct along the length of the urinary tract, resulting in a falsely negative distal Voided Ctx or NGS sample with clinically significant obstructed proximal microorganisms in the urine. Taken together, these limitations highlight uncertainty in how to integrate NGS results into clinical management of patients with urinary-tract stones, and a Voided Ctx with specific antibiotic sensitivities remains the gold standard.

Specific to surgical treatment of nephrolithiasis, there is a lack of consensus regarding the optimal management of patients who have a positive intraoperative Stone culture, and it is unclear how a more sensitive test for microorganism detection could affect this ongoing debate. Additionally, although not the aim of this paper, consideration of healthcare costing for optimal utilization of

NGS will need to be evaluated. Overall, in an effort to continue minimizing postoperative infectious complications while avoiding overtreatment and antibiotic resistance, further studies should be pursued in order to evaluate the best way to utilize NGS for Stone surgery.

In conclusion, NGS has a higher detection rate of microorganisms than standard Ctx for both preoperative Voided and intraoperative Stone Ctx. However, we recognize that over detection of organisms has risks, and a clinical judgment is still required by the treating physician regarding what is considered clinically significant. Preoperative urine NGS yielded a higher sensitivity for the positive Stone culture result (using either Ctx or NGS) when compared with standard Voided Ctx. Further correlation of NGS microorganism detection with objective patient outcomes will determine which clinical situations may benefit most from NGS culture utilization in patients with urinary-tract stones.

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Author Contributions

Conception and Design, A.E.K., T.L., M.E.R. and C.U.N.; Acquisition of Data, C.U.N., A.W.P., T.L., D.K.A., M.E.R. and A.E.K.; Analysis and Interpretation of Data, M.A.A., C.U.N., T.L., M.E.R. and A.E.K.; Drafting of Manuscript, C.U.N. and M.A.A.; Critical Revision of the Manuscript for Important Intellectual Content, C.U.N., M.A.A., T.L., M.E.R. and A.E.K.; Statistical Analysis, C.U.N. and M.A.A; Supervision, T.L., M.E.R. A.E.K.

Conflict of interest statement

Dr Krambeck is a consultant for Boston Scientific and Lumenis and is on the data safety monitoring board for Sonomotion. Dr Rivera is a consultant for Boston Scientific and Lumenis. Dr Large is a consultant for Boston Scientific and Lumenis. No other disclosures or competing financial interests exist.

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References

1. Knopf H-J, Graff HJ and Schulze H. Perioperative antimicrobial prophylaxis in ureteroscopic stone removal. *Eur Urol* 2003; 44: 115–118.
2. Darenkov AF, Derevianko II, Martov AG, *et al.* The prevention of infectious-inflammatory complications in the postoperative period in percutaneous surgical interventions in patients with urolithiasis. *Urol Nefrol (Mosk)* 1994; 2: 24–26.
3. Walton-Diaz A, Vinay JI, Barahona J, *et al.* Concordance of renal stone culture: PMUC, RPUC, RSC and post-PCNL sepsis-a non-randomized prospective observation cohort study. *Int Urol Nephrol* 2017; 49: 31–35.
4. Mariappan P, Smith G, Bariol SV, *et al.* Stone and pelvic urine culture and sensitivity are better than bladder urine as predictors of urosepsis following percutaneous nephrolithotomy: a prospective clinical study. *J Urol* 2005; 173: 1610–1614.
5. Korets R, Graversen JA, Kates M, *et al.* Post-percutaneous nephrolithotomy systemic inflammatory response: a prospective analysis of preoperative urine, renal pelvic urine and stone cultures. *J Urol* 2011; 186: 1899–1903.
6. Liu YQ, Lu J, Hao YC, *et al.* [Predicting model based on risk factors for urosepsis after percutaneous nephrolithotomy]. *Beijing Da Xue Xue Bao Yi Xue Ban* 2018; 50: 507–513.
7. Lai WS and Assimos D. Factors associated with postoperative infection after percutaneous nephrolithotomy. *Rev Urol* 2018; 20: 7–11.
8. Hu M, Zhong X, Cui X, *et al.* Development and validation of a risk-prediction nomogram for patients with ureteral calculi associated with urosepsis: a retrospective analysis. *PLoS One* 2018; 13: e0201515.
9. Tarabichi M, Shohat N, Goswami K, *et al.* Can next generation sequencing play a role in detecting pathogens in synovial fluid? *Bone Joint J* 2018; 100–B: 127–133.
10. Tarabichi M, Alvand A, Shohat N, *et al.* Diagnosis of Streptococcus canis periprosthetic joint infection: the utility of next-generation sequencing. *Arthroplast Today* 2018; 4: 20–23.

11. McDonald M, Kameh D, Johnson ME, *et al*. A head-to-head comparative phase II study of standard urine culture and sensitivity versus DNA next-generation sequencing testing for urinary tract infections. *Rev Urol* 2017; 19: 213–220.

12. Somani BK, Giusti S, Sun Y, *et al*. Complications associated with ureterorenoscopy (URS) related to treatment of urolithiasis: the clinical research office of endourological society URS global study. *World J Urol* 2017; 35: 675–681.

13. Skolarikos A and de la Rosette J. Prevention and treatment of complications following percutaneous nephrolithotomy. *Curr Opin Urol* 2008; 18: 229–234.

14. De la Rosette J, Assimos D, Desai M, *et al*. The clinical research office of the endourological society percutaneous nephrolithotomy global study: indications, complications, and outcomes in 5803 patients. *J Endourol* 2011; 25: 11–17.

15. Zowawi HM, Harris PNA, Roberts MJ, *et al*. The emerging threat of multidrug-resistant gram-negative bacteria in urology. *Nat Rev Urol* 2015; 12: 570–584.