Prevalence, antibiotic sensitivity pattern and genetic analysis of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella* spp among patients with community acquired urinary tract infection in Galle district, Sri Lanka

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Abstract

Introduction Community acquired urinary tract infections (CA-UTI) are commonly caused by *Escherichia coli* and *Klebsiella* spp which are known extended spectrum beta lactamase (ESBL) producers.

Objectives To determine, the prevalence and characteristics of ESBL producing of *E. coli* and *Klebsiella* spp in the community, and the association of risk factors with ESBL CA-UTI.

Methods Descriptive cross-sectional study with urine cultures performed from clinically suspected CA-UTI patients by CLSI standards. Conventional multiplex PCR was performed for gene analysis.

Results Cultures were positive in 178 (38%) patients from 465. Majority were females (103, 58%). Most frequently isolated was *E. coli* (149, 84%) with 68 (46%) ESBL producers followed by 16 (9%) *Klebsiella pneumoniae* with 4 (25%) ESBL producers. Majority of patients with ESBL CA-UTI were >50 yrs (35/72, 49%) and 13 (18%) children <10 years were present. ABST of ESBL producers revealed high resistance rates for quinolones (41%) and >80% sensitivity for nitrofurantoin, fosfomycin, mecillinam, aminoglycosides and carbapenems. Presence of ESBL genes were 83% CTX-M, 71% OXA, 24% TEM and 9% SHV with one organism often producing more than one gene in 29 isolates (71%). Haematuria and structural abnormalities of urinary tract were significantly associated with increased ESBL CA-UTI (p<0.01).

Conclusions ESBL prevalence of this community was 40% in CA-UTI with *E.coli* predominance among female majority. >80% ESBL organisms show high sensitivity for aminoglycosides, carbapenems, nitrofurantoin, mecillinam and fosfomycin. Frequently isolated ESBL gene was CTX-M. Haematuria and structural abnormalities of urinary tract were significantly associated with ESBL CA-UTI.

Introduction

Extended beta lactamase (ESBL) producing organisms were identified in 1960 due to plasmid mediated antibiotic resistance (CTX-1 gene). Since then several other genes causing ESBL resistance were identified such as CTX-M, SHV, TEM, VEB, and PER. ESBLs render resistance to penicillin, cephalosporins and monobactams. Unlike most of such genes, CTX-M type is known to be linked with multi-drug resistance which includes aminoglycosides, quinolones and cotrimoxazole [1] as well.

ESBLs are basically produced by the enterobacteriaceae family; in particular *Klebsiella pneumoniae* and *Escherichia coli* [2]. Other enterobacteriaceae and non-fermenting bacteria like *Acinetobacter* and *Pseudomonas* also produce ESBL [3] but prevalence is low. Most of the urinary-tract infections (UTI) are due to *Escherichia coli* and *Klebsiella* spp and studying community acquired (CA) UTI is more practical with non-invasive urine cultures comparatively. And these results often reflect antibiotic sensitivity pattern in the community setting.

There had been similar studies in the past. However, many previous studies on risk factors for ESBL were based on the samples received in the routine culture laboratory. Information were often taken from the request forms or bed head tickets are highly unsatisfactory when consi-

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dering all potential risk factors for community acquired ESBL infections.

Also, according to Clinical and Laboratory Standards Institute (CLSI 2017 M-1009), ESBL screening in the routine cultures is not recommended, but if done for epidemiological purpose ESBL screening can be done with maximum results with 5 antibiotics such as cefpodoxime10μg, ceftazidime 30 μg, astreonam 30 μg, cefotaxime 30 μg, and ceftriaxone 30 μg. If the screening is positive, confirmatory tests are carried out but not routinely. As most of the previous studies were based on normal laboratory results there can be some deficiencies in ESBL detection.

Further, in Sri Lanka a lot of patients with CA UTI are treated with empirically at the out-patient department and at general practitioners (GP) and therefore most of these patients were not included in the previous studies as they were not admitted. However, this study was designed to include such patients as well with active sampling and direct interviews with the patient (in both OPD and GP centers) to gather data to see the prevalence of community acquired UTI by ESBL producing coliforms and risk factors associated with the food habits, lifestyle and others.

Objectives

In this study, we investigated the prevalence of ESBL producing *Escherichia coli* and *Klebsiella* species in CA UTI. Antibiotic sensitivity pattern and genetic analysis of ESBL producing bacteria, and the association of risk factors and the clinical presentation with CA ESBL UTI were also described.

