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High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK

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Objectives: To determine the proportion of *E. coli* carrying specific CTX-M extended-spectrum β-lactamase (ESBL) genotypes in a community population of East and North Birmingham.

Methods: General practice and outpatient stool samples from 732 individuals submitted for examination for faecal pathogens in 2010 were screened for ESBL-producing *E. coli* using chromogenic agar. Multiplex PCR, denaturing HPLC, DNA sequencing and PFGE were used to determine the CTX-M genotype and clonal subtype. Isolates from people were assigned to 'Europe', 'Middle East/South Asia' (MESA) or 'uncategorized' groups using software to determine probable global origin based on the subject's full name.

Results: Prevalence of CTX-M carriage in the sample population was 11.3%. There was a statistically significant difference ($P<0.001$) between carriage in the Europe group (8.1%) and the MESA group (22.8%). There was also a higher rate of carriage of CTX-M-15-producing *E. coli* ($P<0.001$) in MESA subjects.

Conclusions: The high community carriage rate and the significant difference in carriage between the Europe and MESA subjects may have important consequences for therapy. If the rising trend in carriage of bacteria producing ESBLs continues, guidelines for empirical therapy for patients presenting from the community may need to be modified. The findings also raise the concern that the pattern and routes of spread of CTX-M-15 may be replicated in the future by broader-spectrum β-lactamases, such as New Delhi metallo-β-lactamase ('NDM-1').

Keywords: CTX-M-15, ST131, ethnicity, clonal spread

Introduction

The emergence and global spread of multi-antibiotic-resistant New Delhi metallo-β-lactamase (NDM-1)-producing *Escherichia coli* from the Indian subcontinent via medical tourism and travel threatens to undermine modern medical care across the globe.[1] The prevalence of suspected extended-spectrum β-lactamase (ESBL)-producing invasive *E. coli* infections, in particular bacteraemias, has been rising over the last decade in the UK: it was 2.4% in 2003 and 8.8% in 2008.[2] Over the last 6 years, CTX-M (especially CTX-M-15) β-lactamase-producing *E. coli* have increased rapidly in number both within and outside the hospital environment and make up the vast majority of isolates.[2,3] CTX-M β-lactamases have been shown to be currently the most common cause of multidrug resistance in *E. coli* from other areas of the world, in particular the Far East and South-East Asia, where rates can be as high as 50%–70%,[4] and are a growing problem in some parts of Europe.[5] No definitive studies have been done to determine the prevalence of faecal carriage of CTX-M β-lactamase-producing *E. coli* in the UK population; however, there has been a regional prevalence study in York based on samples from patients with diarrhoea, which estimated the local carriage rate at ~2% in 2003.[6]

Recently, a study from Canada has shown that infections with particular genotypes of CTX-M β-lactamases are associated with recent travel to high-prevalence countries, specifically CTX-M-15 with travel to the Indian subcontinent and CTX-M-14 and -24 with travel to Asia.[7] Birmingham has a diverse population, including communities from Eastern Europe, the Indian subcontinent and Asia. As part of our public health surveillance role, the HPA laboratory undertook an anonymous point prevalence survey of the incidence of CTX-M ESBLs in *E. coli* in community faeces specimens submitted from patients with suspected gastrointestinal disease. The aim of this study was to look at the prevalence of

CTX-M genotypes in *E. coli* and determine whether there are any particular communities with higher prevalence. A secondary aim was to determine the prevalence of the global *E. coli* clone ST131 (where ST stands for sequence type), which has been associated with the carriage of ESBL CTX-M-15 and quinolone resistance.[8]

Methods

Bacterial isolates and analysis

All faeces samples submitted for routine microscopy, culture and susceptibility testing to Birmingham Heartlands Hospital Enterics Laboratory over a period of 4 weeks in 2010 were reviewed. The catchment area of this laboratory consists of East Birmingham (Erdington, Bordesley Green, Hodge Hill, Yardley, Hall Green, Moseley, Kings Heath, Acocks Green and Sheldon) and the towns of Sutton Coldfield and Solihull, with a population of 1.1 million. Samples from inpatients and those with a positive result for a viral/bacterial pathogen were not included. A total of 732 outpatient and general practice samples were selected for further analysis. The study design is summarized in Figure 1.

