Title: Metagenomic sequencing to replace semi-quantitative urine culture for detection of urinary tract infections: a proof of concept.

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ABSTRACT

Semi-quantitative bacterial culture is the standard method to diagnose urinary tract infections (UTI), but bacterial growth rate limits diagnostic speed and it is unreliable when patients have been pre-treated with antibiotics. Metagenomics could increase diagnostic speed and accuracy by sequencing the microbiome and resistome directly from urine samples, bypassing culture. However, a semi-quantitative approach – as needed for diagnosing UTIs – has not been established.

Metagenomics was deployed to identify and semi-quantify bacterial presence indicative of UTI, predict antimicrobial susceptibility (AMR), and results were compared to semi-quantitative culture. Whole genome sequencing of the corresponding uropathogens was done for comparison. Analysis time and cost were tracked.

Forty-one consecutive urine samples underwent metagenomic analysis. All culture positive samples contained >200ng of DNA, suggestive of a threshold below which UTI could be ruled out solely based on DNA quantity. A semi-quantitative Diagnostic Index (DI) was created by multiplying the total DNA quantity by the relative abundance of uropathogens per urine sample. The DI allowed discrimination of UTI from non-UTI samples in all but 1 case. Metagenomic detection of AMR determinants correctly predicted the phenotype of uropathogens in 20 of 32 cases. The metagenomic work-flow was 31h and cost €116 per sample, but could be reduced to 4.5h and €5 for low-DNA-yield non-UTI samples.

The genomic determinants of AMR and their distribution across uropathogens need to be better understood for prediction of AMR phenotypes by metagenomics. The introduction of the

DI demonstrates the potential of semi-quantitative metagenomics to replace culture as rapid diagnostic method for UTI.

INTRODUCTION

Urinary tract infections (UTIs) are amongst the most common infections that require antibiotic prescription, and in catheterized and hospitalized patients UTIs are the most common nosocomial infections(1-3). The high incidence of UTIs, paired with increasing resistance to first and second line antibiotics for common uropathogens, such as *Escherichia coli*, stress the need for rapid diagnostics in aid of appropriate antimicrobial treatment(4). However, the current standard for diagnosing UTIs is time-consuming as it is based on (semi-)quantitative culture of urine, followed by identification and antimicrobial susceptibility testing (AST) of isolated pathogens, which usually takes 1-3 days and for some pathogens up to 7 days. In addition, growth of clinically relevant bacteria may be hampered by prior antimicrobial treatment, which is common practice worldwide, resulting in diagnostic challenges and risks of misdiagnoses.

Dependence on culture for UTI diagnosis can be bypassed by quantitative molecular detection of uropathogens in urine specimens. Multiplex real-time PCR-based methods have shown promise for this purpose but are hampered by exclusive detection of selected micro-organisms and lack of, or incomplete information on antimicrobial resistance (AMR) patterns(5, 6). Direct metagenomic sequencing of microbial communities in urine provides opportunities for unbiased detection of uropathogens, including the presence of AMR genes, while current next generation sequencing (NGS) methods and bio-informatic tools facilitate generation of such results in a timely fashion. Metagenomic approaches have indeed shown promise in identifying

uropathogens and their antimicrobial susceptibility patterns in urine samples(7). However, semi-quantitative detection of uropathogens is essential in order to replace culture-based diagnostics by metagenomic approaches since international clinical guidelines use bacterial loads (e.g. $< 10^3$ or 10^5 CFU/mL) to define evidence of UTI in different patient populations(8, 9). To facilitate such semi-quantitative metagenomic diagnostics for UTI, we developed and tested a metagenomic sequencing-based algorithm for determination of clinically relevant levels of bacterial DNA, indicative of urinary tract infections. Results were compared to the reference test semi-quantitative urine culture followed by species identification by MALDI-TOF, and automated AST. Whole genome sequencing (WGS) of isolated uropathogens served as benchmark for expected AMR gene content in metagenomes. We recorded the time to result and direct costs. The aim of this study was to provide proof of concept for the use of metagenomics as a tool for detecting UTI and predicting antimicrobial susceptibility patterns.