Materials and methods

A descriptive cross-sectional study was carried out for 4 months from December 2016 to March 2017. Study sites were Teaching Hospital Karapitiya (THK) and 10 selected General practitioners' (GP) centers in the Galle district. THK is the 3rd largest tertiary care hospital in Sri Lanka which usually sees about 1500 OPD patients and nearly 1500 newly admitted patients daily.

Study population

Galle district population in the area surrounding Teaching Hospital Karapitiya, who had CA-UTI during this time period considered as the study population.

Inclusion criteria

Patients who came to OPD, clinics and GP centers with clinically suspected CA UTI according to the history were included in the study.

Exclusion criteria

Patients who had hospital admission within the last 3 months, or admitted to the hospital for more than 48 hours or patients on urinary catheters were excluded because we wanted to exclude recurrences due to partially treated urinary tract infections and health care associated infections. Patients who were on antibiotics other than prophylaxis were also excluded.

Sample size

Sample size was calculated as 173 according to the study by Dissanayake et al, (2010) [4] in which the ESBL prevalence among CA-UTI patients was 13%. For the calculation the standard formula $n=Z^2pq/d^2$ was used with margin of error (d) of 5%.

Data extraction sheet

By an interviewer based structured questionnaire, information on basic demography, clinical features, about past history of UTI, prophylactic antibiotics, structural anomalies, and about possible risk factors (diabetes, malignancy, chronic renal failure, steroid drugs, visit to India, fresh recreational swimming, consumption of fish / chicken, family history of UTI, health care worker in the immediate family) were gathered. We assumed that an average person in Galle district would consume about 50g of chicken per week and about 50g of fish 3 times per week according to the household expenditure survey report 2012/13 [5].

Ethical approval

Ethical approval was obtained from ethics review committees of the Faculty of Medicine, Galle and the Medical Research Institute, Colombo 08.

Methodology

With the help of medical professionals all clinically suspected CA-UTI patients were identified in the OPD and the GP centers. GP centers were selected according to the consent of the particular GP, facilities available for on-site collection and the distance to the laboratory. After taking the informed written consent, the questionnaires were completed by the principal investigator. Only patients with the history suggestive of CA-UTI were included and sample collection was carried out throughout the intended 4 months to complete the target sample size. Patients were guided on the correct way of collecting urine for culture. One sample was collected from each consented patient into sterile bottles and were immediately transported to the laboratory during day time or kept in the 4°C refrigerator if collected after 5pm. Storage time did not exceed 12 hours in any case.

Sample processing was done according to the standard laboratory manual [6]. Urine samples were plated on HiCrome UTI Agar and the plates were read after overnight aerobic incubation at 37°C. Pure cultures of colony count >10 were taken as significant as the samples were taken only from the symptomatic patients. Bacterial

species were initially identified using Gram stain and basic laboratory tests and the Gram negatives were identified by using REMEL's RapID™ ONE system to the species level.

Antibiotic sensitivity test (ABST) was done according to CLSI 2017 M-100 (7). All Gram-negative organisms were tested for 1st line antibiotics; ampicillin, cefuroxime, cefotaxime, amoxicillin/clavulanic, gentamicin, ciprofloxacin, co-trimoxazole, norfloxacin, nitrofurantoin, nalidixic acid, and cephalexin.

Also, cefpodoxime 10 μg, ceftazidime 30 μg, aztreonam 30 μg, and ceftriaxone 30 μg were added to the primary ABST. If their zone diameters were less or equal to the standards, ESBL confirmatory test was performed for all *E.coli* and *Klebsiella* spp.

ESBL confirmatory test was done using double disc method with cefotaxime 30 μg, cefotaxime /clavulanic acid 30/10 μg, and ceftazidime 30 μg, ceftazidme /clavulanic 30/ 10 μg. If the zone diameter deference between combined disc and non-combined disk is ≥ 5mm, it was confirmed as ESBL producing organism.

These organisms were further tested with amikacin, imipenem, meropenem, and netilmicin. Only *Escherichia coli* isolates were checked with fosfomycin, and mecillinam. Identified organisms were stored in the nutrient agar broth at -70°C for conventional multiplex PCR later at the Genetech Institute (Colombo) according to the in-house protocols by the principal investigator.

Data analysis

The comparison of bacteriological profiles was analyzed by Statistical Package for Social Sciences (SPSS) software version 22. To analyze the association between categorical variables Pearson's chi-square test was used.

Results

During the study period, data and urine samples were collected from 465 of clinically suspected symptomatic CA UTI patients from OPD (240), GP centers (150) and from hospital clinics (75). We continued to collect samples throughout the intended study period despite the calculated sample size being 173. We had 178 (38%) culture positives and were recruited in the study.

