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RESEARCH ARTICLE

Molecular epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli*

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ABSTRACT

Objectives: *E. coli* O25b-ST131 has disseminated worldwide in hospitals and the community. The objective of this study was to determine the extent to which *E. coli* O25b-ST131 accounts for extended-spectrum beta-lactamase (ESBL)-producing *E. coli* from clinical samples from all sources in this region.

Methods: Between January and June 2010 ESBL-producing *E. coli* were collected from 94 routine samples including 47 from residents of 25 nursing homes, 15 categorized as hospital acquired and 32 others. PCR was performed for detection of bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, bla_{SHV} and for the identification of members of the *E. coli* O25b:ST131 clonal group. PFGE was carried out using *Xba*I in accordance with PulseNet protocols.

Results: The majority (97%) of isolates harbored a bla_{CTX-M} gene. *E. coli* O25b-ST131 accounted for 87% of all ESBL-producing *E. coli* and for 96% of isolates from nursing home residents.

Conclusion: The *E. coli* O25b-ST131 clonal group predominated in the collection of ESBL-producing *E. coli*, particularly in nursing home isolates. *J Microbiol Infect Dis 2014; 4(3): 92-96*

Key words: *Escherichia coli*, O25b-ST131, Resistance, Healthcare, Nursing home

Genişlemiş spektrumlu beta-laktamaz üreten *E. coli* suşlarının moleküler epidemiyolojisi

ÖZET

Amaç: *Escherichia coli* O25b-ST131 dünyanın her tarafında hastanelerde ve toplumda yaygındır. Bu çalışmanın amacı bu bölgedeki farklı kaynaklardan alınan klinik örneklerde genişlemiş spektrumlu beta-laktamaz üreten *E. coli* suşları arasında *E. coli* O25b-ST131 yaygınlığını belirlemektir.

Yöntemler: Ocak-Haziran 2010 arasında toplam olarak 25 bakımevinden 47 suş, hastane kaynaklı 15 suş ve diğer kaynaklardan 32 suş olmak üzere toplam 94 ESBL üreten *E. coli* suşu klinik örneklerden toplandı. PCR kullanılarak bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, bla_{SHV} ve O25b:ST131 klonal grubu üyeleri tanımlandı. PFGE tiplendirmesi PulseNet protokolüne göre *Xba*I kullanılarak yapıldı.

Bulgular: Suşların büyük çoğunluğu (% 97) bla_{CTX-M} geni taşımaktaydı. Bütün ESBL üreten *E. coli* suşlarından % 87'si ve bakımevlerinden gelen suşlardan % 96'sı *E. coli* O25b-ST131 idi.

Sonuç: *E. coli* O25b-ST131 klonal grubu bakımevi kaynaklı olanlarda daha belirgin olmak üzere toplanan ESBL üreten *E. coli* suşları arasında baskındı.

Anahtar kelimeler: *Escherichia coli*, O25b-ST131, Direnç, Sağlık hizmeti, Bakımevi

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INTRODUCTION

The *E. coli* O25b:ST131 clonal group was first reported in 2008 as a major carrier of CTX-M-15 however subsequent studies have identified the presence of this clonal group in earlier isolates dating back to 1967.[1] Since then it has been reported globally in both the hospital and community setting and is associated primarily with urinary tract infections and bacteraemia. Reported risk factors for *E. coli* O25b-ST131 include foreign travel, antimicrobial usage, catheterisation and admission to a nursing home.[2-4] *E. coli* O25b-ST131 are often resistant to fluoroquinolones and cephalosporins such as ceftazidime.[1] The dissemination of $bla_{CTX\text{-}M\text{-}15}$ is closely associated with *E. coli* O25b-ST131 however $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}32}$ have also been observed. In addition, a broad range of other antimicrobial resistance genes ($bla_{OXA\text{-}1}$, bla_{TEM}, *tetA*, *aac(6')-Ib-cr* and aac(3)-II) primarily carried on transferable IncF plasmids are also associated with the group.[5]

An increasing population of older people resides in nursing homes for extended periods. Nursing homes are associated with a significant number of antimicrobial-resistant (AMR) bacteria related to frequent transfer of patients from hospitals and also to factors within the nursing homes including the vulnerability of residents, and antimicrobial consumption.[6] The relative importance of acquisition of AMR bacteria during hospital stay compared with dissemination within nursing homes is not well understood. We have examined the diversity of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolates from all clinical samples submitted to one laboratory over 6 consecutive months to compare isolates from nursing home residents with other isolates.

METHODS

Galway University Hospitals [University Hospital Galway (UHG) and Merlin Park University Hospital (MPUH)] and Roscommon County Hospital (RCH) account for an approximate combined total of 1,052 inpatient beds and about 44 000 admissions per year. The Clinical Microbiology Laboratory service at UHG serves these hospitals and the nursing homes and General Practitioners (GP) throughout the region serving a population of approximately 445 356 (http://www.cso.ie).

