Phylogenetic groups and antimicrobial susceptibility patterns of uropathogenic Escherichia coli clinical isolates from patients at Mulago National Referral Hospital, Kampala, Uganda [version 1; peer review: 2 approved with reservations]

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Abstract

Introduction: Uropathogenic Escherichia coli (UPEC) remains the most common cause of urinary tract infections (UTIs). They account for over 80-90% of all community-acquired and 30-50% of all hospital-acquired UTIs. E. coli strains have been found to belong to evolutionary origins known as phylogenetic groups. In 2013, Clermont classified E. coli strains into eight phylogenetic groups using the quadruplex PCR method. The aim of this study was to identify the phylogenetic groups of UPEC strains in Uganda using Clermont’s quadruplex PCR method and to assess their antibiotic susceptibility patterns in Uganda.

Methods: In this cross-sectional study, 140 stored uropathogenic E. coli isolates from the Clinical Microbiology Laboratory, Department of Medical Microbiology, College of Health Sciences Makerere University were subjected to phylogenetic typing by a quadruplex PCR method. The aim of this study was to identify the phylogenetic groups of UPEC strains in Uganda using Clermont’s quadruplex PCR method and to assess their antibiotic susceptibility patterns in Uganda.

Results: Phylogenetic group B2 (40%) was the most predominant, followed by A (6.23%), clade I and II (5%), D and E (each 2.14%), B1 (1.43%) and F and C (each 0.71%). The most common resistant antibiotic was trimethoprim-sulphamethoxazole (90.71%) and the least was imipenem (1.43%). In total, 73.57% of isolates were multidrug resistant (MDR). Antibiotic resistance was mainly detected in phylogenetic group B2 (54%).

Conclusions: Our findings showed the high prevalence of MDR E. coli...
isolates, with the dominance of phylogenetic group B2. About 9% of E. coli isolates belonged to the newly described phylogroups C, E, F, and clade I and II.

**Keywords**
Uropathogenic E.coli, Phylogenetic groups, Clermont’s, Quadruplex PCR, Antimicrobial resistance.
Introduction
Urinary tract infections (UTIs) remain a major cause of morbidity, with over 1.5 million annual cases reported worldwide (Lee & Neild, 2007)(Neild, 2003). Escherichia coli is the major cause of UTIs accounting for over 80–90% and 30–50% of all community-acquired and hospital-acquired UTIs respectively(Foxman, 2010). Phylogenetic (evolutionary) groups of E. coli strains have been shown to differentiate between pathogenic and commensal strains depending on their fitness landscapes and virulence characteristics. Although multi-locus sequence typing (MLST) and ribotyping are the gold standard methods for phylogenetic typing of E.coli strains, these methods are expensive, time-consuming and require the collection of typed strains (Clermont et al., 2000).

In 2000, Clermont et al. (Clermont et al., 2000) developed a triplex PCR assay that classified E.coli strains into four different phylogenetic groups, A, B1, B2 and D based on the presence or absence of two genes, namely chuA, yjaA, and one DNA fragment TspE4.C2. Since 2000, growing knowledge of MLST for E.coli from different habitats has made it possible to validate the triplex PCR method (Gordon et al., 2008). The validation studies found that only 80–85% of all E. coli phylogenetic groups were assigned correctly(Gordon et al., 2008)(Clermont et al., 2013).

In 2013 Clermont et al. (Clermont et al., 2013) added an additional gene target, arpA, to the three candidate markers (chuA, yjaA and TspE4.C2) and developed a quadruplex PCR assay to classify E. coli isolates into eight phylogroups: A, B1, B2, C, D, E, F, and clade I/II. The use of this quadruplex PCR phylotyping method has been found to correctly assign 95% of all E. coli strains. Phylogenetic analysis has demonstrated a relationship between different E. coli phylogenetic groups, antimicrobial resistance and other virulence characteristics (Iranpour et al., 2015). Different studies have shown that the most virulent and antimicrobial-resistant extra-intestinal E. coli strains belong mainly to group B2 and, to a lesser extent, to group D (Iranpour et al., 2015)(Bashir et al., 2011)(Liu et al., 2014). In contrast, most of the commensal strains are associated with group A or group B1 (Clermont et al., 2000). In this study, we aimed to determine the prevalence of the different phylogenetic groups of Uropathogenic E. coli strains using the new Clermont quadruplex PCR phylotyping method and their antimicrobial susceptibility patterns.

Methods
Study design and setting
This was a cross-sectional laboratory-based study carried out in the Clinical Microbiology and Molecular Biology Laboratories in the Department of Medical Microbiology, College of Health Sciences, Makerere University (Kampala, Uganda).

