Diagnostic Microbiology and Infectious Disease xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

The population structure of clinical extra-intestinal *Escherichia coli* in a teaching hospital from Nigeria

Jeremiah Seni [a,b,1], Giselle Peirano [c,d,1], Kenneth Okwong Okon [e,1], Yusuf Bara Jibrin [f], Alkali Mohammed [f], Stephen E. Mshana [a,2], Rebekah DeVinney [b,2], Johann D.D. Pitout [b,c,d,g,*,2]

[a] Department of Microbiology and Immunology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania
[b] Department of Microbiology, Immunology and Infectious Diseases, University of Calgary,Calgary, Alberta, Canada
[c] Department of Pathology & Laboratory Medicine, Cumming School of Medicine, University of Calgary,Calgary, Alberta, Canada
[d] Division of Microbiology, Calgary Laboratory Services, Calgary, Alberta, Canada
[e] Department of Medical Microbiology, Federal Medical Centre, Makurdi
[f] Department of Internal Medicine, Abubakar Tafawa Balewa University teaching hospital Bauchi
[g] Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa

ARTICLE INFO

Article history:
Received 1 March 2018
Received in revised form 1 April 2018
Accepted 5 April 2018
Available online xxxx

Keywords:
ExPEC
Population structure
Nigeria
Molecular typing

ABSTRACT

Limited information is available regarding the population structure of extra-intestinal pathogenic *Escherichia coli* (ExPEC) in Africa. Antimicrobial resistance profiles, sequence types (STs) and *fimH* types were determined on 60 clinical ExPEC from Nigeria using a 7-single nucleotide polymorphism quantitative PCR and sequencing of certain genes. Different ST131 clades were identified with a multiplex PCR. The isolates were mostly obtained from urines (58.3%). Not-susceptibility rates were as follows: trimethoprim-sulfamethoxazole (98%), cefotaxime (68%), gentamicin (55%), ciprofloxacin (62%) and piperacillin-tazobactam (2%). Dominant STs were associated with CTX-M-15 and included ST131-*fimH*30 (23%), ST457-*fimH*145 (20%), ST405-*fimH*27 (13%) and ST95-*fimH*41 (10%). We found the 7-SNP qPCR to be simple and cost-effective that can be utilized to tract different ExPEC clones on a global scale. This study provided insight into the population structure of ExPEC from Nigeria showing high prevalence of the rarely reported ST457 and the presence of multidrug resistant ST95.

1. Introduction

Extrapathogenic *Escherichia coli* (ExPEC) is the most common cause of urinary tract infections (UTIs) and the most common Gram-negative bacterium associated with bloodstream infections in both developed and developing countries (Johnson and Russo, 2002; Pitout, 2012). Molecular surveillance has shown that ExPEC are over presented by certain global clones (e.g. ST69, ST73, ST95, ST131, ST393) suggesting that these clones are more "successful" than other ExPEC (Riley, 2014). Of special interest is that certain clones (e.g. ST131 and its clades, namely clade C1/H30R and clade C2/H30Rx) are mainly responsible for global increase of antimicrobial resistance among ExPEC while other clones (e.g. ST95) remain mostly susceptible (Mathers et al., 2015).

There is an need to evaluate simple, reliable and cost effective epidemiological laboratory tools in resource limited countries such as those in Africa, where limited information exist regarding the population structure of ExPEC. Previous studies from Lagos and Oyo states in Nigeria have revealed presence of *E. coli* ST131, but did not determine the presence of other clones among ExPEC (Adenipekun et al., 2016; Aibinu et al., 2012; Inwezerua et al., 2014). A study was designed to determine antimicrobial susceptibility patterns and the population structure of ExPEC isolates (ST/*fimH* types) in a tertiary care center from North-eastern Nigeria.