Between January 1st and June 30th 2010, approximately 3 599 clinical isolates of *E. coli* were reported by the Laboratory with urine, blood, wound and respiratory samples accounting for approximately 3337, 82, 74 and 66 isolates, respectively. All *E. coli* isolates are tested for susceptibility to cefpodoxime (Oxoid Ltd, UK) and cefpodoxime non-susceptible isolates were initially assessed for ESBL production using cefpodoxime/clavulanic acid discs in accordance with Clinical Laboratory Science Institute (CLSI) methods and criteria. Ninety four ESBL-producing *E. coli* were collected from urine (n=80), blood (n=3), sputum (n=9), drainage fluid (n=1), and tissue (n=1).

Forty seven ESBL-producing *E. coli* isolates were from residents of 25 nursing homes (21 private and 4 public; NH=47), and 47 were non-nursing home isolates. Of the 25 nursing homes, 18 were represented by a single isolate, 2 by 2 isolates and the remaining five nursing homes by 3 or more isolates. The number of beds in each nursing home varied from 28 to 62. The nursing home samples were submitted in the course of routine investigation of clinical episodes of suspected infection and the interval between admission to the nursing home and date of sample collection was not available. Fifteen ESBL-producing *E. coli* were categorized as likely hospital associated (HA) on the basis that the clinical samples were collected more than 48 hours after admission (range 3-79 days). Of the remaining 32 isolates of ESBL-producing *E. coli*, 4 were from General Practitioners and 28 were from hospitalized patients but with samples collected (a) at or within 48 hours of admission (n= 16) or (b) submitted from a hospital but with interval from admission to sampling not accessible (n=12). For purposes of this study these 32 isolates are classified as "others". Thirty of the 32 "others" had previous hospital contact (out-patient and in-patient). There was no record of nursing home admission for those patients hospitalized at time of sampling.

The *E. coli* ST131 clonal group was identified by PCR specific to the detection of the *pabB* gene (specific marker the *E. coli* O25b-ST131 clonal group) and the *trpA* gene (to act as a control and to ensure quality of DNA).[7] All ESBL isolates were also tested for bla_{TEM}, bla_{SHV}, $bla_{OXA\text{-}1}$ and $bla_{CTX\text{-}M}$ as previously described and representative bla genes were sequenced using primers specific to the entire coding region of $bla_{CTX\text{-}M\text{–group 1}}$, bla_{TEM} and bla_{SHV} (Sequiserve, Vaterstetten, Germany).[8,9] Pulsed field gel electrophoresis (PFGE) using *Xba*I was performed by the Pulse-Net protocol with analysis of profiles (PFPs) using the Dice coefficient with clustering by the unweighted pair group method with arithmetic averaging (UPGMA).[10] Three major UK ST131

variants (Strains A, C and D) were included in the PFGE analysis for comparison.[5] Chi Square tests were applied to compare demographic and clinical data from the hospital and nursing homes collections.

RESULTS

Table 1 shows results for ST131 status, bla_{CTX-M} genes, bla_{OXA-1}, bla_{TEM} and bla_{SHV} gene detected in the 3 categories of isolates. All isolates were confirmed as carrying one or more ESBL encoding genes. The ST131 clonal group accounted for 82 of the 94 isolates with bla_{CTX-M} in 81 of 82 ($bla_{CTX-M-group1}$ n=73; $bla_{CTX-M-group\ 9}$ n=8) and bla_{TEM-10} in the remaining isolate. Sequencing of a $bla_{CTX-M-group1}$ gene from an ST131 isolate showed $bla_{CTX-M-15}$ while the $bla_{CTX-M-group1}$ gene in a non ST131 isolate was $bla_{CTX-M-61}$. One NH *E. coli* harbored bla_{SHV-2}, $bla_{CTX-M-group\ 1}$, bla_{OXA-1} and bla_{TEM}. A higher percentage of nursing home isolates were ST131 (96%) compared with hospitals associated (87%) and "other" ESBL-producing *E. coli* (75%) although the differences did not reach statistical significance (Table 1). Among the 12 non-ST131 bla_{CTX-M} was also predominant (n=10) with the remaining isolates being bla_{SHV-2} (n=1) in one case and both bla_{SHV-12} and bla_{TEM} in the other.

Table 1. Characteristics of nursing home, hospital acquired and other ESBL-producing *E. coli* isolates detected from all sample types submitted over a 6 month period.

Isolate Characteristics	Nursing Home, *n*=47 (%)	Hospital, *n*=15 (%)	Others, *n*=32 (%)	Total Isolates, n=94 (%)
ST131	45 (96)	13 (87)	24 (75)	82 (87)
bla_{CTX-M}	47 (100)	15 (100)	29 (91)	91 (97)
$bla_{CTX-M\ Group\ 1}$	42 (89)	12 (80)	26 (81)	80 (85)
$bla_{CTX-M\ Group\ 9}$	5 (11)	3 (20)	3 (9)	11 (12)
bla_{OXA-1}	39 (83)	12 (80)	20 (63)	71 (76)
bla_{TEM}	20 (43)	4 (27)	8 (25)	32 (34)
bla_{SHV}	1 (2)	0 (0)	2 (6)	3 (3)

ST=sequence type; bla=beta-lactamase

Table 2. Molecular typing of ESBL-producing *E. coli* and source of isolates.

