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Short Communication

# Shotgun metagenomics for microbiome and resistome detection in septic patients with urinary tract infection ☆

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ABSTRACT

In sepsis, early and appropriate antibiotic therapy is key but is frequently challenging due to the increasing incidence of multidrug-resistant bacteria. The feasibility of shotgun metagenomics (SM) has been scarcely assessed in urinary tract infections (UTIs). In this study, the feasibility of SM to detect both the microbiome and the resistome in patients with confirmed UTI-related sepsis was evaluated. Urine samples were obtained from 40 adult patients with UTI-related sepsis. Conventional culture was used as a reference. Following total DNA extraction and depletion of human DNA, SM was performed using Ion Proton™ technology. Bioinformatics analysis was conducted using Geneious® software as well as online tools from the Center for Genomic Epidemiology. For the microbiome, SM was consistently concordant when urine culture was positive with only one bacterium (mainly *Escherichia coli*). For the resistome, results were in agreement with antimicrobial susceptibility testing with no major discrepancies. SM consistently identified $bla_{CTX-M}$ genes responsible for resistance to third-generation cephalosporins. Resistance to aminoglycosides and fluoroquinolones was identified in all patients. This pilot study confirms that SM can provide clinically relevant information both on the microbiome and the resistome from urine samples of patients with UTI-related sepsis.



## 1. Introduction

Sepsis is a leading cause of mortality worldwide and remains a major health burden since its incidence is increasing over time [1]. Early identification of the causative micro-organism and determination of its antimicrobial resistance phenotype are key [2]. Unfortunately, conventional culture usually requires 36–48 h to identify the causative pathogen of sepsis and to determine its antimicrobial susceptibility. In addition, the choice of empirical antibiotic therapy may be challenging because of increasing antimicrobial resistance rates, especially of Gram-negative bacteria (GNB) with the emergence of multidrug-resistant bacteria producing extended-spectrum $\beta$-lactamases (ESBLs) or carbapenemases [3].

To shorten this delay, molecular biology techniques have been developed [4,5]. However, they provide little information on antimicrobial susceptibility. In the field of clinical microbiology, next-generation sequencing (NGS) had brought new insights. Two different NGS approaches can be used [6]. Metagenomics targets the 16S rDNA and can deeply describe the microbial community within a sample; however, this targeted amplicon approach does not provide access to the resistome. Shotgun metagenomics (SM) is another approach able to sequence the whole DNA content of a sample allowing access both to the microbiome, with a higher taxonomic resolution, and to the resistome. Besides its expensive cost, its main issue lies in the fact that large quantities of eukaryotic DNA can be present and sequenced, requiring a preliminary step of human DNA depletion. Although clinical metagenomics reports are increasing exponentially [6], use of SM on urine samples has been scarcely studied [7,8]. The epidemiology of urinary tract infections (UTIs) is quite stable with regard to causative micro-organisms,

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with *Escherichia coli* remaining the main species responsible [9]; however, the resistance profiles of GNB are challenging to predict [3]. Thus, we sought to determine the feasibility of SM to detect both the microbiome and the resistome directly from urine samples of septic patients with UTI, using conventional culture as a reference.

## 2. Materials and methods

### 2.1. Study population

This was an ancillary study of an observational project accepted by the Ethics Committee of Limoges University Hospital (Limoges, France). A total of 40 adult patients with UTI-related sepsis were enrolled from 2012–2014 in emergency departments and intensive care units from eight French hospitals [10]. Patients with a history of antibiotic treatment in the last 10 days were excluded. The diagnosis of UTI was confirmed by two blinded intensivists. For each patient, a urine sample was collected before any antibiotic treatment.

### 2.2. Conventional culture and antimicrobial susceptibility testing (AST)

Microbiological analysis of urine samples was performed as recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (http://www.escmid.org/escmid_publications/manual_of_microbiology). Briefly, 10 µL of urine was inoculated onto CLED (cystine–lactose–electrolyte-deficient agar) agar plates (bioMérieux, Marcy-l'Étoile, France) and was incubated for 16–24 h at 35 ± 2 °C in an aerobic atmosphere. Negative culture plates were incubated until 48 h. The bacterial count was determined from $10^2$ CFU/mL to $>10^6$ CFU/mL. Bacterial identification and AST were performed using a VITEK™ MS and VITEK®2 system (bioMérieux), respectively.