METHODS

Urine Samples

During two days, all urine samples obtained from patients with suspected UTIs that were assessed at the clinical microbiology laboratory for culture, identification, and AST, were included in the study. We collected up to a maximum of 20ml of surplus urine. Urine samples, corresponding routine culture plates and AST results were collected and anonymized, according to local and national ethical requirements for diagnostic studies using surplus routine care patient samples.

Urine Culture

Urine was cultured according to local standard operating procedures (see supplementary data for summarized SOP). Bacterial isolates were identified morphologically and by MALDI-TOF MS (MALDI Biotyper, Bruker, Karlsruhe, Germany) and were reported in a semi-quantified way as the number of colony forming units (CFU) per ml.

A culture-positive urine was defined as growth of clinically relevant bacteria at numbers $>10^3$ CFU/ml and for the purpose of this study all culture-positive urine samples were assigned to the UTI group. Growth of 10^3 CFU/ml or less was reported as “no significant growth”, while mixed growth of commensal vaginal, rectal, or mucocutaneous bacteria in any numbers or absence of any growth were reported as “commensal flora” or “no growth”, respectively. The latter three culture results together formed the non-UTI group. The clinically relevant bacteria from culture-positive urines were subcultured on Columbia agar plates with 5% sheep blood (Biomérieux, Marcy-l’Étoile, France) prior to DNA extraction and WGS.

Antimicrobial susceptibility testing

AST was done directly on urine by disc diffusion if Gram staining of the urine revealed the presence of abundant bacteria of a single morphology, or on identified bacterial isolates using VITEK 2 (version 06.01; Biomérieux), both according to EUCAST guidelines and breakpoints(see Supplementary data, Summary of SOPs)(10).

DNA extraction and sequencing

DNA was extracted from 20 ml of urine. If <20 ml of urine was available, the sample was supplemented with PBS. The urine was first centrifuged for removal of human cells (2000 g, 30 s), followed by centrifugation of the supernatant for pelleting of bacterial cells (8000 g, 10 min). The bacterial pellet was pre-lysed with an in-house enzyme cocktail comprising achromopeptidase, mutanolysine, lysostaphine (1000:100:3) and lysozyme (1mg/ml) (Sigma-Aldrich, St. Louis, MO, USA) in TE buffer. This was followed by lysis using proteinase K and an in-house lysis buffer (Sodium-docecyl-Sulphate (1 %), Tween-20 (0.5 %) and Sarkosyl (0.5 %) in TE-buffer), after which automated DNA extraction was performed immediately using the NucliSENS easyMag platform (Biomérieux) following manufacturer's instructions. DNA from the cultured bacteria was extracted with the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). The Qbit dsDNA HS Assay Kit (ThermoFisher, Waltham, Massachusetts, USA) was used to measure DNA concentration. The Ion Xpress™ Plus Fragment Library Kit (Thermo Fisher Scientific) was used for PCR-free, manual library preparation according to manufacturer's specifications. Library quantification was performed with the Ion Library TaqMan Quantification Kit (Thermo Fisher Scientific). DNA was sequenced on the Ion Torrent Proton platform, set to produce an average of 1 million 200 base-pair length single-end reads per sample. Trimmomatic was used to remove low quality reads (settings WGS: headcrop 15, sliding window 4:20, Phred score 15; settings metagenomics: headcrop 15, crop 270, sliding window 4:20, Phred score 10, minlen 50)(11).

Metagenomic analysis

Human sequences in the metagenomic datasets were removed with Deconseq version 0.4.3, human genome version 37(12). The remaining reads were analyzed for identification of microbial DNA at subspecies level and determination of the organism's relative abundance using the CosmosID bioinformatics software package (CosmosID Inc., Rockville, MD)(13-16). A cloud version is accessible at https://app.cosmosid.com. The relative abundance of each bacterial organism per sample was expressed as a percentage of the total number of bacterial reads belonging to that organism, normalized for organism-specific genome length. Reads identified as eukaryote, viral, or archaeal were excluded.

All bacteria identified by metagenomics were classified as commensal or uropathogen. Commensal bacteria comprised members of the genito-urinary tract and skin microbiota generally not considered as urinary pathogenic. Uropathogens comprised all *Enterobacteriaciae,* other species known as common causative agents of UTI and putative uropathogens that have been described to cause UTI in rare cases(17). A full list of bacterial species detected and their designation is provided in the Supplementary data, table 1.