Faeces samples were directly plated onto a chromogenic ESBL selective medium, chromID (bioMérieux, Basingstoke, UK) and incubated at 37°C in air for 24 h. Colonies from the chromogenic media that were consistent with ESBL-producing *E. coli* were selected for further identification with API 20E (bioMérieux) and ESBL testing with a BSAC combination disc method.[9]

The criteria for selection of isolates for PCR analysis were a community faecal isolate positive for ESBL detected by chromogenic agar and combination disc testing and identified as *E. coli* by API 20E. These isolates were then analysed to detect CTX-M β-lactamases using a multiplex PCR.[10] The bla_{CTX-M} genotype was determined using denaturing HPLC (dHPLC) and full sequencing to determine the exact CTX-M genotype of the isolate.[11]

PFGE was performed using XbaI digestion.[12] PFGE was carried out on all CTX-M-15-producing isolates. Profiles were compared digitally using Bionumerics™ software (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis of Dice similarity indices based on an unweighted pair group method with arithmetic mean (UPGMA) was used to generate a dendrogram describing the relationships among PFGE profiles. Isolates were considered to belong to the same PFGE group if their Dice similarity index was ≥85%.

One isolate in each cluster defined by 85% similarity level was further analysed by multilocus sequence typing (MLST) carried out as previously described,[13] using the University College Cork MLST database.[14] Six isolates falling outside the PFGE clusters were selected for MLST to give an insight as to the variety of clones within the dataset. All the isolates were also analysed with a rapid O25b-ST131 PCR screen.[12]

Determination of global origin and statistical analysis

The isolates were analysed and grouped into categories according to the global origin of the name of the patient from which they were

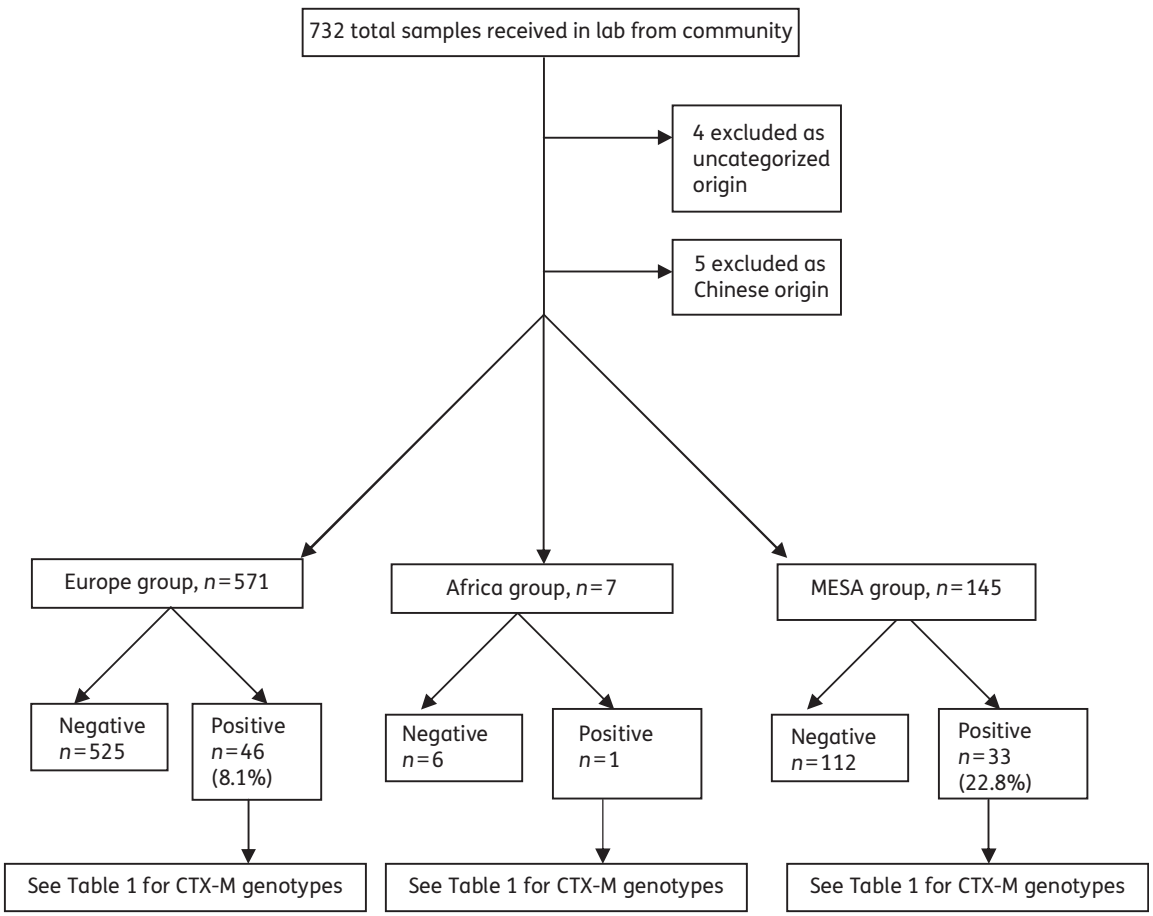

Figure 1. Study design.

