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Correspondence

Do the clonally different *Escherichia coli* isolates causing different infections in a HIV positive patient affect the selection of antibiotics for their treatment?

Sir,

In HIV patients, bacterial infections are mostly caused by the pathogenic bacteria harbouring multidrug-resistance genes[1]. While carrying out a study (2014-2015) on infections caused by drug-resistant bacteria in HIV positive patients attending YRG Centre for AIDS Research and Education (YRG CARE), Chennai, Tamil Nadu, India, we found a 35 yr old male patient who had complaints of severe fever, irritation during micturition, vomiting and body chills. Hence, the urine and blood specimens were collected from this patient, and were subjected to bacterial culture and identification of the isolates. This patient was also infected with leptospirosis, *Pneumocystis jirovecii* pneumonia and pulmonary tuberculosis. He was under antiretroviral therapy with the following regimen: tenofovir, emtricitabine and efavirenz and was hospitalized for nine weeks. The CD4 cell count of this patient at the time of admission was 19 cells/µl. The bacterial isolates from both the specimens were identified as *Escherichia coli* based on the standard cultural and biochemical characteristics. Both these *E. coli* isolates were subjected to polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD) analysis to determine their clonal relationship and molecular detection of drug-resistance genes using PCR and DNA gene sequencing. The PCR-RAPD reaction was performed using a 10 base pair primer with the sequence of 5'-AGC GTC ACT G-3' (Eurofins, India)[2]. Antibiotic susceptibility patterns of these *E. coli* isolates were studied using the Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[3]. The genes responsible for the production of extended spectrum β-lactamases (ESBL), such as bla_{TEM}[4], bla_{CTX-M}[5], bla_{SHV}[4] and bla_{OXA}[4], metallo β-lactamases[6], AmpC β-lactamases[7], Class 1 and Class 2 integrons[8] and sulphamethoxazole-trimethoprim (TMP-SMX)[9-11], were detected using PCR technique. Both the *E. coli* isolates showed different clonal patterns which indicated that the patient had blood and urinary tract infections caused by two different *E. coli* strains (Figure). The molecular characterization revealed that the *E. coli* isolate from urine sample harboured the genes bla_{TEM}, bla_{CTX-M} and bla_{OXA} for ESBL and s*ul1* and s*ul2* genes for SMX and none for AmpC and Class 1 and Class 2 integrons. The phenotypic characterization showed that the isolate had resistance to ampicillin, doxycycline, gentamicin, cefpodoxime, cefoperazone, ceftriaxone, ciprofloxacin, trimethoprim-sulphamethoxazole, piperacillin, piperacillin-tazobactam, tetracyclin, trimethoprim, cefotaxime, ceftazidime and cefoxitin and sensitivity to amikacin, chloramphenicol, ertapenem and imipenem. On the other hand, the *E. coli* isolate from blood sample was found to harbour the genes bla_{CTX-M} for ESBL, bla_{CIT-M}

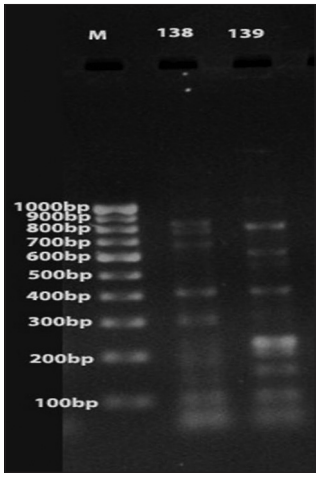

Figure. *Escherichia coli* isolates from urine and blood samples of a HIV patient showing different gene patterns by polymerase chain reaction-random amplified polymorphic DNA analysis. M, marker; 138 - *E. coli* isolate from urine; 139 - *E. coli* isolate from blood.

for AmpC, *sul1, sul2* and *dfrA7* for TMP-SMX and also for Class 2 integron by molecular characterization and also showed resistance to ampicillin, aztreonam, cefpodoxime, cefoperazone, ceftriaxone, imipenem, piperacillin, piperacillin-tazobactam, trimethoprim, trimethoprim-sulphamethoxazole, cefotaxime, ceftazidime and cefoxitin and sensitivity to amikacin, doxycycline, chloramphenicol, ciprofloxacin, ertapenem, gentamicin and tetracycline by phenotypic characterization (Table). Both the isolates were negative for MBL-producing genes bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{SPM}, bla_{GIM} and bla_{NDM}, TMP resistance genes *dfrA1, dfrA5* and *dfrA17* and for Class 1 integron gene. In our study, β-lactamases-producing genes from both the *E. coli* isolates were sequenced and identified as $bla_{TEM-116}$, $bla_{CTX-M-15}$, bla_{OXA-1} and bla_{CMY-30} using BLAST and phylogenetic analyses[12]. Similar to our study, the co-positivity of ESBL along with AmpC and other drug resistance genes in bacterial isolates from HIV patients was observed by Padmavathy *et al*[13].