### 2.3. Shotgun metagenomics

DNA was extracted using a NucliSENS® easyMAG™ system (bioMérieux) from 1 mL of urine with an elution volume of 50 µL. Depletion of human DNA was performed using a NEBNext® Microbiome DNA Enrichment Kit (New England Biolabs, Évry, France) according to the manufacturer's recommendations with an initial quantity of DNA between 250 ng and 1 µg.

An Ion Proton™ system (Thermo Fisher Scientific, Illkirch-Graffenstaden, France) was used for SM. For each sample, 50–100 ng of DNA was submitted to enzymatic shearing using an Ion Shear™ Plus Reagent Kit (Thermo Fisher Scientific). Libraries from four samples were mixed in equimolar proportions, i.e. 100 pM, and were loaded on an Ion PI Chip v3 microarray (Thermo Fisher Scientific). Reads of approximately 200 bp were obtained. Reads were first aligned against the human genome hg19. Contigs were obtained from unaligned reads following de novo assembly using MIRA (Mimicking Intelligent Read Assembly) software.

### 2.4. Bioinformatics analysis

The microbiome was determined using three different methodologies. (i) All contigs were analysed using SpeciesFinder 1.2 software (http://www.genomicepidemiology.org). (ii) Contigs were aligned using Geneious® software (Biomatters Ltd., Auckland, New Zealand) with the whole sequence of the 16S rDNA of an *E. coli* strain (**FN554766**). Overlapping parts of contigs aligned with this reference sequence were extracted and were compared with the GenBank database. Bacterial species with a percentage homology >99.9% on >500 bp were considered. And (iii), contigs longer than 10 000 bp were compared with the GenBank database using BLAST software (https://blast.ncbi.nlm.nih.gov). When query coverage and identity were both >99% for a complete genome, bacterial species were considered. Results from these three methodologies were pooled and were compared with those of bacterial species identification obtained from conventional culture.

For the resistome, acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations were identified using ResFinder 3.0 (http://www.genomicepidemiology.org) [11]. Genes with a 40% minimum length and a percentage of identity >98% were considered. For $bla_{AmpC}$ genes that are not present in the ResFinder database, their presence in urine samples for which a *Klebsiella aerogenes* or *Enterobacter cloacae* was present was manually checked using Geneious. The $bla_{AmpC}$ sequences of *K. aerogenes* EA1509E (**NC_020181**) and *E. cloacae* ATCC 13047 (**NC_014121**) were used as references. As currently accepted [12], strains with one mutation in the quinolone resistance-determining regions (QRDRs) of the *gyrA, gyrB, parC* or *parE* genes were considered resistant to nalidixic acid. Strains with at least two mutations in the QRDRs were considered resistant to fluoroquinolones. The PointFinder module of ResFinder 3.0 allowed detection of QRDR mutations only for *E. coli*. For urine samples with other bacterial species, mutations were checked manually with Geneious using the gyrase genes of *E. coli* str. K-12 substr. MG1655 (**NC_000913**) as reference. Results from the resistome were compared with AST results used as reference.

Initially, the investigator who analysed SM data was aware of the results of conventional microbiology (Group 1). Subsequently, patients were independently selected at random by a physician, and the investigator who analysed SM data had no access to microbiological results (Group 2). This study design aimed at confirming the results obtained in Group 1 in a blinded manner in Group 2.

## 3. Results

The characteristics of the 40 enrolled patients are presented in Supplementary Table S1, and raw data from SM are presented in Supplementary Table S2. Regarding culture (Table 1; Supplementary Tables S3 and S4), one bacterium was isolated in 28 patients and two bacteria were isolated in 7 patients; one urine sample was polymicrobial with four different bacterial species and four urine samples were negative. *Escherichia coli* was the most frequently isolated species.