taken. This information was obtained using publicly available software, OriginsInfo.[15] The principles behind this software are described elsewhere,[16] as is its use in infectious disease epidemiology.[17] In this study we used OriginsInfo as an epidemiological tool for study at the population level, not for the investigation of individual patients. The isolates were grouped into three broad categories: Europe ($n=571$), Middle East/South Asia (MESA; $n=145$) and Africa ($n=7$). A decision was made not to have more categories (e.g. China), as these numbers were even smaller ($n=5$) and so were excluded from the analysis. There were a small number of patients ($n=4$) whose global origins could not be determined by the software used or placed in the three groups. These 'uncategorized' samples were also excluded from the study, leaving a total of 723 samples. Statistical analysis of the data was performed using Fisher's exact test. Ethics approval for this project was gained from the local research ethics committee, reference no. 10/H1207/33.

Results

Of the 723 samples screened, 80 were identified as ESBL-producing *E. coli* and were genotyped. Only CTX-M group 1 and CTX-M group 9 were found on the initial multiplex PCR. The CTX-M genotype distribution is shown in Table 1. The frequency of CTX-M carriage was 11.3% for the 723 samples. CTX-M-15 was the dominant genotype (8.0%) and accounted for 72.5% ($n=58$) of all positive samples. Analysis by PCR showed that 14 (24.1%) of the 58 CTX-M-15-positive isolates belonged to the global clone ST131. The only other ST131 isolate carried the CTX-M-14 gene and was in the MESA group. There was a significantly higher proportion of CTX-M-carrying isolates from the MESA group compared with the Europe group ($P<0.001$). In addition, there was also a significantly higher proportion of carriage of CTX-M-15 in the MESA group ($P<0.001$). The numbers within the other genotypes were too small to apply statistical analysis, as were the numbers within the Africa group.

Banding patterns were obtained for 58 of the 59 CTX-M-15-producing *E. coli* (Figure 2); eight PFGE clusters with a similarity index of ≥85% were identified. Unique PFGE profiles were seen in 41 isolates. The 14 isolates identified as O25b-ST131 formed one large group at the 65% similarity level, clustering with the local UK epidemic O25b-ST131 strain A. One isolate (F024) was assigned a new ST (ST2076) and one isolate (F104) was untypeable by MLST.

Discussion

The results from this study show that there is a significant difference in faecal carriage of ESBL-producing *E. coli* between our two study groups. Overall, the point prevalence rate of 11.3% ($n=80$) of community samples carrying an ESBL-producing *E. coli* is far higher than that in the previous regional UK study.[6] Global origin determination from patients' names has been applied in other fields of healthcare. For example, it has been used to enhance cluster studies in tuberculosis by investigating potential cultural links for transmission.[17] There are also studies recognizing the potential risk that increasing travel abroad poses to the acquisition of antimicrobial-resistant bacteria.[7,18–20] One prospective study from Sweden looking at gut colonization found an ESBL-producing *E. coli* acquisition rate of 24% in travellers who had been screened before departure.[19] Another study[18] looked at ESBL-producing Enterobacteriaceae carriage in patients who had travelled abroad and submitted stool samples for diagnosis of traveller's diarrhoea on their return. A total carriage rate of 24% was determined, and a distinction was made between those who had travelled to Europe (carriage rate 3%) and those who had travelled outside Europe (36%).

The rapid spread of the genotype CTX-M-15 has been the subject of worldwide concern in the microbiology community.[21,22] The genotype was originally described in six isolates from one hospital in Delhi, India.[23] Subsequently, a genotyping survey of 130 cefotaxime-resistant *E. coli* and *Klebsiella* species from three widely dispersed locations in India showed it to be the only CTX-M genotype found, constituting 73% of the isolates.[24] In particular, the uropathogenic ST131 clone is known to carry CTX-M-15 frequently and has been successful in spreading across the world in a relatively short period of time.[8,25] The overall rate of ST131 clones in our CTX-M-15-positive population was 24.1% ($n=14$), which is similar to the rate reported in 2007 for 127 ST131/CTX-M-15 isolates identified in a collection of 1596 non-duplicate *E. coli* isolates from 33 widely dispersed US medical centres.[26] Although urinary tract infections caused by *E. coli* usually arise from prior faecal colonization with uropathogenic strains, rates of faecal carriage of the ST131 clone are largely unknown and comparison with studies using clinical isolates may not therefore be valid. However, as our study is the only contemporary UK survey of ESBL faecal carriage in a

