Human carriage of cefotaxime resistant *Escherichia coli* in North-East India: an analysis of sequence types and associated resistance mechanisms.

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SYNOPSIS

Objectives: To determine the prevalence of *E. coli* sequence types and associated resistance mechanisms carried by the community in North-Eastern India.

Methods: *E. coli* (108) were isolated from sewage collected from 19 sites across the city of Silchar by plating on MacConkey agar with/without selection (50mg/L ceftotaxime). Species identification was confirmed by MALDI-TOF MS for 82 isolates. Common resistance mechanisms were determined by WGS of pooled *E. coli* isolates. PFGE combined with specific probes determined the presence of common resistance mechanisms in all isolates. Phylotype, MLST, cgMLST, resistance gene and virulence gene content were determined by in silico analysis of 38 genomes.

Results and conclusions: Analysis of isolates collected without selection (n=33) indicated that CTX resistance in *E. coli* was 42% (14/33) and estimated meropenem resistance at 9%. The remaining 58% (19/33) were additionally sensitive to ampicillin, trimethoprim, ciprofloxacin and aminoglycosides. The most common ST among the CTX resistant *E. coli's* was ST167 (29%) followed by ST410 (17%) and ST648 (10%). *E. coli* ST131 was absent from the collection. Sixty-three isolates were resistant to cefotaxime, and harboured *bla*$_{\text{CTX-M-15}}$, 54% (34/63) or *bla*$_{\text{CMY-42}}$, 46% (29/63) of which 10% (6/63) harbored both genes. Carbapenem resistance was due to *bla*$_{\text{NDM-5}}$, found in 10/63 CTX resistant isolates and/or *bla*$_{\text{OXA-181}}$ found in 4/63 isolates. NDM-5 was encoded by IncX3 and/or IncFII plasmids and CMY-42 was mostly encoded by IncI plasmids. NDM-5 appears to have replaced NDM-1 in this region and CMY-42 appears to be in the process of replacing CTX-M-15.

INTRODUCTION

Escherichia coli is universally carried in the human gut and is one of the most common bacterial pathogens causing a range of disease manifestations.[1] Importantly, the main source of infection is the patients' own digestive tract with the main site of infection the urinary tract. *E. coli* is the leading cause of urinary tract infections [2] and subsequent septicaemia's in many nations.[3, 4] *E. coli* has an "open genome" meaning that as a species it easily gains and loses genetic information.[5] Thus comparison of *E. coli* genomes has revealed that the core genome only consists of c. 1000 genes (1/5th of the genome of each isolate).[6] This diverse genetic make-up means that any individual isolate may be a serious pathogen or an innocuous commensal and therefore typing of common community carriage isolates is useful to understand the link between infection and prevalence of gut carriage of virulent and/or antibiotic resistant clones. Antibiotic resistance is also an important marker since increasing antibiotic resistance prevalence is associated with both increasing bacteraemia rates[3] and associated mortality due to delay in appropriate therapy.[7] Furthermore, rise in the prevalence of gut carriage of a virulent and antibiotic resistant strain of *E. coli* has a compounded effect.[3] This is important since European bacteraemia rates have substantially increased over the last 20 years, partially due to rising carriage rates of a virulent and often multi-drug resistant *E. coli* strain, ST131.[3] We have previously shown that common *E. coli* sequence types and resistance mechanisms can vary dramatically in different human communities.[8] To further understand this observation we sought to determine these in the city of Silchar, North East India.

Materials and Methods:

Collection of samples

Human sewage samples (30mL) were collected in January 2018 from 19 sites across the city of Silchar, Assam, India.

Bacterial Identification

Bacteria were pelleted by centrifugation and resuspended in 500µL LB broth. 50µL of several serial dilutions were then spread on MacConkey agar plates with and without 50mg/L cefotaxime. Up to 10 colonies with typical *E. coli* morphology were randomly collected from each site, 5 with and 5 without selection (55 and 53 with and without selection, respectively) of which 49 and 33 were confirmed as *E. coli* by MALDI-TOF MS.

Genomic DNA extraction

Genomic DNA was extracted using the Qiagen genomic DNA kit.

MiSeq sequencing

DNA libraries were prepared using the Nextera XT sample kit and sequenced at 30X coverage with a standard 2X 100 base protocol on a MiSeq instrument (illumina, San Diego, CA, USA).