Finally, the resistome i.e., the pool of resistance genes present in the microbial community, was characterized from the metagenome with the CosmosID (CosmosID Inc., Rockville, MD) metagenomic software package using CosmosID's curated antibiotic resistance gene database. Resistome predictions were compared with results of AST and WGS of the corresponding isolated bacteria.

Whole genome sequencing

SPAdes/3.6.0 was used for read-assembly (settings: --iontorrent, -k 21,33,55 --careful)(18). KmerFinder version 2.1 identified the bacterial species(19). If KmerFinder was inconclusive, a BLAST search of the NCBI database was done, using the assembled genome(20).

ResFinder version 2.1 was used with default settings (90% identity match, 60% coverage) for detection of acquired antimicrobial resistance (AMR) genes (https://cge.cbs.dtu.dk/services/ResFinder/ analysis date: 14th June 2017)(21). Additional acquired and chromosomal genes and mutations were detected from the assembled reads using the web-based "Resistance Gene Identifier" tool for searching the Comprehensive Antibiotic Resistance Database (CARD) using "strict" and "perfect" matches only with identity match of ≥99%(https://card.mcmaster.ca/analyze/rgi analysis date: 14th June 2017)(22). AMR genes identified by WGS served as benchmark of expected AMR gene content in metagenomic sequence data.

Metagenomic quantification

In order to integrate both DNA quantity and relative abundance in a single diagnostic measure, thus emulating the qualitative and quantitative properties of the reference test culture, we created the diagnostic index (DI). The DI is the product of the total quantity of extracted DNA in nanograms (D) and the relative abundance of uropathogens (RA) for each urine sample: DI = D * RA. Semi-quantitative culture and metagenomics results were compared one on one per urine sample for species identification and quantification. The median and interquartile ranges (IQR) for DNA yield, RA and DI for the groups UTI and non-UTI were compared. Thus, we assessed

which metagenomic measure had best discriminatory power, displaying the least overlapping values between groups UTI and non-UTI.

Statistics

Differences in relative abundance between uropathogens, as well as differences in total DNA yield between specimens were analyzed using the Mann-Whitney-U test (RStudio version 0.99.902). A significant difference was defined as a p-value <0.05. Graphs were designed with RStudio version 0.99.902. Sensitivity, specificity, and 95% confidence intervals (95% CI) were calculated using MEDCALC (https://www.medcalc.org/calc/diagnostic_test.php).

RESULTS

Culture and WGS of isolates

Forty-six consecutive urine samples collected for routine culture were included, results of which were reported as no-, commensal- or no significant ($\leq 10^3$ CFU/ml) growth for respectively 12, 19 and 4 urines, hence classified as the non-UTI group. Eleven samples were classified as culture-positive and constituted the UTI-group. From these 11 specimens, 15 uropathogens were identified in total: 2 *Enterobacter cloacae,* 6 *Escherichia coli,* 2 *Klebsiella pneumoniae,* 2 *Staphylococcus aureus* and 1 each of *Proteus mirabilis, Morganella morganii,* and *Pseudomonas aeruginosa.* Of these, 14 were available for WGS. Species identification by WGS confirmed identification by MALDI-TOF in all 14 cases.

Metagenomics of urine samples

Metagenomic sequencing and analysis was performed for 41 of the 46 included urine samples. In the UTI group, one sample did not contain surplus urine for metagenomic analysis, and DNA extraction was unsuccessful for another due to technical difficulties. In the non-UTI group, zero DNA was extracted from three samples for which culture showed no growth for one and commensal flora in 10^2CFU/ml for two samples. Thus, 9 UTI and 32 non-UTI urine samples, containing 5.7-1210.0 ng of DNA, were used for metagenomic sequencing and analysis.

Non-UTI group

The 11 sequenced urine samples yielding no growth in culture, showed at least one bacterial species to be present by metagenomics. In these samples, the most frequently detected genera were *Gardnerella*, *Bifidobacterium*, *Enterococcus*, and *Lactobacillus* (Supplementary data table 2-C). Of the 19 urine samples that grew commensal flora in culture, 17 were analyzed by metagenomics and the most frequently observed bacterial taxa were *Bifidobacterium*, *Lactobacillus*, *Prevotella*, *E. coli*, *Staphylococcus epidermidis*, and *Gardnerella vaginalis* (Supplementary data table 2-B). All four samples with no significant growth in culture, respectively showed *E. coli*, *Bifidobacterium* and *Lactobacillus* as most abundant taxa (Supplementary data table 2-D).