From that collection, 72 (40.4%) ESBL forming *Escherichia coli* and *Klebsiella pneumoniae*, 93 non-ESBL *Escherichia coli* and *Klebsiella pneumoniae* and 13 other bacterial species were identified (Table 1).

Table 1. Isolated organisms from culture positive CA UTI patients (Total=178)

Isolates	Number
Non ESBL *Escherichia coli*	81 (45.5%)
ESBL *Escherichia coli*	68 (38.2%)
Non ESBL *Klebsiella pneumoniae*	12 (6.7%)
ESBL *Klebsiella pneumoniae*	4 (2.2%)
Enterobacter spp	2 (1.1%)
Enterococcus spp	6 (3.4%)
Proteus spp	2 (1.1%)
Pseudomonas aeruginosa	2 (1.1%)
Coagulase negative *Staphylococcus* spp	1 (0.6%)

Majority was females among the total positives (103/ 178, 58%) and among the total ESBL positives (40/72, 56%) but the association was not statistically significant (p=0.637). The mean age was 39.58 years with 90yrs being the maximum and most of ESBL producing organisms were isolated from age between 51-60 years (Figure 1).

Table 2. BST of ESBL *E.coli* and *Klebsiella* spp isolates (n=72)

| Antibiotic	Sensitive	Intermediate	Resistance
Gentamicin 10 μg	57 (79%)	0	15 (21%)
Ciprofloxacin 5μg	15 (21%)	0	57 (79%)
Co-trimoxazole 1.25/23.75μg	29 (40%)	0	43 (60%)
Norfloxacin 10μg	11 (15%)	0	61 (85%)
Nitrofurantoin 300μg	64 (89%)	1 (1%)	7 (10%)
Nalidixic acid 30μg	9 (12%)	0	63 (88%)
Amikacin10μg	72 (100%)	0	0
Imipenem 10μg	72 (100%)	0	0
Meropenem 10μg	70 (97%)	0	2 (3%)
Netilmicin 30μg	68 (94%)	0	4 (6%)
Fosfomycin 200μg – only for *E.coli*	68 (100%)	0	0
Mecillinam 10μg – only for *E.coli*	62 (91%)	4 (6%)	2 (3%)
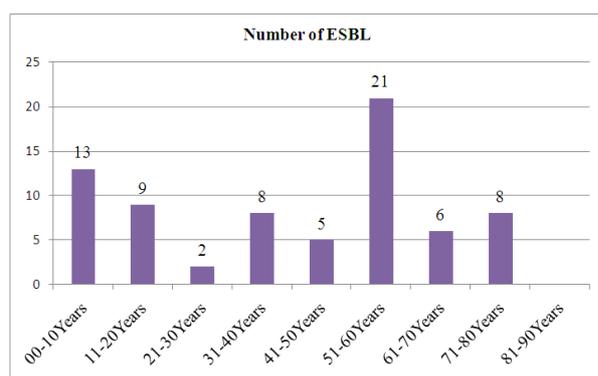
Figure 1. **Isolation of ESBL *E.coli* and *Klebsiella* spp among age groups.**

Antibiotic sensitivity pattern

Oral antibiotics which are being commonly used empirically in the community set-up were noted to have high resistance rates for *E.coli* and *Klebsiella pneumonia*e; co-amoxiclav 59% (97/165 of total *E.coli* and *Klebsiella*), norfloxacin 48.5% (80/165), co-trimoxazole 43.6% (72/165), ciprofloxacin 41% (68/165) and nalidixic acid 28% (46/165).

All *E.coli* and *Klebsiella* were fully sensitive to imipenem and amikacin (100% sensitivity) while gentamicin and nitrofurantoin had 89.7% (148/165) and 91.5% (151/165) sensitivity respectively. ESBL *E.coli* showed 100% sensitivity to fosfomycin and 91% sensitivity to mecillinam.

ESBL gene analysis

Only 43 samples were tested by conventional PCR due to limited funds. Therefore, only 39 ESBL *E.coli* isolates (randomly selected) out of 68 totals, and all 4 ESBL *Klebsiella* isolates were analysed.

According to gene analysis, 83% CTX-M (34/41), 70.73% OXA (29/41), 24.4% TEM (10/41) and 9.8% SHV (4/41) were found. PCR was not successful in two *E.coli* samples.