Initial WGS analysis

Isolates were pooled into ten pools consisting of 8 isolates per pool and sequenced at 30X coverage by illumina Miseq to give an indication of the range of resistance mechanisms in the samples.

PFGE and specific probing

PFGE was performed as described previously.[9] Gels were probed directly using radio-labeled probes for bla_{NDM}, $bla_{\mathrm{CTX\text{-}M\text{-}15}}$, $bla_{\mathrm{CMY\text{-}42}}$, *qnrS1*.

ST and virulence gene detection

The MLST of sequenced strains was determined with StringMLST using short read data in FASTQ format and Ridom Seqsphere + (version 3.5.0) using assembled data in FASTA format.

CH typing (*fum*C/*fim*H) was used to indicate the MLST group of non-sequenced strains.[10] *E. coli* strains were clustered based on core genome MLST (cgMLST). Antimicrobial resistance genes were detected using CLC Biogenomic workbench. CH types, plasmid and virulence genes were determined online using CH typer, Plasmid finder and Virulence finder (http://www.genomicepidemiology.org/).

Phylogroup analysis

The *E. coli* phylotypes were determined with in-silico searches for *chuA*, *yjaA*, *tspE4C2*, *arpAgpE* and *tnpAgpC* using geneious software based on the Clermont method.[11, 12]

Results and discussion

We isolated 82 *E. coli* collected from 19 sites across the city of Silchar to determine the *E. coli* ST and associated resistance mechanisms carried by the local population. The 82 *E. coli* included 33 isolates collected without selection and 49 by selection on 50mg/L cefotaxime. Fifty-eight percent of isolates collected without selection (19/33) were cefotaxime susceptible and also susceptible to all tested antimicrobials including ciprofloxacin, trimethoprim and gentamicin. Sixty-three isolates were cefotaxime resistant including 14/33 collected without selection, thus 42% of carriage *E. coli* in Silchar are cefotaxime resistant. Twenty-percent (10/49) of cefotaxime resistant isolates were also resistant to carbapenems giving an estimate of carriage of carbapenem resistance in *E. coli* of 9% (42/100 X 10/49). Cefotaxime and carbapenem resistant isolates were found at 100% and 47% (9/19) of sample sites, respectively (Figure 1, Table S1). The total complement of resistance mechanisms was initially determined by MiSeq sequencing of ten pools of eight isolates at 30X coverage. This indicated a high prevalence of resistance genes *bla*$_{CTX-M-15}$, *bla*$_{CMY-42}$, *bla*$_{NDM-5}$ and *qnrS1* and allowed us to target their presence by PCR/sequencing and genomic location by in-gel radio-labeled probing of PFGE gels (Table S1). The most common gene conferring cefotaxime resistance was *bla*$_{CTX-M-}$

15 found in 34/63 cefotaxime resistant isolates followed by *bla*$_{CMY-42}$ (29 isolates) and *bla*$_{NDM-5}$ (10 isolates). One isolate contained all three ß-lactamases and six isolates contained both *bla*$_{CTX-M-15}$ and *bla*$_{CMY-42}$ genes, several isolates contained multiple ß-lactamase genes (Figure 1, Table S1). The *bla*$_{NDM-5}$ and *bla*$_{CMY-42}$ genes were found on plasmids ranging in size from 85-140kb and 25-130kb (Table S1), respectively. However, *bla*$_{CTX-M-15}$ and *qnrS1* genes were found mostly on the chromosome 66% (22/33) or on plasmids of 40-175kb. 38 individual isolates were further chosen for whole genome sequencing to determine the MLST types and resistance and virulence gene complements (Figure 1, Figure S1, Table S1). In addition, a further 10 isolates were typed by the Weissman 2 locus scheme. MLST was determined for 48 isolates (Figure 1, Figure S1, Table S1), the most prevalent being ST167, 29% (14/48), followed by ST410 17% (8/48), ST648 10% (5/48), ST224 (2/48), ST609 (2/48), ST973 (2/48), ST2083 (2/48) and single isolates of ST46, ST84, ST101, ST156, ST215, ST315, ST361, ST617, ST405, ST2521, ST3268 and ST4450 and one new ST (Figure 1, Table S1). ST167 was found at 9/19 sites and ST410 was found at 6/19 sites. Over half of all of isolates (56%) belonged to ST167, ST410 and ST648 (Figure 1). In a recent UK nationwide study of cefotaxime resistant *E. coli* isolated from human faeces (360 isolates), sewage (65 isolates) and bacteraemia's (293) [13, 14] the most prevalent *E. coli* were ST131 and ST38 (44%, 31% and 70% of all isolates from faeces, sewage and bacteraemia's, respectively). Interestingly, in this study both ST38 and ST131 were absent. Thus, the prevalence of *E. coli* ST carriage varies greatly by geographic location. In Silchar, the common sequence type ST167 was closely associated with *bla*$_{NDM-5}$, 50% (7/14) and both ST167 and ST410 with *bla*$_{CMY-42}$, 65% (9/14) and 100% (8/8), respectively (Figure 1). This high prevalence of *bla*$_{CMY-42}$ has not been documented before in India or elsewhere and appears to be in the process of replacing *bla*$_{CTX-M-15}$ at this location. This may be related to the wider spectrum of ß-lactam hydrolysis of CMY-42 as compared to CTX-M-15. Similarly *bla*$_{NDM-5}$ appears to have replaced *bla*$_{NDM-1}$ in Silchar as no isolates were detected with *bla*$_{NDM-1}$. Whilst