UTI group

In 9 of 11 urine samples from the UTI group that underwent metagenomic sequencing and analysis, 12 of the 13 cultured uropathogens were identified amongst the most abundant species by metagenomics, but some differences were observed (Table 1). Where *S. aureus* (sample 30), present in mixed growth with *E. coli*, was cultured at a concentration of 10^4CFU/ml, it was not

found in the metagenomic dataset. Sample 19 showed *Klebsiella pneumoniae* dominance (90.4% relative abundance) with only 0.9% relative abundance of *E. coli*, whilst culture suggested equal growth of $>10^5$CFU/ml of both species. Similarly, culture of sample 49 suggested equal growth of *Enterobacter cloacae* and *Pseudomonas aeruginosa*, whilst metagenomics revealed *E. cloacae* dominance (relative abundance 53.9%) with *P. aeruginosa* present at 7.8% relative abundance. Additionally *E. coli* was detected at a RA of 29.4%. Only *S. aureus* (10^4 CFU/ml) was cultured from sample 8 whilst *Klebsiella species* was additionally detected by metagenomics (Table 1) in a patient pre-treated with trimethoprim. Metagenomics identified additional taxa compared to culture in 6 other UTI samples including *Bifidobacterium species, Shigella species, Lactobacillus species, Bacillus cereus, Anaerococcus lactolyticus, Peptoniphilus harei* and *Atopobium vaginae.*

Metagenomic quantification

The median relative abundance (RA) of uropathogens in urine samples from the UTI-group (93.6%; IQR 7.4) was significantly different ($p<0.001$) from that of the non-UTI group (0.3%; IQR 11.2) (Supplementary data, figure 1). The median DNA yield from urine samples of the UTI-group (642.4ng; IQR 456.5; range 203.5 – 1210.0ng) was and significantly different ($p<0.001$) from the non-UTI group (127.3ng; IQR 295.3; range 0.0-734.8 ng)(Supplementary data, Figure 2). Where the non-UTI samples had a variable DNA yield, all urine samples from the UTI group, yielded > 203 ng of DNA, forming a clear threshold below which no UTIs were found. However, both RA and DNA yield lacked discriminatory power as individual sample values were overlapping (Supplementary Data, figures 1 and 2).

Integrating RA and DNA yield per urine sample in the diagnostic index (DI) discriminated all 9 culture positive urine samples of the UTI-group from the remaining 32 non-UTI samples except for one. This sample corresponded to a culture showing no growth whilst metagenomics identified *Aerococcus urinae* as being present (RA 88%, DNA yield 374 ng) (Figure 1 and Supplementary Data, Figure 3. The median difference in DI for groups UTI and non-UTI was significant ($p<0.0001$), with only 1 overlapping value between groups. The sensitivity and specificity of the DI for correctly allocating a urine sample to the UTI or non-UTI group were respectively 100% (95%-CI: 66.4-100%) and 96.7% (95%-CI: 83.8-99.9%)(23).

Antimicrobial susceptibility

Phenotypic AST of the isolated uropathogens showed resistance against 0 to 6 drug classes per isolate, with resistance against an average of 3 drug classes per isolate, cumulating to 38 cases of AMR against a drug class for all 15 uropathogens together(Table 2, Supplementary data Table 3). Combined Resfinder and CARD analyses of WGS gave genotype-phenotype agreement in 22 of 38 resistant phenotypes.

Nine of 11 urine samples from the UTI group were available for metagenomic resistome analysis. These 9 samples contained 13 of the 15 cultured uropathogens, comprising 32 cases of resistance against an antimicrobial drug class, as per phenotypic AST. Metagenomic resistome analysis and phenotypic AST were concordant in 20 out of 32 cases (Table 2).