### 3.1. Microbiome detection (Table 1; Supplementary Table S3)

The three bioinformatics methodologies provided different results (Supplementary Table S3). Using SpeciesFinder, identification of a single bacterium was achieved in 24 patients (60%). One or several bacteria were identified in 38 patients (95%) by 16S rDNA sequence analysis and in 34 patients (85%) by analysis of contigs longer than 10 000 bp. The three methodologies provided identical results in 19 patients (48%). Altogether, pooled analysis yielded bacterial documentation in 38 patients (95%). The microbiome was fully concordant with conventional culture in 27 patients (68%), reaching 78% (31 patients) when only considering bacterial species able to grow on CLED agar plates. Some anaerobes (e.g. *Bacteroides*) and fastidious bacteria (e.g. *Actinotignum*) were only detected by SM since they are unable to grow on CLED agar plates. When culture identified a single GNB ($n = 28$), data were consistently concordant. In four of these patients, SM revealed the presence of one or two additional micro-organisms. When culture was positive for two bacteria ($n = 7$), SM correctly identified both bacteria in two patients (including one with a third GNB) and only one of two bacteria in the remaining patients. When urine culture

**Table 1**
Results from shotgun metagenomics (SM) compared with conventional urine culture and antimicrobial susceptibility testing (AST)

| Patient | Bacterial culture (CFU/mL) | Microbiome | Concordance: culture vs. microbiome | Resistance by AST[a] | Resistome: associated ARGs | Concordance: AST vs. resistome |
|---|---|---|---|---|---|---|
| A007 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, CTX, CAZ (ESBL) | $bla_{CTX-M-15}$ | Total |
| | | | | Aminoglycosides: GEN, TOB | *aac(3)-IIa* | |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile; Glu84Val), *parE* (Ile529Leu) | |
| | | | | SXT | *dfrA17, sul1* | |
| A022 | Negative | *Escherichia coli* | No[b] | Not performed | NA | NA |
| A027 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, TZP | $bla_{TEM-1A}$ | Total |
| A028 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC | $bla_{TEM-1B}$ | Total |
| | | | | SXT | *dfrA7, sul1, sul2* | |
| A029 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC | $bla_{TEM-1B}$ | Total |
| G003 | *Escherichia coli* ($>10^4$) | *Escherichia coli* | Total[c] | β-Lactams: AMX, TIC, AMC, CTX, CAZ (ESBL) | $bla_{CTX-M-3}$, $bla_{OXA-1}$, $bla_{TEM-1A}$ | Total |
| | | | | Aminoglycosides: TOB, AMK | *aac(6′)-Ib*-cr | |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile; Glu84Val), *parE* (Ile529Leu), *aac(6′)-Ib*-cr | |
| | | *Lactobacillus gasseri* | | | | |
| G046 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC | $bla_{TEM-213}$ | Total |
| G085 | *Klebsiella oxytoca* ($10^6$) | *Klebsiella oxytoca* | Total[b] | β-Lactams: AMX, TIC (intrinsic resistance) | $bla_{OXY-2-10}$ | Total |
| | | *Pseudomonas aeruginosa* | | | | |