Table 1. Distribution of CTX-M genotypes according to global origin

Global origin	$bla_{\text{CTX-M}}$ PCR	Both CTX-M-9 and CTX-M-15	CTX-M-1	CTX-M-9	CTX-M-14	CTX-M-15		
						total	ST131	other ST
Europe ($n=571$)	46 (8.1)[a]	1 (0.18)	1 (0.18)	9 (1.6)	4 (0.7)	31 (5.4)[b]	8	23
MESA ($n=145$)	33 (22.8)[a]	0	2 (1.4)	4 (2.8)	1 (0.69)[c]	26 (17.9)[b]	5	21
Africa ($n=7$)	1	0	0	0	0	1	1	0
Total ($n=723$)	80 (11.1)	1	3	13	5	58 (8.0)	14	44

Values in parentheses indicate percentages of each genotype with respect to global origin or total.
[a]$P<0.001$, significant difference between proportions.
[b]$P<0.001$, significant difference between proportions.
[c]This isolate belonged to the ST131 clonal subtype.

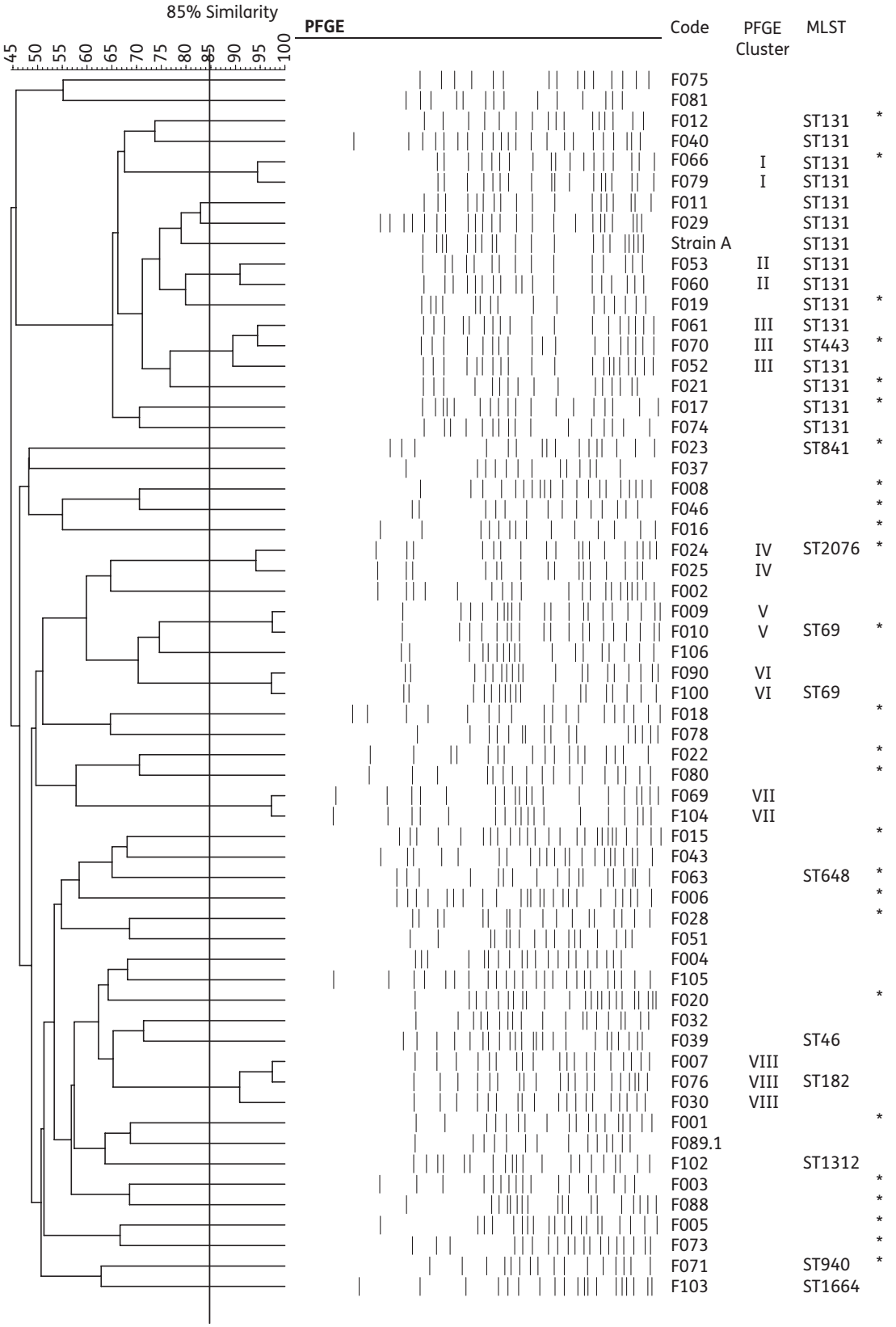

Figure 2. XbaI-PFGE dendrogram for 58 *E. coli* isolates harbouring the $bla_{CTX\text{-}M\text{-}15}$ gene. The dendrogram, as produced by the UPGMA algorithm on Dice similarity coefficients, included eight PFGE clusters and 41 unique PFGE profiles based on ≥85% similarity. MLST identified one large ST131 cluster at the 65% similarity level. Isolates with an asterisk belong to the MESA group; those without an asterisk belong to the Europe group. F079 belongs to the Africa group.

non-hospitalized population, the only comparison that can be made is with clinically significant isolates. Interestingly, the ST131 clone rate from the USA is much lower than rates reported from more limited recent surveys in the UK (one from clinical isolates in the West Midlands[12] and one concerning faecal carriage[27]) and elsewhere, e.g. Canada.[28] One of the UK studies[27] looked at faecal carriage amongst nursing home residents only in Belfast, Northern Ireland, and the high rate (40.5%) may reflect cross colonization in a high-risk population. The reason for this disparity in UK prevalence may be due to the local population—there is a significant community originating from the Middle East and Asian subcontinent in and around Birmingham, and there is frequent travel to countries of origin, where the incidence of CTX-M-15-producing *E. coli* ST131 is not known and may be lower. This may have resulted in a skewed distribution of clones that does not necessarily represent the rest of the UK. Also, these other studies have tended to look at clinical isolates (usually urinary) causing disease rather than faecal carriage. ESBL-producing organisms are known to easily spread between healthy household contacts,[29] and this may well have contributed to the high rates in MESA subjects when one or more family members have travelled to the MESA. We acknowledge that there are a number of limitations to our study: (i) we recognize that the use of the surname to categorize according to global