Bacterial strains
A total of 140 Uropathogenic E. coli strains that belonged to the bacterial collection of the Department of Medical Microbiology, College of health sciences Makerere University were studied. These strains had been isolated and stored at -80°C during a 12-month period (between January and December 2016). These isolates had been recovered from samples with a bacterial count of 10^5 CFU/ml of midstream urine samples of patients with a suspected UTI. The samples were selected by consecutive sampling and all samples that were poorly labeled and lacked traceable laboratory request form were excluded from the study. All isolates were plated on MacConkey agar and incubated at 37°C aerobically for 18–24 hours to obtain pure growth.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was done using the Kirby Bauer disk-diffusion method. Briefly, between one and five colonies of the isolate from the MacConkey agar were emulsified in saline and adjusted for turbidity to obtain a 0.5 McFarland standard. A sterile cotton swab on a stick was dipped in the colony-saline mixture, excess saline was then squeezed out by pressing the swab against the test tube, and the cotton swab gently applied onto the surface of the Mueller-Hinton-2- agar to obtain a uniform lawn. Up to six antibiotic impregnated discs were gently placed on the agar surface, at a minimum distant of 25 mm from each other, and the plates incubated at 37°C aerobically for 18–24 hours. The zones of inhibition diameters around each disc were measured using a ruler and compared against the zone diameter interpretative standards according to CLSI (2014). A panel of 12 antibiotics were used: ampicillin (30 μg), cefuroxime (30 μg), amoxicillin/clavulanic acid (20/10 μg), gentamycin (10 μg), trimethoprim/sulphamethoxazole (1.25/23.5 μg), chloramphenicol (5 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), cefazidime (30 μg), imipenem (10 μg), nalidixic acid (30 μg) and nitrofurantoin (300 μg). E. coli ATCC 25922 was used as a control strain.

ESBL detection
All isolates that showed an area of inhibition of diameter <22 mm for cefazidime and <25 mm for ceftriaxone, which were selected for phenotypic detection of The test isolate was mixed in saline to obtain a suspension of 0.5 McFarland standard. The suspension was later swabbed to make a uniform lawn on Mueller-Hinton agar (MHA) plate. An amoxicillin-clavulanate (Augmentin) (20 μg/10 μg) disk was placed in the center of the plate with a 30-μg disk of a third-generation cephalosporin (cefazidime and cefotaxime) at a distance of 20 mm from center to center on a Mueller Hinton agar (MHA) plate on opposite sides. The plate was later incubated aerobically at 37°C for 18–24 hours. All isolates that showed a clear extension of the edge of inhibition zone of the third-generation cephalosporin toward the augmentin disk were interpreted as positive for ESBL production.

Carbapenemase detection
Detection of carbapenemases was determined by a positive Modified Hodge test (MHT) as described by CLSI (2014); In brief, E. coli ATCC 25922 was streaked for confluent growth on Mueller-Hinton II agar plates. A disk saturated with 10 μg of imipenem was placed in the center of the plate, and each sample was then subsequently streaked from the disk to the edge of the plate. The presence of a distorted inhibition zone after overnight incubation was interpreted as a positive result. Klebsiella pneumoniae ATCC BAA-1705 and K. pneumoniae ATCC
BAA-1706 were used as positive and negative controls, respectively. Phenotypic detection of Metallo-beta-lactamases (MBL), *K. pneumoniae* producing carbapenems (KPC) and AmpC was carried out as described by Andrea Bartolini *et al.* and Tsakris *et al.* (Bartolini *et al.*, 2014)(Tsakris *et al.*, 2008).

**DNA extraction**
DNA for amplification was extracted from whole cells by the boiling lysis method, as explained briefly below. A full loop of pure colonies from fresh pure cultures was suspended in 1 ml of sterile distilled water. The cells were lysed by heating at 95°C for 10 minutes. The cells were then vortexed for 5 seconds. Centrifugation was later done at 13,000 rpm for 5 minutes at room temperature and the sample kept at -20°C to harvest the supernatant containing the DNA. The supernatant was subsequently used for PCR as template DNA. The integrity of extracted DNA was evaluated by electrophoresis on a 1% agarose gel. The purity of DNA was also determined by the ratio A260/A280 using a spectrophotometer.