Five isolates (2 *E. cloacae*, 1 *K. pneumoniae*, 1 *M. morganii* and 1 *P. mirabilis*) derived from individual urine samples showed a nitrofurantoin resistance phenotype, but a nitrofurantoin resistance conferring gene was not detected, neither by metagenomic resistome nor by WGS

analysis. Metagenomics did not detect a matching AMR gene to explain seven additional resistance phenotypes in five samples (Table 2) whilst AMR genes corresponding to these phenotypes were detected in the WGS assembly of the isolates.

In four samples, different AMR genes were identified by metagenomics and WGS of the corresponding isolate, although both could explain the phenotype (Table 2). In 2 of these samples (*bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes detected by WGS in sample 19 and *bla*$_{\text{DHA}}$ genes in sample 45), coverage of the metagenomic datasets was too low to distinguish the highly homologous gene variants (5-10 SNP differences between identified genes) (Supplementary data, Figure 4). The third, a *bla*$_{\text{ACT-28}}$ gene detected by WGS in sample 7 was absent in the CosmosID database and was instead identified as the *bla*$_{\text{ACT-9}}$ gene (a close variant with 5 SNPs difference) in the corresponding metagenomic sample. Finally, in sample 30, containing *E. coli* phenotypically resistant to 3$^{\text{rd}}$ generation cephalosporins, metagenomic analysis identified an *ampC* gene whilst the *bla*$_{\text{CTX-M-27}}$ ESBL-gene was identified in the WGS of the isolate.

Metagenomics outperformed WGS in three cases. In sample 28, containing *E. coli* phenotypically resistant to 3$^{\text{rd}}$ generation cephalosporins, metagenomics identified an *ampC* gene, whilst WGS detected *bla*$_{\textit{TEM-1B}}$, which encodes resistance against penicillins, but not 3$^{\text{rd}}$ generation cephalosporins. In two instances a resistance encoding genotype was identified by metagenomics but an equivalent coding gene was not detected in the corresponding WGS analysis. Both genotypes matched to a resistant phenotype: *oqxA* and *oqxB* coding for norfloxacin resistance in sample 45 and *ampC* for amoxicillin resistance in sample 23.

Cost and timing

The time from urine arrival at the laboratory to obtaining results by culture was approximately 24h for non-UTI samples and 48-72h for culture and AST for UTI samples. The cost of this analysis per sample was approximately €4 for a non-UTI and €10 for a urine sample from the UTI group, excluding cost of staffing.

Total time required for metagenomic analysis was approximately 31h per sample, which included 4.5h for sample centrifugation, DNA extraction, and DNA shearing. A total of 15.5h were required for library preparation. Four hours were needed for sequencing. The computation time for CosmosID analysis for all 41 datasets was 3.88 min per sample using a Linux analysis server with two physical processors, a total of 12 cores (non-multithreaded) and 64GB of memory. The cost of library preparation and sequencing was €116 per sample, excluding cost of staffing. Should a screening be made based on quantity of extracted DNA per sample, non-UTI samples would have a processing time of approximately 4h and a cost of €5, namely the cost of DNA extraction only.

DISCUSSION

This study demonstrates the potential of metagenomics for rapid detection of clinically relevant levels of uropathogens, thus distinguishing UTI from non-UTI urine samples.

Whilst several reports were previously published on the microbial community composition of urine(24, 25), successful metagenomic identification of uropathogens present in clinical samples has only been reported once to the best of our knowledge(7). However, that

study did not include the semi-quantitative analysis required to establish a diagnosis of UTI(2, 3, 26). If metagenomics is to replace culture, a semi-quantitative analysis of clinically relevant bacteria present within the urine microbial community is critical to obtain a diagnostic test result, as has been done in this study.

Whilst all samples of the UTI group yielded >203.5 ng of DNA, 12 of 21 samples above this threshold were non-UTI urine samples, reflective of the fact that the procedure of urine sample collection is highly prone to bacterial contamination and that urine itself is not sterile(27). However, for none of the UTI samples was a low amount of DNA (<200 ng) associated with clinically relevant bacterial growth in culture, independent of the volume of urine from which DNA was extracted which ranged between 2 and 20 ml (data not shown). This observation suggests that establishing an unambiguous threshold of DNA extraction yield per urine volume is feasible due to the fact that in the presence of a UTI the bacterial DNA load in the urine increases exponentially. Thus, DNA quantity alone could serve as an initial screening step for a diagnostic algorithm, directing sequencing only those samples with a high DNA yield.

