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

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Highlights

- The majority of ExPEC isolated from patients attending ATBUTH in Nigeria belonged to multidrug resistant ST131-*fimH*30, ST457-*fimH*145, ST405-*fimH*27 and ST95-*fimH*41 that were associated with $bla_{\text{CTX-M-15}}$.
- The ExPEC ST131-*fimH*30 clades C1 and C2 were fluoroquinolone resistant while C2 was also

positive for *bla*$_{\text{CTX-M-15}}$.

- The 7-SNP qPCR combined with sequencing of *gyrB* is a simple, cost-effective approach that can be utilized in AMR surveillance programs to tract different ExPEC STs on a global scale.

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%).This study provided insight into the population structure of ExPEC from Nigeria showing high prevalence of the rarely reported ST457. The 7-SNP qPCR combined with sequencing of *gyrB* is a simple and cost-effective approach that can be utilized in surveillance programs to tract different ExPEC clones on a global scale.

Keywords: ExPEC, population structure, Nigeria, molecular typing

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 November 2014 at Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), a 750-bed hospital in Bauchi, North-eastern Nigeria. A total of 60 non-repeat sequential ExPEC isolates were obtained from patients using conventional methods. 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 CLSI disk method (CLSI, 2015). PCR amplification and sequencing for $bla_{\text{CTX-Ms}}$ 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 (Figure 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, Figure 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, Figure

1)) (Weissman et al., 2012). The ExPEC typing workflow undertaken in this study is shown in Figure 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).