**New Clermont’s quadruplex PCR Phylotyping**
The distribution of phylogenetic groups amongst *E. coli* isolates was determined by the New Clermont Quadruplex PCR phylotyping method of 2013 (Clermont *et al.*, 2013). Briefly, a single reaction mixture contained 2 μL of 10x buffer (supplied with Taq polymerase), 2 μL of DNA (approximately 100 ng), 20 pmol of each appropriate primer (except for AceK.f (40 pmol), ArpA1.r (40 pmol), trpBA.f (12 pmol), and trpBA.r (12 pmol)) (Shanghai Generay Biotech Co., Ltd.), 2mM of each dNTP, and 2U of Taq DNA polymerase (Fermentas, Lithuania) in a total volume of 20 μL. Primer sequences for the new Clermont’s quadruplex PCR phylogroup assignment method are as shown in Table 1. PCR amplifications were carried out on a thermal cycler Master-cycler gradient (Eppendorf, USA) under the following conditions: initial denaturation at 94°C for 4 min and 30 cycles for each denaturation at 94°C for 5 sec, annealing at 57°C for 20 sec (group E) or 59°C for 20 sec (quadruplex and group C), amplification at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis with Qiaxel machine and a 1% agarose gel. The purity of DNA was also determined by the ratio A260/A280 using a spectrophotometer.

**Statistical analysis**
The data was entered in an excel spreadsheet, cleaned and double-checked for missing variables duplicate entries and values out of range. The data was then exported to STATA version 14 for statistical analysis. Means and proportions were obtained. Chi-square test or the Fisher exact test was applied to compare categorical variables. *P* values < 0.05 were considered to be statistically significant.

**Ethical considerations**
Ethical approval (SBS-HDREC- 487) and a waiver of consent were sought to use stored clinical isolates from the higher degrees Research and Ethics Committee of the School of Biomedical Sciences, College of Health Sciences Makerere University and the Uganda National Council of Science and Technology.

**Results**

**Demographics from the isolate data**
Of the 140 *E. coli* isolates, 102 (72.9%) were females and 38 (27.1%) were males. The age of the patients was ranging from 2 to 91 years. The mean age was 36.27 with a standard deviation of 18.98.

**Table 1. Primer sequences that were used in the Clermont’s quadruplex phylotyping method.**

<table>
<thead>
<tr>
<th>PCR Reaction</th>
<th>Primer ID</th>
<th>Target</th>
<th>Primer Sequence</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadruplex</td>
<td>chuA.1b</td>
<td>chuA</td>
<td>5-ATGGTACC CGGACGAACACAC-3</td>
<td>288</td>
</tr>
<tr>
<td></td>
<td>chuA.2</td>
<td>chuA</td>
<td>5-TGCCGCGCAGTACAAAGACA-3</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>yjaA.1b</td>
<td>yjaA</td>
<td>5-CAAACGTAAGTGTCCAGGAG-3</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>yjaA.2b</td>
<td>yjaA</td>
<td>5-AGTTTATGCCTCGGGGTAGC-3</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>TspE4C2.1b</td>
<td>TspE4.C2</td>
<td>5-CACTATTCGTAAGGTCTACCC-3</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>TspE4C2.2b</td>
<td>TspE4.C2</td>
<td>5-CAGTATTTCGCTCCGCGGTCGC-3</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>AceK.f</td>
<td>arpA</td>
<td>5-AATGCGTATCCTCACCCAGCTG-3</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>ArpA.r</td>
<td>arpA</td>
<td>5-TCTCCCGATCGCTACGCTA-3</td>
<td>301</td>
</tr>
<tr>
<td>Group E</td>
<td>ArpAGpE.f</td>
<td>ArpA</td>
<td>5-GATTTCCATTTGTAAATAATGCC-3</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>ArpAGpE.r</td>
<td>ArpA</td>
<td>5-GAAAAGAAAAAGAAATTCCCAAGG-3</td>
<td>301</td>
</tr>
<tr>
<td>Group C</td>
<td>trpAgpC.1</td>
<td>trpA</td>
<td>5-AGTTTTATGCCCCAGTGCAAG-3</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>trpAgpC.2</td>
<td>trpA</td>
<td>5-TCTGGCGCGGTCAGGCCC-3</td>
<td>489</td>
</tr>
<tr>
<td>Internal control</td>
<td>trpBA.f</td>
<td>trpA</td>
<td>5-CGCGGATAAGACATCTTCAC-3</td>
<td>489</td>
</tr>
<tr>
<td></td>
<td>trpBA.r</td>
<td>trpA</td>
<td>5-GCAACGCGGCGCTGCGGAGA-3</td>
<td>489</td>
</tr>
</tbody>
</table>
Culture and susceptibility results
Resistance was highest to trimethoprim-sulphamethoxazole 127/140 (90.71%) followed by ampicillin 122/140 (87.14%) while resistance to nitrofurantoin 16/140 (11.43%) and imipenem 2/140 (1.43%) was minimal. Resistance to other antibiotics were as follows; cefuroxime 72/140 (51.43%), amoxicillin-clavulanic acid 46/140 (32.86%), gentamycin 52/140 (37.14%), chloramphenicol 35/140 (25%), ciprofloxacin 80/140 (57.14%), ceftriaxone 69/140 (49.29%), ceftazidime 50/140 (35.71%) and nalidixic acid 94/140 (67.14%). Figure 1 contains a bar chart depicting percentage resistance to different antibiotics. Data from disc-diffusion assays are available as Underlying data (Katongole, 2019).