The total amount of time needed to detect and identify uropathogens and AMR genes in the UTI samples was significantly less (31h vs. 48-72hs) for metagenomics than for standard culture whilst metagenomics provided greater detail. Automated library preparation will further reduce processing time of UTI samples by several hours. Metagenomic costs were €116 vs. €10 for culture per sample. Although metagenomic sequencing is more expensive at the present time, sequencing costs are continuing to decline(28). At present €4 and 24h are required for ruling out a UTI by culture. Should a validated metagenomic diagnostic algorithm be in place, a UTI could be ruled out in a low yield DNA sample within 4.5 hours and costs would be reduced to the cost of DNA extraction – approximately €5 – making metagenomics highly competitive.

Overlapping values for the UTI and non-UTI samples were observed for both DNA quantity and relative abundance of uropathogens, indicating these parameters are unsuitable for identifying urine samples from patients with UTI. However, the DI discriminated UTI and non-UTI samples extremely well. The single outlier was a sample containing *Aerococcus urinae* in high relative abundance and high DNA yield, that had been reported to be culture negative, even though Gram-staining revealed Gram-positive cocci. *A. urinae* can be overlooked in cultures because of its morphological similarity to coagulase negative staphylococci(29), leading to false negative culture results.

Metagenomic analysis was successfully employed to identify uropathogens in all but two of the culture positive urine samples (Table 1). In the initial analysis *Morganella morganii* in sample 45 was not identified. The species was successfully identified after addition of the genome to CosmosID's reference database, highlighting the importance of well-curated and complete databases. In sample 30, *S. aureus* was cultured at a concentration of $>10^4$ CFU/ml. It is unlikely that insufficient DNA extraction due to poor lysis of Gram-positive bacteria can explain the apparent lack of *S. aureus* DNA, since an extensive pre-lysis protocol was used, although this cannot be ruled out(30). Alternatively, contamination of the agar plates may have occurred.

CosmosID metagenomic bioinformatics system identified AMR encoding genes that were confirmed to be present by pure isolate WGS analysis. However for a selected number of cases, metagenomic and WGS analyses identified a highly similar, but non-identical AMR gene. An explanation for this discrepancy can be the different databases used in the metagenomic and WGS analysis. For WGS, it has been shown that different bioinformatic AMR identification tools produce different results and that databases are often incomplete(31). In addition, low coverage of gene segments by metagenomic reads resulting in less accurate gene calling could explain

differences between metagenomics and WGS. Interestingly, even though metagenomic and WGS methods identified different gene variants in those few instances, the variants were predicted to encode the same phenotype. However, accurate phenotype prediction on the basis of genomic information, whether WGS or metagenomics, remains a challenge. Phenotype clearly depends on more than the presence or absence of a resistance encoding gene. Whether antibiotic resistance mechanisms are expressed, depends on complex interplay between accumulating mutations or acquisition of resistance encoding genes and as yet unknown regulators, as well as environmental factors(32).

In conclusion, we provided a proof of concept of semi-quantitative metagenomic diagnostics for UTI, including the development of a “diagnostic index” based on uropathogen relative abundance and total DNA yield per sample, to facilitate rapid classification of urine samples to UTI and non-UTI groups.. In future studies, clinical characteristics of patients and prior antibiotic use should be included to determine the sensitivity, specificity, and the positive and negative predictive value of this metagenomics analysis approach applied to urine samples using culture as the reference test.