this may be a local phenomenon, it is interesting that many nations have reported increased detection of *bla*$_{NDM-5}$ over the last few years. [15, 16, 17, 18] Many isolates harboured other resistance mechanisms (Figure S1, Table S1) and chromosomal mutations conferring high level fluoroquinolone resistance making many strains MDR (Figure S1, Table S1). However, fosfomycin and chloramphenicol resistance was low (only 3 strains produced a full-length chloramphenicol resistance gene) suggesting that these antibiotics could be useful treatment options in this locale. Phylotype and virulence gene analysis indicated that most isolates belonged to non-pathogenic phylogroups A and B1, which included the majority of isolates carrying carbapenemase genes (ST167 and ST410). Six isolates belonged to the pathogenic phylotype D (ST268, ST315, STNEW, ST405, 2X ST 973, Figure S1) yet none belonged to the B2 group. Many of the isolates (20/48) including 2x ST648 and the majority of ST167 isolates were missing the type 1 fimbrial adhesion *fimH* which has been shown to be essential for colonizing the urinary tract. The cgMLST (Figure S1, Figure 2) agreed with the phylogroup analysis and demonstrated that all sequenced strains were unique confirming the random nature of the selection process. The within ST SNP analysis further demonstrated this and highlighted that differences within ST648 and ST167 were more numerous than within ST410, perhaps suggesting that the ST410 have expanded within the Silchar population in more recent history.

In conclusion, our survey has revealed that the majority of *E. coli* strains in this area are fully sensitive to antibiotics. However, 42% and 9% of isolates are cefotaxime and carbapenem resistant, respectively. We also found that the majority of resistant strains belonged to just three prevalent ST, which are different to the prevalent *E. coli* ST in Europe. Notably, *E. coli* ST131, the dominant cefotaxime resistant strain found throughout Europe and North America was absent from our study. Since UTI infections typically originate in the community and from the human gut, the survey of resistant human carriage isolates is a useful surveillance approach to quickly identify common ST and resistance mechanisms and guide local therapy.

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Transparancy declaration

All authors have none to declare.

References

1. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect* 2003; **5**: 449-56.
2. Schito GC, Naber KG, Botto H *et al.* The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 2009; **34**: 407-13.
3. de Kraker ME, Jarlier V, Monen JC *et al*. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin Microbiol Infect* 2013; **19**: 860-8.
4. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. *Clin Microbiol Infect* 2013; **19**: 492-500.
5. Lukjancenko O, Wassenaar TM, Ussery DW. Comparison of 61 sequenced *Escherichia coli* genomes. *Microb Ecol* 2010; **60**: 708-20.

6. Land M, Hauser L, Jun SR *et al.* Insights from 20 years of bacterial genome sequencing. *Funct Integr Genomics* 2015; **15**: 141-61.

7. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *J Antimicrob Chemother* 2007; **60**: 913-20.

8. Zahra R, Javeed S, Malala B *et al.* Analysis of *Escherichia coli* STs and resistance mechanisms in sewage from Islamabad, Pakistan indicates a difference in E. coli carriage types between South Asia and Europe. *J Antimicrob Chemother* 2018.

9. Toleman MA. Direct in Gel Genomic Detection of Antibiotic Resistance Genes in S1 Pulsed Field Electrophoresis Gels. *Methods Mol Biol* 2018; **1736**: 129-36.