Multi-drug resistant (MDR) organisms
In the study, 103/140 isolates (73.57%) were found to be MDR. In addition, 4/140 isolates (2.85%) were resistant to all antibiotics tested.

ESBL, AmpC, and carbapenem detection
In this study, 61/140 (43.57%) were positive for ESBL production. Out of all the ESBL positive isolates, (57/61) 93.44% were

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Percentage resistance of uropathogenic *E. coli* clinical isolates to different antibiotics (N=140).

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Quaxel gel Picture of Clermont’s Quadruplex PCR of some Uropathogenic *E. coli* isolates. Shown are bands for different genes for phylogenetic typing.
Phylogenetic groups and Antimicrobial resistance

Phylogenetic group B2 was the most frequently resistant (38.57%). Phylogenetic groups B1 and F were the least resistant with a recorded highest resistance rate of 0.71% (Table 3).

Discussion

The clinical management of UTIs is becoming a major burden due to the emergence of MDR uropathogens (Shabbir et al., 2018) (Flores-Mireles et al., 2015). Currently, third-generation cephalosporins are the most commonly used drugs in the management of complicated and uncomplicated UTIs (Stiller et al., 2017) (Bonkat et al., 2017). This study aimed to identify the phylogenetic groups of UPEC clinical isolates based on the Clermont quadruplex PCR method and to assess the relationship between these phylogroups and antibiotic susceptibility patterns in Uganda. In this study majority of the E. coli strains belonged to B2 (40%). This was similar to other studies worldwide (Iranpour et al., 2015) (Basu et al., 2013) (Moreno et al., 2008) (Takahashi et al., 2006). Other studies have, however, the most predominant phylogenetic group was group A (Ejrnæs et al., 2011) (Zhao et al., 2015). Phylogenetic group B2 has been associated with MDR-UPEC strains and increased expression of virulence factors (Ochoa et al., 2016) (Molina-López et al., 2011) (Nüesch-Inderbinen et al., 2017). Persistent and recurrent UTIs have also been associated with phylogenetic group B2 and this has been implicated in the pathogenesis of pyelonephritis (Ejrnæs et al., 2011) (Kudinha et al., 2013) (Luo et al., 2012). A similar study by Ramos et al. of pregnant mothers at Mulago National Referral Hospital found B1 to be the most predominant phylogenetic group (Ramos et al., 2012). The difference could be explained by the different study

### Table 2. Prevalence of the phylogenetic groups of Uropathogenic E. coli isolates.

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Prevalence, n (total 140)</th>
<th>Prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>6.23</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td>1.43</td>
</tr>
<tr>
<td>B2</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0.71</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>2.14</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>2.14</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0.71</td>
</tr>
<tr>
<td>Unknown</td>
<td>58</td>
<td>41.43</td>
</tr>
<tr>
<td>Clade I or II</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 3. Prevalence of resistance among different phylogenetic groups of E. coli isolate.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Phenylogenetic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A N (%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>3 (2.14)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>2 (1.43)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>3 (2.14)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4 (2.86)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>4 (2.86)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4 (2.86)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>4 (2.86)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
In this study, we did not find any carbapenemases on phenotypic and genotypic screening. This was, however, different from other studies conducted in a similar setting (Okoche et al., 2015) (Kateete et al., 2016).

This study showed that group B2 isolates were the most resistant to most antimicrobials (38.5%). This was similar to other similar studies (Iranpour et al., 2015) (Massot et al., 2016). In addition, groups D, B1, and F were least resistant, again similar to other studies (Iranpour et al., 2015) (Massot et al., 2016).

In conclusion, our findings showed that group B2 (40%) were the most predominant and most resistant phylogenetic group among UPEC clinical isolates. About 9% of E. coli isolates belonged to the newly described phylogroups C, E, F, and clade I. We recommend routine surveillance of antibiotic resistance patterns in the region to help clinicians make the treatment options for patients with UTIs. We recommend a longitudinal study employing whole-genome sequencing of E. coli strains in relation to UTI acquisition this will provide more insight on the role of Phylogenetic groups in the pathogenesis of UTIs.