| | | *Citrobacter amalonaticus* | | | | |
| G096 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC | $bla_{TEM-1A}$ | Total |
| | *Klebsiella pneumoniae* ($10^6$) | *Klebsiella pneumoniae* | | β-Lactams: AMX, TIC, AMC, TZP, CTX, CAZ (ESBL) | $bla_{CTX-M-15}$, $bla_{OXA-1}$ | |
| | | | | Aminoglycosides: GEN, TOB | *aac(3)-IIa, aac(6′)-Ib*-cr | |
| | | | | Quinolones: fluoroquinolones | *aac(6′)-Ib*-cr | |
| | | | | SXT | *dfrA14, sul2* | |
| G107 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC | $bla_{TEM-1B}$ | Total |
| | | | | SXT | *dfrA17, sul2* | |
| L021 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| L029 | *Klebsiella pneumoniae* ($>10^6$) | *Klebsiella pneumoniae* | Total | β-Lactams: AMX, TIC, AMC, TZP, CTX, CAZ (ESBL) | $bla_{CTX-M-15}$, $bla_{OXA-1}$, $bla_{TEM-1B}$ | Total |
| | | | | Aminoglycosides: GEN, TOB, AMK | *aac(3)-IIa, aac(6′)-Ib*-cr | |
| | | | | Quinolones: fluoroquinolones | *parC* (Ser80Ile), *aac(6′)-Ib*-cr, *oqxA, oqxB* | |
| | | | | SXT | *dfrA1, dfrA14, sul2* | |
| L030 | Polymicrobial[d] | *Pseudomonas aeruginosa* | Partial[b] | Not performed | NA | NA |
| | | *Providencia stuartii* | | | | |
| L034 | *Klebsiella aerogenes* ($>10^6$) | *Klebsiella aerogenes* | Total | β-Lactams: AMX, AMC (intrinsic resistance) | $bla_{AmpC}$ | Total |
| L049 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, CTX (ESBL) | $bla_{CTX-M-14}$, $bla_{TEM-1B}$ | Total |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu), *parC* (Ser80Arg) | |
| | | | | SXT | *dfrA12, sul1, sul2* | |
| L063 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| L067 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total[c] | No acquired resistance | None detected | Total |
| | | *Bacteroides fragilis* | | | | |
| L082 | *Enterobacter cloacae* ($10^4$) | *Enterobacter cloacae* | Total | β-Lactams: AMX, TIC, AMC, TZP, CTX, CAZ (ESBL) | $bla_{AmpC}$, $bla_{CTX-M-15}$, $bla_{ACT-16}$, $bla_{OXA-1}$, $bla_{TEM-1B}$ | Total |
| | | | | Aminoglycosides: GEN, TOB, AMK | *aac(3)-IIa, aac(6′)-Ib* | |
| | | | | Quinolones: fluoroquinolones | *parC* (Ser57Thr; Ser80Ile), *qnrB1* | |
| | | | | SXT | *dfrA1, dfrA14, sul1, sul2* | |
| L090 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, CTX, CAZ (ESBL) | $bla_{CTX-M-15}$, $bla_{TEM-1B}$ | Total |
| | | | | Aminoglycosides: GEN, TOB | *aac(3)-IId* | |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile; Glu84Val), *parE* (Ile529Leu) | |
| | | | | SXT | *dfrA17, sul1, sul2* | |
| L098 | *Escherichia coli* ($>10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, TZP | $bla_{TEM-1C}$ | Total |
| L136 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| L154 | Negative | Clostridiales bacterium | No[c] | Not performed | NA | NA |
| L158 | *Klebsiella pneumoniae* ($>10^6$) | *Klebsiella pneumoniae* | Partial | β-Lactams: AMX, TIC (intrinsic resistance) | $bla_{SHV-1}$ | Total |
| | *Escherichia coli* ($10^4$) | | | No acquired resistance | None detected | |