MLST Type [a]	PFGE Grouping [b]	Nursing home, n (%)	Hospital, n (%)	Other n (%)	Total, n (%)
ST131	X [c]	19 (41%)	9 (60%)	17 (53%)	45 (47%)
ST131	Y [c]	9 (19%)	3 (20%)	1 (3%)	13 (14%)
ST131	Z [c]	10 (21%)	1 (7%)	2 (6%)	13 (14%)
ST131	Diverse [a d]	7 (15%)	0 (0%)	4 (13%)	11 (12%)
Non ST131	Diverse [ad]	2 (4%)	2 (13%)	8 (25%)	12 (13%)

a MLST= Multilocus sequence typing, inferred from PCR specific assay
b PFGE= Pulsed Field Gel Electrophoresis
c X, Y, Z= Represent randomly assigned Pulsed Field Gel Electrophoresis clusters based on based on a similarity of > 85%
Diverse [ad] = Represents isolates not in clusters X, Y and Z based on < 85%

PFGE analysis identified 65 pulsed field profiles (PFPs) within the 94 isolates and the reference Strain A. PFP's of the *E. coli* ST131 were > 78% similar. The ST131 isolates formed 3 clusters X to Z, based on a similarity of > 85%. Cluster X included the pandemic UK Strain A and 45 local isolates (NH n=19; OT=17; HA n=9; UK Strain A control n=1). Cluster Y comprised mainly nursing home isolates (NH n=9, HP n=3; OT=1). Most cluster Z isolates (NH n=10; OT=2; HP n=1) were from nursing homes and in particular one nursing home provided 7 (54%) of all cluster Z isolates (Table 2).

The two non-ST131 nursing home isolates were from residents of the same nursing home and were indistinguishable by PFGE, and one of these was confirmed by sequencing to harbor $bla_{CTX\text{-}M\text{-}61}$. The 10 non-ST131 isolates from 2 hospital patients and 8 from the OT category were all quite distinct (61% similar) on PFGE and were 58% similar to the group of ST131 isolates.

DISCUSSION

The association of AMR with nursing homes is well described however the relative importance of dissemination within nursing homes compared with acquisition during episodes of care for nursing home residents at acute hospitals is not well understood. Rooney et al (2009) reported that CTX-M - producing *E. coli* O25b-ST131 accounted for 49% of ESBL-producing *E. coli* from nursing homes in Northern Ireland.[3] Burke et al. (2012) reported *E. coli* O25b-ST131 represented 85% and 51% respectively of nursing home and hospital inpatient ESBL-producing *E. coli* isolated from Dublin, Ireland.[6] *E. coli* O25b-ST131 was reported to account for 21%, 43% and 41% of ESBL-producing *E. coli* isolates from Japan, South Africa and Canada, respectively.[4] In addition, *E. coli* O25b-ST131 was identified in 27% of isolates in the United States; however *E. coli* O25b-ST131 was detected in 76% of isolates from long-term care facilities in this region of the United States.[2] Our data confirm other reports of global dissemination of $bla_{CTX\text{-}M\text{-}15}$ carrying *E. coli* O25b-ST131. Our study also demonstrates the striking predominance of this clonal group in this region of Ireland. Even by comparison with Burke and colleagues the predominance we observe (87% of all ESBL-producing *E. coli*) in this collection of consecutive clinical isolates from all sources is exceptionally high. In 2012 ESBL-producing *E. coli* accounted for 8.8% of all *E. coli* blood stream infections in Ireland as a whole. In comparison, ESBL-producing *E. coli* accounted for approximately 21.5% of *E. coli* (20-23%) blood stream infections for most hospitals in the West of Ireland where this study was performed (S. Murchan, Health Protection Surveillance Centre, personal communication, January 8, 2013). It is possible that there are differences between regions of Ireland with respect to case ascertainment related to differences indications for performing blood cultures and or in laboratory protocols for processing blood cultures and susceptibility testing. However we are aware of no systematic regional bias with respect to any of these factors therefore the differences are not readily explained as ascertainment bias. There are also some differences in population profile (higher mean age in the region with higher proportion of ESBL-producing *E. coli*) though it is not clear that this is sufficient to explain the difference.[11] It is interesting to speculate if the prevalence of a virulent clonal group in this region may contribute to the high incidence of ESBL-producing *E. coli* blood stream infection.

In conclusion this study demonstrates that a single clonal group, *E. coli* O25b-ST131 accounts for the vast majority of ESBL-producing *E. coli* in the West of Ireland, with a particular predominance (96%) in nursing home isolates. The ST131 variant designated as UK strain A represents almost half of all ESBL-producing *E. coli* from all sources. A unique aspect of this study is the comparison of the homogeneity of ESBL-producing *E. coli* in consecutive isolates from nursing home and other settings in the same region during a defined time period. Although this study is not sufficient to form firm conclusions we suggest that there is a trend towards greater homogeneity of ESBL-producing *E. coli* within the nursing home isolates. Given the growing nursing home population in developed countries and the practical challenges of implementing effective measures to control dissemination of AMR bacteria in nursing homes more comprehensive studies comparing nursing home isolates with non-nursing home isolates are required.

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