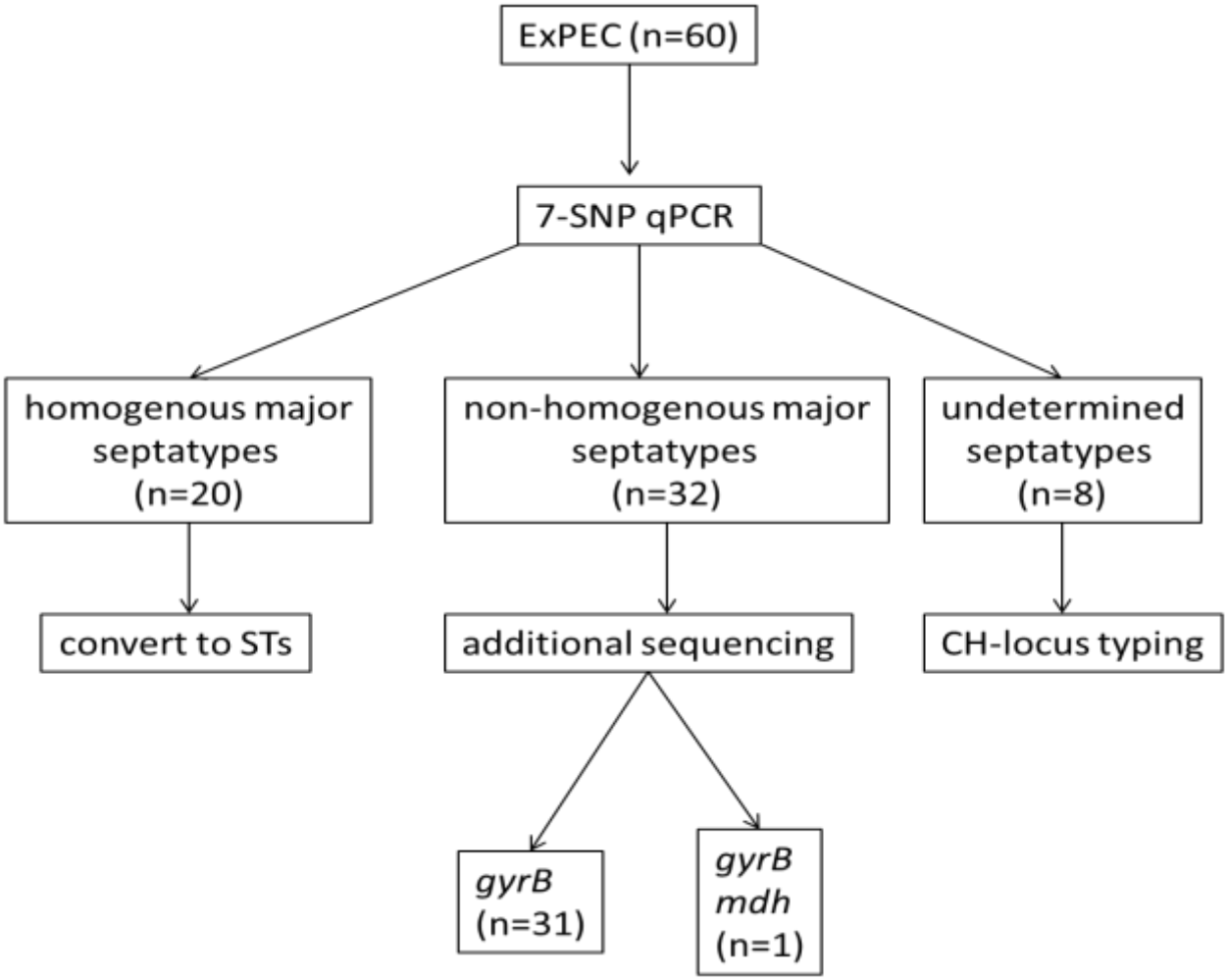

Figure 1: Flow diagram for the molecular characterization of ExPEC from Bauchi, Nigeria

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)

Table 1. Susceptibility patterns and molecular characterization of ExPEC from Bauchi, Nigeria

No	Septatype	Additional gene sequenced	Sequence type	*fimH*	CTX-M-15 (no)	Non-susceptible (%)
14	561	N/I	131	*fim*H30	6	SXT (100%); CIP (100%); GENT (93%); CTX (43%)
6	760	N/I	95	*fim*H41	5	SXT (83%); CIP (33%); GENT (0%); CTX (83%)
12	360	*gyrB*	457	*fim*H145	12	SXT (100%); CIP (0%); GENT (16%); CTX (100%)
4	360	*gyrB*	297	*fim*H38	2	SXT (100%); CIP (100%); GENT (50%); CTX (50%)
1	360	*gyrB*	101	*fim*H86	1	SXT (100%); CIP (100%); GENT (100%); CTX (100%)
8	371	*gyrB*	405	*fim*H27	7	SXT (100%); CIP (100%); GENT (88%); CTX (88%)
3	351	*gyrB*	58	*fim*H54	3	SXT (100%); CIP (100%); GENT (0%); CTX (100%)
3	361	*gyrB*	2536	*fim*H31	3	SXT (100%); CIP (100%); GENT (100%); CTX (100%)
1	361	*gyrB* and *mdh*	354	*fim*H58	0	SXT (100%); CIP (100%); GENT (100%); CTX (0%)
2	300	CH-locus typing	10	*fim*H0	0	SXT (100%); CIP (0%); GENT (0%); CTX (0%)
2	100	CH-locus typing	12	*fim*H0	0	SXT (100%); CIP (0%); GENT (0%); CTX (0%)
2	260	CH-locus typing	38	*fim*H65	0	SXT (100%); CIP (100%); GENT (0%); CTX (0%)
1	371	CH-locus typing	410	*fim*H24	1	SXT (100%); CIP (100%); GENT (100%); CTX (100%)
1	771	CH-locus typing	617	*fim*H27	1	SXT (100%); CIP (100%); GENT (100%); CTX (100%)

SXT; trimethoprim-sulfamethoxazole, CIP; ciprofloxacin, GENT; gentamicin, CTX; cefotaxime

N/I; not indicated; sequence type was inferred from the septatype.

CH locus typing involved the sequencing of *fumC* and *fimH* (Weissman et al., 2012)

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. 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%]).

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 $bla_{\text{CTX-M-15}}$. 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 $bla_{\text{CTX-M-15}}$ while the remaining six were positive for clade C2 and contained $bla_{\text{CTX-M-15}}$.

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

3.4. Characteristics associated with ST131 and non-ST131 ExPEC attributable infection

There was no significant difference between ST131 and non-ST131 ExPEC with median age, sex or sample types. ExPEC ST131 strains were significantly more resistant than non ST131 strains to ciprofloxacin (p = 0.003) and gentamicin (p = 0.001). Interestingly the presence of CTX-M-15 was higher among non–ST131 ExPEC (76.1%) compared to ST131 (35.7%), with the association being protective [OR: 0.17, 95% CI: 0.04 – 0.75; p=0.005].