(*continued on next page*)

**Table 1** (*continued*)

| Patient | Bacterial culture (CFU/mL) | Microbiome | Concordance: culture vs. microbiome | Resistance by AST[a] | Resistome: associated ARGs | Concordance: AST vs. resistome |
|---|---|---|---|---|---|---|
| L182 | *Escherichia coli* (>$10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, CTX (ESBL) | $bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1B}$ | Total |
| | | | | SXT | *dfrA1, sul1, sul2* | |
| L200 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Partial | β-Lactams: AMX, TIC, AMC | $bla_{TEM\text{-}1B}$ | Total |
| | | | | SXT | *dfrA8, sul2* | |
| | *Streptococcus agalactiae* | | | Not performed | NA | |
| L207 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total[c] | β-Lactams: AMX, TIC | $bla_{TEM\text{-}1B}$ | Total |
| | | | | SXT | *dfrA1, dfrA5, sul2* | |
| | | *Bacteroides* sp. | | | | |
| | | *Ruminococcus* sp. | | | | |
| M005 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| P008 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Partial | β-Lactams: AMX, TIC | $bla_{SHV\text{-}1}$ | Partial |
| | | | | Quinolones: nalidixic acid | None detected | |
| | *Proteus mirabilis* ($10^6$) | | | β-Lactams: AMX, TIC, AMC | $bla_{SHV\text{-}1}$ | |
| P021 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, TZP, CTX, CAZ (ESBL) | $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1B}$ | Total |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile) | |
| | | | | SXT | *dfrA17, sul2* | |
| P022 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total[b] | β-Lactams: AMX, TIC, AMC | $bla_{TEM\text{-}1C}$ | Total |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu), *parE* (Ile529Leu) | |
| | *Acinetobacter baumannii* ($10^5$) | *A. baumannii* | | No acquired resistance | None detected | |
| | | *Proteus mirabilis* | | | None detected | |
| P034 | *Escherichia coli* (>$10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, TZP, CTX (ESBL) | $bla_{CTX\text{-}M\text{-}3}$, $bla_{TEM\text{-}1B}$ | Total |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile) | |
| | | | | SXT | *dfrA17, sul1, sul2* | |
| P036 | *Escherichia coli* (>$10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC, AMC, TZP, CTX, CAZ (ESBL) | $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$ | Total |
| | | | | Aminoglycosides: GEN, TOB | *aac(3)-IIa, aac(6′)-Ib*-cr | |
| | | | | Quinolones: fluoroquinolones | *gyrA* (Ser83Leu; Asp87Asn), *parC* (Ser80Ile; Glu84Val), *parE* (Ile529Leu), *aac(6′)-Ib*-cr | |
| | | | | SXT | *dfrA17, sul1* | |
| P038 | *Escherichia coli* (>$10^6$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| R007 | *Klebsiella oxytoca* ($10^5$) | *Klebsiella oxytoca* | Total | β-Lactams: AMX, TIC (intrinsic resistance) | $bla_{OXY\text{-}2\text{-}2}$ | Total |
| R009 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total | No acquired resistance | None detected | Total |
| T010 | Negative | Negative | Total | Not performed | NA | NA |
| T034 | *Escherichia coli* ($10^5$) | *Escherichia coli* | Partial[c] | No acquired resistance | None detected | Total |
| | *Enterococcus faecalis* | | | Not performed | NA | |
| | | *Actinotignum schaalii* | | | | |
| T039 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Total | β-Lactams: AMX, TIC | None detected | No |
| T048 | *Escherichia coli* ($10^6$) | *Escherichia coli* | Partial | No acquired resistance | None detected | No |
| | *Klebsiella pneumoniae* ($10^5$) | | | β-Lactams: AMX, TIC (intrinsic resistance) | None detected | |
| T050 | Negative | Negative | Total | Not performed | NA | NA |

AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMX, amoxicillin; ARG, antimicrobial resistance gene; CAZ, ceftazidime; CTX, cefotaxime; ESBL, extended-spectrum β-lactamase; GEN, gentamicin; NA, not applicable; SXT, trimethoprim/sulfamethoxazole; TIC, ticarcillin; TOB, tobramycin; TZP, piperacillin/tazobactam.

[a] Resistance to fluoroquinolones refers to resistance to ofloxacin ± ciprofloxacin. When present, an ESBL phenotype is indicated.

[b] SM detected additional bacterial species compared with conventional culture.

[c] SM detected additional bacterial species compared with conventional culture that were not able to grow (fastidious bacteria, anaerobic bacteria, etc.).

[d] New urine culture revealed the presence of *Pseudomonas aeruginosa, Providencia stuartii, Staphylococcus aureus* and *Enterococcus faecalis.*

was polymicrobial ($n = 1$), SM identified two different species. Finally, when urine culture was negative ($n = 4$), SM identified GNB in one patient (*E. coli*) and an anaerobic bacterium (Clostridiales) in another patient; no bacteria were detected in the two remaining patients.

### 3.2. Resistome detection (Table 1; Supplementary Table S4)

AST was not performed in 5 patients having negative urine cultures ($n = 4$) or polymicrobial cultures ($n = 1$); thus, AST was performed in 35 of the 40 patients. SM was fully concordant in 32 (91%) of the 35 patients, whereas discrepancies were observed for 3 patients. In one patient with UTI related to *Klebsiella pneumoniae* (T048), the chromosomal $bla_{SHV}$ gene was not detected. In two other patients, SM failed to explain acquired resistance, including one to nalidixic acid (P008) and the other to amoxicillin and ticarcillin (T039). For the 32 concordant patients, ARGs were in agreement with AST and explained the observed resistance to β-lactams, aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole (SXT). When strains were fully susceptible to these four antibiotic families, no ARGs was detected. All ESBL phenotypes conferring resistance to third-generation cephalosporins were due to the presence of $bla_{CTX\text{-}M}$ genes, and most resistance to fluoroquinolones was due to mutations in GyrA and/or ParC. Other ARGs conferring resistance to non-tested and non-clinically relevant antibiotics were identified.