2. Methods

This was a hospital-based study conducted between June and July 2014 at Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), a 750-bed hospital in Bauchi, North-eastern Nigeria. A total of 60 non-repeat unique sequential ExPEC isolates were obtained from patients over a 2 month period using conventional methods. Most of the isolates were obtained from urine (35, [58.3%]), followed by intra-abdominal (13 [21.7%]), blood (6, [10%]) and wound swabs (6, [10%]). Isolates were recovered from Antimicrobial susceptibility was determined by disc diffusion method using the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2015). The antimicrobial drugs tested were ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, piperacillin-tazobactam, cefoxitin, cefotaxine, ceftazidime, and ertapenem. The phenotypic presence of ESBLs was detected using the

* Corresponding author. Tel.: +1-403-770-3309; fax: +1-403-770-3347.
E-mail address: johann.pitout@cls.ab.ca (J.D.D. Pitout).
[1] Equal contributors.
[2] Co-senior authors.

https://doi.org/10.1016/j.diagmicrobio.2018.04.001
0732-8893/

CLSI disk method (CLSI, 2015). PCR amplification and sequencing for $bla_{CTX\text{-}Ms}$ alleles performed using conditions and primers as previously described (Peirano et al., 2010).

The 7-single nucleotide polymorphism quantitative PCR (7-SNP qPCR) method was used to type ExPEC into different septatypes by assessing the presence or absence of 7 SNPs in the *fimH* and *fumC* genes as described before (Tchesnokova et al., 2016). The assay also included primers for *uidA* gene as an internal control for confirmation of *E. coli*. A septatype with the subsequent conversion to a ST and *fimH* were done as previously described (Tchesnokova et al., 2016).

In the present study, certain septatypes (referred to as "homogenous major" in the original Tchesnokova publication) were directly converted into a single ST-*fimH* lineage (Fig. 1, Table 1). E.G. septatypes 561 and 760 were converted into ST131 and ST95. For some septatypes (called "non-homogenous major" in the original Tchesnokova publication), additional sequencing of certain MLST housekeeping genes was performed to identify the ST. E.G. septatypes 360, 371, 351, 361 underwent additional sequencing of *gyrB* to identify ST457, ST297, ST101, ST405, ST58 and ST2536 (Table 1, Fig. 1). One isolate (setatype 361) also underwent sequencing of *mdh* and was identified as ST354 (Table 1). For "undetermined" septatypes (from the original Tchesnokova publication) such as 300, 100, 260, 371 and 771, the CH locus typing that involved the sequencing of *fumC* and *fimH* was performed (Table 1, Fig. 1)) (Weissman et al., 2012). The ExPEC typing workflow undertaken in this study is shown in Fig. 1. The identification of the dominant STs (i.e. ST131, ST457, ST405 and ST95) was confirmed by multilocus sequence typing ((http://mlst.ucc.ie/mlst/dbs/Ecoli). A multiplex PCR was used to identify different *E. coli* ST131 clades using a previously described method (Matsumura et al., 2017).

Ethical clearance was obtained from the ATBUTH Ethical Clearance Committee. Analysis of patients' demographics, clinical and laboratory data was done using STATA version 13.0 software (College Station, Texas, USA) according to the objectives of the study.

3. Results

3.1. Baseline demographic and clinical information of patients

Of 60 patients included, 38 (63.3%) were female and the median age (IQR) was 23 (18–33.5) years; ranging from 2 years to 85 years.

3.2. Antimicrobial resistance patterns of ExPEC strains

Proportions of ExPEC strains not-susceptible [NS] (i.e. intermediate or resistant) to ampicillin, trimethoprim-sulphamethoxazole, ciprofloxacin, cefotaxime, ceftazidime, gentamicin and piperacillin-tazobactam, were 100%, 98%, 62%, 68%, 68%, 55% and 2%, respectively. All isolates were sensitive to cefoxitin and ertapenem.