10. Weissman SJ, Johnson JR, Tchesnokova V *et al.* High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. *Appl Environ Microbiol* 2012; **78**: 1353-60.

11. Clermont O, Christenson JK, Denamur E *et al.* The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep* 2013; **5**: 58-65.

12. Beghain J, Bridier-Nahmias A, Le Nagard H *et al.* ClermonTyping: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. *Microb Genom* 2018; **4**.

13. Livermore DM, Day M, Cleary P *et al.* OXA-1 beta-lactamase and non-susceptibility to penicillin/beta-lactamase inhibitor combinations among ESBL-producing *Escherichia coli. J Antimicrob Chemother* 2019; **74**: 326-33.

14. Sattar H, Toleman M, Nahid F *et al.* Co-existence of bla(NDM-1) and bla(KPC-2) in clinical isolates of *Klebsiella pneumoniae* from Pakistan. *Journal of Chemotherapy* 2016; **28**: 346-9.

15. Li X, Fu Y, Shen M *et al.* Dissemination of blaNDM-5 gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control* 2018; **7**: 59.

16. Huang Y, Yu X, Xie M *et al*. Widespread Dissemination of Carbapenem-Resistant *Escherichia coli* Sequence Type 167 Strains Harboring blaNDM-5 in Clinical Settings in China. *Antimicrob Agents Chemother* 2016; **60**: 4364-8.

17. Howard JC, Creighton J, Heffernan H *et al*. Evidence of transmission of an NDM-5-producing *Klebsiella pneumoniae* in a healthcare facility in New Zealand. *J Antimicrob Chemother* 2017; **72**: 949-51.

18. Reynolds ME, Phan HTT, George S *et al.* Occurrence and characterization of *Escherichia coli* ST410 co-harbouring blaNDM-5, blaCMY-42 and blaTEM-190 in a dog from the UK. *J Antimicrob Chemother* 2019.

Legend Figure 1

This figure appears in colour in the online version of JAC and in Black and hite in the printed version of JAC.

The association of ß-lactamase resistance mechanism with *E. coli* ST for 48 carriage isolates. The Inner ring corresponds to the percentage of cefotaxime resistant *E. coli* belonging to each sequence type. MLST was determined in silico for 38 isolates that had been whole genome sequenced (Strain ID and isolation site in bold) and a further ten isolates by CH typing (normal font). The outer ring indicates the ß-lactamase resistance mechanisms associated with each isolate. Isolate ID and isolation site is indicated outside the second ring. Isolates coloured yellow and green belong to commensal phylotype A. Isolates coloured white-blue belong to phylotype B1 and isolates coloured orange to red belong to pathogenic phylogroups F and D.

Legend figure S1

This figure appears in colour in the online version of JAC and in Black and hite in the printed version of JAC.

Core genome analysis of sequenced isolates (38) with detail of non-ß-lactam resistance mechanisms. Chromosomal mutations in gyrase A and parC genes rthat result in amino acid substitutions known to confer ciprofloxacin resistance in *E. coli* are given in the quinolone column along with acquired genes that also confer quinolone resistance. Virulence genes and *fim*H types as well as phylogroup are given for each strain. Isolates coloured yellow and green belong to commensal phylotype A. Isolates coloured white-blue belong to phylotype B1 and isolates coloured orange to red belong to pathogenic phylogroups F and D.

Legend figure 2

This figure appears in colour in the online version of JAC and in Black and hite in the printed version of JAC.

SNP variation found among and between sequence types. Isolates belonging to the same sequence type are highlighted as clusters 1-6. The number of SNP's found between members of the same sequence types and between sequence types are given in bold adjacent to each isolate pair. Cluster 2 ST410 isolates shared the least within ST SNP variation. Colours represent different ST groups as in other figures.

Table S1

Table gives ST and CH data of all 63 cefotaxime resistant *E. coli* isolates collected in this study together with entire resistance gene complements and plasmid size, incompatibility group and genomic location of the most prevalent resistance genes. Whole genome sequenced isolates are highlighted in bold. Isolates with *bla*$_{CMY}$ genes other than *bla*$_{CMY-42}$ are highlighted with an asterix in the table ie DJ-95 produces *bla*$_{CMY-145}$ and isolate DJ58 produces *bla*$_{CMY-4}$. The genomic location of resistance genes is highlighted by plasmid size and chromosomal location of CTX-M-

15 genes by c. Selection or non selection is indicated by CTX (50mg/L cefotaxime) or NS, respectively.