4. Discussion

The global rise of antimicrobial resistance (AMR) is a global crisis which negatively affects human health and the economy (http://www.who.int/antimicrobial-resistance/en/). The past decade had seen a rising trend in AMR, resulting into limited treatment options, and is disproportionately to the rate of antimicrobial drugs development (Silver, 2011). Extended spectrum β-lactamase (ESBL) producing Gram negative bacteria are spreading across the globe at alarming rates in humans, animals and various environmental sources (Mathers et al., 2015, Sonda et al., 2016).

Extraintestinal pathogenic *E. coli* from this Nigerian study showed very high levels of resistance to ampicillin, trimethoprim-sulphamethoxazole, gentamicin, ceftriaxone and ciprofloxacin. These agents are often used to empirically treat infections due to ExPEC (Pitout et

al., 2016). This may be partly related to the readily availability of these agents and more likely to be misused especially in African countries where stringent measures to ensure rational use of antimicrobial agents is still limited. Of note, just over two thirds of the ExPEC strains from this study were CTX-M-15 producers as opposed to only 12.8% in 2012 in the same country (Aibinu et al., 2012). This may be explained by either variation in the occurrence of ESBLs across regions/states or the rising trend of ESBLs in Nigeria. This study and other studies from Nigeria are showing low levels of resistance of *E. coli* to piperacillin-tazobactam and carbapenems (Adenipekun et al., 2016, Iliyasu et al., 2016), underscoring the need to strengthen antimicrobial stewardship programs to preserve them as potential therapeutic options now and for future generations.

E. coli with $bla_{\text{CTX-M-15}}$ is a global phenomenon and has been linked to the presence of ST131 (Mathers et al., 2015). The predominance of ExPEC ST131 among CTX-M-15-producing *E. coli* in this study is similar to previous studies from Nigeria (35.7%), Tanzania (24% to 37%) and South Africa (45%) (Aibinu et al., 2012, Mshana et al., 2016, Mshana et al., 2011, Peirano et al., 2011). All the ST131 with $bla_{\text{CTX-M-15}}$ belonged to clade C2/H30Rx.

In 2014, the World Health Organization released a report that specifically noted that surveillance for antibacterial resistance (especially in resource-limited countries) is neither coordinated nor harmonized and there are many gaps in information on bacteria of major public health importance (Organization, 2014). That report also revealed that key tools to tackle antibiotic resistance, such as basic systems to track and monitor the problem, do not exist in many countries. We report on a study that used a cost-effective, rapid and user friendly 7-SNP qPCR typing method to describe the population structure of a clinical collection of ExPEC

obtained in North-Eastern Nigeria. This method was able to characterize 33% of the ExPEC isolates into STs and *fimH* types. The additional sequencing of 1 gene (namely *gyrB*) was able to further characterize an additional 53% (for a total of 86%) of this population (Table 1). This approach is feasible, cost effective and a relative simple epidemiological tool that was able to identify all dominant clones (i.e. ST131-*fimH*30, ST457-*fimH*145, ST405-*fimH*27 and ST95-*fimH*41) within this Nigerian collection. It can easily be utilized in surveillance programs to tract different ExPEC STs on a global scale.

This study revealed some interesting results. 1. We did not detect AmpC-producing isolates among this non-biased clinical collection. 2. A rarely report clone, ST457-*fimH*145 was the 2nd most common clone identified. ST457 from this study was associated with $bla_{\text{CTX-M-15}}$ and had previously only been reported from Italy and England (Accogli et al., 2014, Lau et al., 2008). The ST457 isolate from Italy was positive for the carbapenemase, KPC-3 (Accogli et al., 2014). 3. The majority of ST95-*fimH*41 from this study was positive for $bla_{\text{CTX-M-15}}$ which is in stark contrast to other reports showing that ST95 is a relative antimicrobial susceptible clone (Kallonen et al., 2017, Stephens et al., 2017, Yamaji et al., 2018).

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Ethical approval: Ethical clearance was sought and obtained from Abubakar Tafawa Balewa University Teaching Ethical Clearance Committee.

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