Data availability


This project contains the disc-diffusion antibody sensitivity data and phylogenetic group for samples taken from each patient in this study.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).


The study evaluated the Phylogenetic groups and antimicrobial susceptibility patterns of uropathogenic *Escherichia coli* clinical isolates from patients at Mulago National. The topic is very interesting, however, the manuscript needs to be reviewed and there are also some important questions that require consideration.

**Specific Comments:**

**Abstract:**
- It was not clear the results about ESBL, AmpC, and carbapenem detection. Also, it should be emphasized that 41.3% of the isolates belonged to the unknown Phylogenetic group.

**Introduction:**
- It is clear. However, I would suggest include more recent references.

**Methods:**
- Why CLSI (2014) was used? There is probably a more recent edition of CLSI.
- It was not included how the identification/classification of MDR was done. Also, it is necessary to include the Demographics data analyses (gender, age...).

**Results:**
- In case, 41.3% of the isolates that belonged to the unknown Phylogenetic group it is an impressive result. Is there *E. coli* control for each Phylogenetic group? It deserves more attention and discussion. It seemed to be a high number considering the fact to be new strains or the un-typable by PCR

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology and molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 07 August 2020

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Comments to the manuscript

Abstract
This is a study of frequencies in UPEC strains, recovered from patients with UTIs, whose purpose was the association of phylogenetic groups (PG) with antimicrobial resistance, using the Quadruplex-PCR suggested by Clermont et al., 2013.

Although the approach to PG and resistance has been widely discussed, the information from other countries can support treatment and the epidemiological studies. However, I believe that
more information can be extracted from the data already presented, which supports these results and an adequate discussion.

**Introduction**
The introduction is clear and contains enough information for the article to be read clearly.

**Material and Methods**
It is important to indicate the origin of the strains since it is not indicated what type of UTI they are related to, that is, if they are community UTIs, hospital UTIs, complicated or uncomplicated UTIs.

The authors describe the presence of UPEC-MDR strains; however, it is important to define the significance of an MDR strain.

As a suggestion for the authors, it is important to add the identification of UPEC-XDR and PDR strains. Based on these data, it is necessary to add 2 more categories of antibiotics, such as monobactams and tetracycline.

Check the name of the authors.

The purpose of the work was: "to typify the PGs, in a collection of UPEC clinical strains, by the new scheme of Clermont et al., 2013 and to relate it to antibiotic susceptibility". However, the results showed that 58/140 (They are many) strains were not typed by this method. This is a result that needs to be discussed in more detail.

According to the analysis made by the authors, does it mean that the Clermont system implemented does not work on Ugandan strains?

Regarding the DNA extraction method? Raw or boiled extracts are generally not suitable for multiplex PCR assays. Is DNA quantification better with this scheme? I suggest that in untyped strains, PCR be performed again using purified DNA (manual or with a commercial kit), quantified and adjusted to a standard concentration. This experiment will validate the negative results.

As a suggestion to the authors, it would be interesting to integrate an analysis of PG, resistance, and the age of the patients (pediatric and adult) for the improvement of the manuscript.

Considering the enzyme production, GF, age, characteristic of UTI as related to the 4 strains that were characterized as R to all antibiotics, is it an XDR or PDR strain?. Under this same context, how were the phenotypic AmpC-producing strains?

In various populations, the phylogenetic group D is highly prevalent, why is it not mentioned in the article and it is not discussed either?

How is the MDR profile (73%) in relation to the PG? This information is also important for inclusion in the manuscript.

Fig. 2 may be supplemental material. I suggest adding a caption, indicating the following: 1) The corresponding to each lane, 2) the presence of molecular size, 3) the presence of results of the clinical strains, and 4) the presence of positive control strains.
In the discussion, the authors justify the presence of strains that did not typify, data that correlate with the article by Clermont et al., 2013, in addition to this, that other studies show this same trend.

The manuscript requires a more solid conclusion, regarding whether the new PG scheme of Clermont et al., 2013, is optimal or not for this study.

Although many results were disputed, I consider that this manuscript requires a more detailed analysis.

In the discussion, the authors described: “In this study, we did not find any carbapenems on phenotypic a genotypic screening”. The methodology and results, only indicate that they carried out the phenotypic screening, they do not indicate how they carried out the genetic typing. Correct or add this information in the methodology and results section.

In the results section, I suggest that the authors describe in which groups the statistical test was performed, for example, if the statistics were performed only for PG vs antibiotic resistance or considering the MDR groups, or the presence of ESBL, carbapenems, and AmpC.

It is not clear whether the identification of AmpCs was chromosomal or plasmid. Please indicate.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Bacterial pathogenesis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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