### 3.3. Analysis of shotgun metagenomics data: Group 1 and Group 2

When blinded for Group 2 analysis, the investigator efficiently determined both the microbiome and resistome. Overall performance of SM was similar to that observed in Group 1 (Supplementary Tables S3 and S4).

## 4. Discussion

To date, few studies have applied SM on biological samples from infected patients, [7,8,13,14], most of them focusing on 16S rDNA targeted amplicon sequencing [6]. The current pilot study purposely targeted patients with UTI-related sepsis, which is frequently due to resistant GNB, including multidrug-resistant bacteria [15]. Since SM still requires mastering of bioinformatic tools, a two-step pilot study was designed. Urine samples of patients from Group 1 allowed for the lay user to fully master technical and bioinformatical requirements, whilst urine samples of patients from Group 2 allowed subsequent validation of the approach and to determine its feasibility in reproducing similar results with the same investigator blinded from the results of conventional culture.

For microbiome detection, the three methodologies gave different results, with SpeciesFinder being the least sensitive, suggesting the need to use different complementary overlapping methodologies. Once pooled, methodologies provided a total concordance for the detection of bacterial species in the 28 patients with a single GNB. SM detected only one microorganism in five of seven patients with two isolated strains. This lack of sensitivity can be explained by (i) differences in CFU between the two bacteria, (ii) the difficulty of assembling reads to reconstitute two different contigs of 16S rDNA sequences from two phylogenetically close bacteria or (iii) an insufficient number of reads and contigs. In a 94-year-old patient (T034), SM allowed the detection of *Actinotignum schaalii*. This Gram-positive bacterium is an emerging uropathogen in elderly patients and thus was considered of clinical significance [16]. Of note, SM identified bacteria in two of four patients with negative urine culture. This ability of SM has been reported previously [7]. Overall, SM analysis appears to be as sensitive as conventional urine culture for monomicrobial cultures and more sensitive than negative cultures, however the main weakness is that SM is less sensitive when dealing with polymicrobial cultures. This could have been circumvented by a 16S rDNA-targeted sequencing strategy [17] but, first, we wanted to avoid the risk of false-positive identifications and second, our main objective was to focus on the resistome that is the clinically relevant feature for UTI-related sepsis. In all cases, detection of bacterial gene material should be carefully interpreted in light of its clinical relevance to avoid excessive antibiotic therapy.

For resistome detection, we purposely focused on the most clinically relevant antibiotics used in the treatment of UTIs [15]. SM consistently identified all of the $bla_{\text{CTX-M}}$ genes responsible for resistance to third-generation cephalosporins; moreover, all mechanisms involved in resistance to aminoglycosides, fluoroquinolones and SXT were also identified. These results validate the reliability of SM in detecting resistance to clinically relevant antibiotics. The three discrepancies observed were minor and without clinical consequence, since they involved resistance to narrow-spectrum $\beta$-lactams or nalidixic acid that are not used empirically. Chromosomal $\beta$-lactamase not detected by SM could have been anticipated based on the bacterial species (*K. pneumoniae*).

SM has limitations that currently preclude its routine use in clinical practice. It requires a long turnaround time of 3–4 days [18] and a major human intervention with the use of several software hardly accessible for lay users. The duration of SM should dramatically decrease in the near future and promises to be available in a few hours owing to technological advancements, such as third-generation sequencing and faster ready-to-use bioinformatics tools [8,19]. Presumably, the current fairly long turnaround time will be dramatically reduced with the use of automatic sample processing systems as well as faster assemblers and standalone software; other important considerations such as harmonisation, validation and quality assurance also promise to be solved [20]. Overall, we can reasonably anticipate that the SM timeframe will be shorter than conventional culture, allowing clinicians to prescribe earlier a more targeted therapy in the near future.

## 5. Conclusions

This pilot study confirms the feasibility of using SM directly on urine samples. Microbiome analysis was in agreement with conventional culture for all monomicrobial cultures. Resistome analysis accurately identified clinically relevant ARGs. These promising results remain to be confirmed by larger-scale studies involving other sites of infection.

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**Ethical approval:** This was an ancillary study of an observational project accepted by the Ethics Committee of Limoges University Hospital (Limoges, France) [No. 99-2012-24].

**Competing Interest:** None declared.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.09.009.

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