3.3. Molecular characterization of ExPEC

Of the 60 ExPEC included in this study, 41 (68%) were phenotypically positive for ESBLs and all contained $bla_{CTX\text{-}M\text{-}15}$. A total of 11 different septatypes were obtained and were converted to 14 STs (Table 1). The dominant STs (i.e. containing 6 isolates or more) were ST131-*fimH*30 (14 [23%]), ST457-*fimH*145 (12 [20%]), ST405-*fimH*27 (8 [13%]) and ST95-*fimH*41 (6 [10%]); these STs represented 67% of the total ExPEC population (Table 1). Eight of the ST131-*fimH*30 isolates were positive for clade C1 and tested negative for $bla_{CTX\text{-}M\text{-}15}$ while the remaining six were positive for clade C2 and contained $bla_{CTX\text{-}M\text{-}15}$.

ST131-*fimH*30 clade C2 and ST405-*fimH*27 were ciprofloxacin and gentamicin resistant clones and were associated with $bla_{CTX\text{-}M\text{-}15}$. ST131-*fimH*30 clade C1 had similar susceptibility patterns but was negative for $bla_{CTX\text{-}M\text{-}15}$. ST457-*fimH*145 and ST95-*fimH*41 was positive for $bla_{CTX\text{-}M\text{-}15}$ but remained mostly sensitive to ciprofloxacin and gentamicin (Table 1).

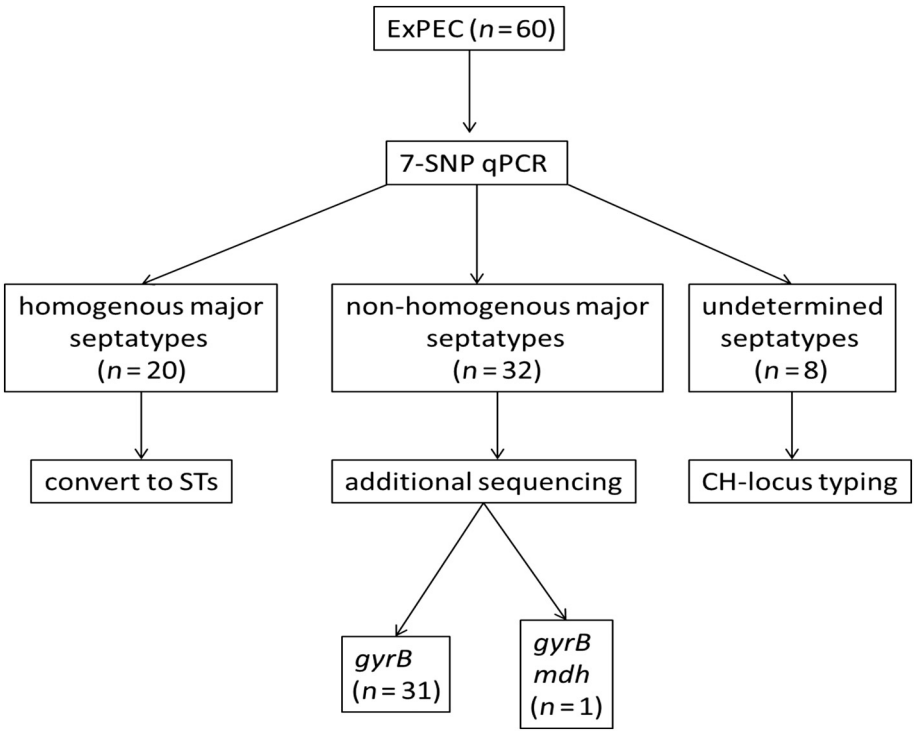

Fig. 1. Flow diagram for the molecular characterization of ExPEC from Bauchi, Nigeria. 7-SNP qPCR: 7-single nucleotide polymorphism-based quantitative polymerase chain reaction. CH locus typing involved the sequencing of *fumC* and *fimH* (Weissman et al., 2012).

Please cite this article as: Seni J, et al, The population structure of clinical extra-intestinal *Escherichia coli* in a teaching hospital from Nigeria, Diagn Microbiol Infect Dis (2018), https://doi.org/10.1016/j.diagmicrobio.2018.04.001