In this study, *E. coli* isolate from urine sample of HIV positive patient was found to be resistant to at least any one antibiotic of the classes β-lactams, aminoglycosides, tetracyclines, quinolones, pyrimidines and sulphonamides. It was also observed that the *E. coli* isolate from urine was phenotypically positive for ESBL, MBL and AmpC production. Vignesh *et al*[14] reported that 80.6 per cent of the *E. coli* isolates from urine specimens from HIV patients were multidrug resistant and among them 83.3 per cent showed resistance to TMP-SMX, 94.4 per cent to ampicillin and 100 per cent sensitivity to imipenem and 44.4 per cent sensitivity to amikacin. They also reported that 25 per cent of the isolates showed positive for β-lactamase production. Phe *et al*[15] found that antibiotic resistant *E. coli* was the main contributor of bloodstream infection in HIV patients which corroborated this finding. In this study, *E. coli* isolate from urine sample was positive for bla_{TEM}, bla_{CTX-M} and bla_{OXA} genes related to ESBL production, and these

Table. Demographic data, positivity of drug-resistance genes and antibiotic susceptibility patterns of clonally different *Escherichia coli* isolates from urine and blood samples of a HIV patient

Parameters studied	Positivity of drug-resistance genes and antibiotic susceptibility	
Demographic data		
Age and sex	35 and male	
CD4 cell count	19 cells/µl	
Sample	Urine	Blood
Organism	*E. coli*	*E. coli*
Phenotypic production of β-lactamases	ESBL, MBL and AmpC	ESBL and AmpC
Resistance genes		
ESBL	bla_{TEM}, bla_{CTX-M}, bla_{OXA}	bla_{CTX-M}
AmpC	Not detected	bla_{CIT-M}
MBL	Not detected	Not detected
Sulphamethoxazole	*sul1* and *sul2*	*sul1, sul2*
Trimethoprim	Not detected	*dfrA7*
Integrons		
Class 1 integron	Not detected	Not detected
Class 2 integron	Not detected	Detected
Resistance to antibiotics	Ampicillin, doxycycline, gentamicin, cefpodoxime, cefoperazone, ceftriaxone, ciprofloxacin, trimethoprim- sulphamethoxazole, piperacillin, piperacillin-tazobactam, tetracyclin, trimethoprim, cefotaxime, ceftazidime and cefoxitin	Ampicillin, aztreonam, cefpodoxime, cefoperazone, ceftriaxone, imipenem, piperacillin, piperacillin-tazobactam, trimethoprim, trimethoprim- sulphamethoxazole, cefotaxime, ceftazidime and cefoxitin
Sensitive to antibiotics	Amikacin, chloramphenicol, ertapenem and imipenem	Amikacin, doxycycline, chloramphenicol, ciprofloxacin, ertapenem, gentamicin and tetracycline

ESBL, extended spectrum β-lactamases; MBL, metallo-beta-lactamases; AmpC, ampC beta-lactamases

findings were also in line with Lin *et al*[16] who reported the coexistence of two or more ESBL genes in about 40 per cent of *E. coli* isolates. In our previous study[17], we reported that Gram-negative bacteria harbouring β-lactamases-producing genes along with TMP-SMX resistance, and Class 1 and Class 2 integrons might make the treatment to bacterial infections more complicated in clinical settings. The probable source for the urinary tract and bloodstream infections of the HIV patient in this study may be from his own gut flora. An earlier study from India, reported that the endogenous translocation of gut flora was one of the major causes of infections of the urinary tract and bacteraemia[18].

In conclusion, the present study showed the clonally different *E. coli* isolates causing blood and urinary tract infections in HIV patient from India and also the isolates harboured multiple drug-resistance genes. In this study, it is demonstrated that differences in antibiotic resistance and susceptibility profile of clonally different *E. coli* isolates causing different infections in an HIV patient may affect the selection of proper antibiotics for their treatment. This study also suggests that for effective treatment of bacterial infections, the proper antibiotic susceptibility testing should be carried out even for two bacterial isolates belonging to the same genus and species and isolated from two different infection sites of a patient.

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