origin is only an approximation—there may be errors, e.g. married names and name changes; (ii) the sample population was limited to patients in East/North Birmingham who had faecal samples sent for investigation—the results may not be applicable to wider populations; and (iii) due to restrictions imposed by the local research ethics committee, we were unable to collect detailed case information on postcode, recent foreign travel and social gatherings amongst the subjects, and other risk factors, such as recent hospitalization and antibiotic exposure. There is an urgent need for a more detailed study to address these issues and elucidate the movement of ESBLs in family groups, expanding the findings of our preliminary study. The issue of increasing antimicrobial resistance worldwide has been recently raised with a study looking at NDM-1 in Enterobacteriaceae in India and the UK.[30] It is notable that CTX-M-15, which is currently the most dominant ESBL genotype in Western Europe[31]—possibly originating in India, where it is very common[24]—might be a paradigm for the spread of the currently much rarer NDM-1 carbapenemase.[32]

In conclusion, we have found that there is a significantly higher rate of faecal carriage of bacteria producing ESBLs in individuals in the community originating from the MESA. The most prevalent CTX-M genotype was CTX-M-15 in the entire sample population, which was again of significantly higher proportion in those same communities, consistent with other studies worldwide. Our study provides evidence of significant faecal ESBL carriage in the community and raises concern that, in the not too distant future, empirical therapy of patients presenting from the community with Gram-negative sepsis may need to be modified to account for ESBL-producing organisms.[33] Most importantly, our findings corroborate suggestions that the spread of antibiotic resistance and long-term carriage in the community is related to increasing population exchange between Europe and the MESA. The clinical implications of this, considering recently discovered enzymes such as NDM-1, are extremely worrisome.

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Transparency declarations

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Author contributions

N. H. W. and T. S. identified faeces samples and undertook culture and bacterial identification. L. X., A. E. and S. S. carried out PCR analysis, PFGE and antimicrobial susceptibility testing. L. X. and A. E. undertook origin assignment, dHPLC genotyping and DNA sequencing. P. M. H. designed the study, was responsible for data interpretation and, with N. W. and L. X., prepared the manuscript.

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