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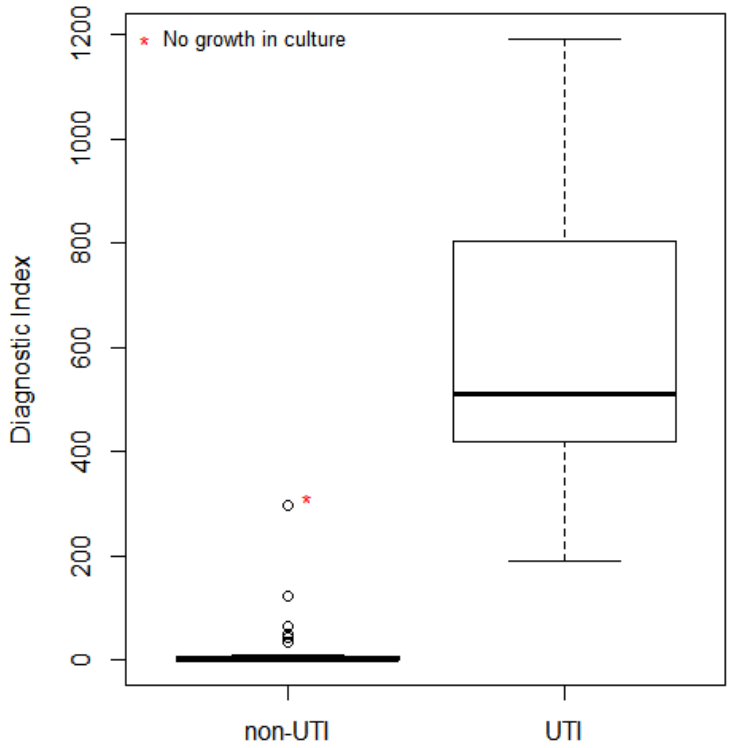
Rita R. Colwell is Founder and Chairman of the Board of CosmosID®, a bioinformatics company, and some of the other authors are employees of the company. Affiliation with CosmosID does not alter the authors' adherence to all JCM policies as detailed in the online instructions for authors. Authors declare no conflict of interest with regards to this manuscript.

AUTHORS CONTRIBUTION

V. A. J. wrote the manuscript. V. A. J., S. M. and M. E. J. performed the experiments. V. A. J., S. M., N. W., P. S. and N. A. H. performed the bio-informatics analysis. C. E. V. supervised the clinical diagnostic analysis. S. M., V. A. J., C. E. V., B. d W., R. R. C., M. D. d J., and C. S. designed the study. All authors provided critical review of the data and manuscript.

376 Figure 1. Diagnostic Index (DI) for urine samples in the groups UTI and non-UTI



377
378 Legend Figure 1. The "Diagnostic Index" (DI), the product of the relative abundance of
379 uropathogens, and total DNA quantity per sample was computed for the groups UTI and non-
380 UTI. The median DI for the UTI group was 512.0 (IQR 382.8) versus 0.11 (IQR 5.4) for the
381 non-UTI group, which was a significant difference ($p < 0.00001$, Mann Whitney-U test). The red
382 asterisk represents urine sample 44 showing *Aerococcus urinae* in metagenomic analysis (88%
383 relative abundance, 325.6ng DNA extracted from that sample), which was classified as a
384 uropathogen in this study. Culture showed no growth.

385

386 Table 1. Comparing species identification by semi-quantitative culture and metagenomics'

387 relative abundance (Rel. ab.) of organisms per urine sample of the UTI group.

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Sample ID	Culture		Metagenomics	
	Species	**CFU/ml**	**Species**	**Rel. ab. (%)**
7	*Enterobacter cloacae*	10^4	*Enterobacter cloacae*	58.8
			Enterobacter species	34.8
			Bacillus cereus	6.4
8	*Staphylococcus aureus*	$>10^4$	*Staphylococcus aureus*	76.3
			Klebsiella species	23.7
18	*Escherichia coli and skin flora*	$>10^4$	*Escherichia coli*	55.8
			Bifidobacterium pseudocatenulatum	25.0
			Bifidobacterium longum	11.4
			Shigella flexneri	3.1
			Other	4.7
19	*Escherichia coli*	$>10^5$	*Klebsiella species*	90.4
	Klebsiella pneumoniae	$>10^5$	*Klebsiella pneumoniae*	8.1
			Escherichia coli	0.9
			Other	0.7
23	*Escherichia coli*	10^4	*Escherichia coli*	100.0
28	*Escherichia coli*	10^4	*Escherichia coli*	86.6
			Shigella sonnei	6.6
			Other	6.8
30	*Escherichia coli*	$>10^5$	*Escherichia coli*	80.4
	Staphylococcus aureus	10^4	*Anaerococcus lactolyticus*	9.3
			Escherichia species	5.3
			Peptoniphilus harei	3.6
			Staphylococcus aureus	0.0
			Other	1.5
45	*Proteus mirabilis*	$>10^5$	*Proteus mirabilis*	66.4
	Morganella morganii	$>10^5$	*Morganella morganii*	26.9
			Klebsiella variicola	2.1
			Klebsiella pneumoniae	1.7
			Other	2.8
49	*Enterobacter cloacae*	$>10^4$	*Enterobacter cloacae*	53.9
	Pseudomonas aeruginosa	$>10^4$	*Escherichia coli*	29.4
			Atopobium vaginae	8.9
			Pseudomonas aeruginosa	7.8

390 Table 2. Phenotypic AST per drug class compared to genotypic AMR gene detection using the

391 detection tools ResFinder and CARD (WGS), and CosmosID (metagenomics).

392

		CULTURE	WHOLE GENOME SEQUENCING		METAGENOMICS
Sample ID	**Species**	**Antimicrobial drug class resistance phenotype**	**ResFinder[1]**	**CARD[2]**	**CosmosID**
7	***E. cloacae***	2nd gen. cephalosporin		blaACT-28	blaACT-9, fosA
		Pip/taz[3]		blaACT-28	
		Fosfomycin	fosA		
		Nitrofurantoin			
8	***S. aureus***	None	NA[4]	NA[4]	None
9	***E. coli***	Folic acid synthesis inhibitors	sul2-like	sul3	NA[4]
17	***K. pneumoniae***	Penicillins	blaCTX-M-15, blaSHV-26, blaTEM-1B	blaCTX-M-15, blaSHV-26, blaTEM-1	NA[4]
		3rd gen. cephalosporin	blaCTX-M-15	blaCTX-M-15	
		Fluoroquinolones	oqxA-like, oqxB-like, QnrB66-like	QnrB1	
		Folic acid sysnthesis inhibitors	dfrA14-like	dfrA14	
		Nitrofurantoin			
18	***E. coli***	Folic acid synthesis inhibitors		sul3	None
19	***K. pneumoniae***	Penicillins	blaSHV-1	blaSHV-1	blaTEM-128 , blaSHV-69, AmpC, oqxA, oqxB, drfA14
		Nitrofurantoin			
	E. coli	Penicillins (amoxicillin)	blaTEM-1B	blaTEM-206	
		Fluoroquinolones		parC, gyrA, mfd	
		Folic acid synthesis inhibitors	dfrA1, drfA14-like, sul1, sul2	dfrA1	
23	***E. coli***	Penicillins (amoxicillin)			AmpC
28	***E. coli***	Penicillins	blaTEM-1B	blaTEM-1	AmpC, dfrA5, strB, aadA1
		3rd gen. cephalosporin			
		Folic acid synthesis inhibitors	dfrA5, Sul2	sul2	
30	***E. coli***	Penicillins	blaCTX-M-27-like		AmpC
		3rd gen. cephalosporin	blaCTX-M-27-like		
		Pip/taz[3]	blaCTX-M-27-like		
		Fluoroquinolones		gyrA	
	S. aureus	Penicillins (penicillin)	blaZ-like		
45	***M. morganii***	Penicillins	blaDHA-1-like	blaDHA-14	blaDHA-2, QnrD, oqxA, oqxB, FosA
		2nd gen. cephalosporins	blaDHA-1	blaDHA-14	
		Fluoroquinolones	QnrD-like		
		Fosfomycine			
		Tobramycine			
		Nitrofurantoin			
	P. mirabilis	Penicillins (amoxicillin)			
		Nitrofurantoin			
49	***E. cloacae***	2nd gen. cephalosporin	blaACT-15-like	blaACT-15	blaACT -15
		Nitrofurantoin			
	P. aeruginosa	3rd gen. cephalosporins	blaOXA-50-like, blaPAO-like	PDC-7	
		Carbapenems (imipenem)	blaOXA-50-like, blaPAO-like	PDC-7	
		Fosfomycin	fosA-like	fosA	

Legend Table 2. If only 1 antibiotic was tested per antimicrobial drug class, the tested drug was noted between brackets. [1] Database contains acquired AMR determining genes only. [2] Database contains chromosomal and acquired AMR determining genes and mutations.

[3]Piperacillin/tazobactam. [4] Not available.

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