Out of total ESBL *Klebsiella pneumoniae*, TEM in 25%, SHV in 75%, OXA in 75% and CTX-M in 75% were identified. ESBL *E.coli* genes were identified as; SHV in 2.7% (1/37), TEM in 24.32% (9/37), OXA in 70% (26/37) and CTX-M in 83.8% (31/37). More than one gene was identified in 64.86% (24/37) ESBL *E.coli* and 75% (3/4) ESBL *Klebsiella pneumoniae*. One *E.coli* isolate and one *Klebsiella* isolate had all 4 genes in each and showed similar antibiotic sensitivity pattern.

Clinical features

Statistical significance was analysed for features of UTI such as fever, dysuria, frequency, abdominal pain, renal angle tenderness, haematuria and anuria. There was significant association between haematuria and ESBL CA-UTI.

Table 3. Significance of clinical features and ESBL CA UTI (ESBL=72, non-ESBL=106)

Symptoms/ Signs	ESBL UTI		Non-ESBL UTI		P value
	Positive	Negative	Positive	Negative	
Fever	54	18	71	35	0.251
Dysuria	59	13	87	19	0.928
Frequency	59	13	78	28	0.194
Abdominal pain	45	27	68	38	0.822
Renal-angle tenderness	13	59	26	80	0.306
Hematuria	15	57	12	94	0.032
Anuria	2	70	6	100	0.362

Risk factors for ESBL CA-UTI

Among the risk factors we analysed, only structural abnormalities of the urinary tract was significantly associated with ESBL UTI in the community.

Table 4. Significance of risk factors and ESBL CA UTI (ESBL-72, non-ESBL=106)

Symptoms/ Signs	ESBL UTI		Non-ESBL UTI		P value
	Positive	Negative	Positive	Negative	
Above 65 years	13	59	19	87	0.982
Diabetes	16	56	16	90	0.224
Structural abnormalities of the urinary tract	8	64	2	104	0.022
Visit to India	1	71	1	105	1.000
Steroid use	0	72	1	105	0.654
Fresh-water recreational activities	2	70	1	105	0.734
Chicken consumption (at least 50g weekly)	43	29	68	38	0.637
Fish consumption (50g 3 times per week)	62	10	82	24	0.260
Family history of other infections	0	72	0	106	-
Health care worker in the immediate family	4	68	6	100	1.000
Chronic renal failure	0	72	0	106	-
Malignancy	0	72	0	106	-
Past history of UTI	22	50	22	84	0.137
Antibiotic prophylaxis	0	72	0	106	-

Discussion

Community acquired urinary tract infection is one of the major reasons to attend to the medical practitioners. Due to antibiotics resistance of major causative organisms of UTI the antibiotic options in the community has been rendered limited.

Only 39.03% of clinically suspected CA-UTI urine samples yielded positive cultures with *Escherichia coli* (83%) as the main pathogen. Majority of culture positive patients were females (57.86%). These facts are compatible with most of previous CA-UTI studies [4].

In this community, ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* prevalence was 40.44% while Mohomed et al in India [8] found almost same in 2007. However, according to the local study done by Dissanayake et al in 2010 [4], the ESBL prevalence found among the CA-UTI patients was 13%.

We noted that, many primary antibiotics such as co-amoxiclav (59% resistance), norfloxacin (48.5%) and cotrimoxazole (43.6%) were shown resistant for *E.coli* and *Klebsiella* spp. According to the standard guidelines, to use an antibiotic as an empirical antibiotic it must have been tested >90% sensitive on the causative *E.coli* of that community [9]. According to that criterion, only mecillinam, nitrofurantoin and fosfomycin can be used as oral empirical antibiotics in this community. However, fosfomycin is currently unavailable in Sri Lanka.

When comparing the current study and the study by Dissanayake et al [4], while imipenem and amikacin sensitivity has been preserved same, nitrofurantoin and ciprofloxacin sensitivity has decreased by 7% and 24% respectively during the 8 years gap. No previous studies had tested fosfomycin or mecillinam in Sri Lanka.

It had been identified that most of ESBL producing organisms are having CTX-M gene. We found CTX-M gene on 83% of samples while OXA (70.73%), TEM (24.4%) and SHV (9.8%) were also detected. High prevalence of CTX-M can explain the high rates of multi-resistance to antibiotics [1].

According to the clinical features we analysed, statistically significant relationship was noted only between haematuria and ESBL CA-UTI. However, several studies have concluded that the clinical picture of UTI cannot exactly predict whether it is ESBL UTI or not [10].

Among risk factors, only structural abnormality of urinary tract was statistically significant in the association with ESBL UTI. In 2013 Søraas et al [11] found several independent risk factors increased the probability of ESBL UTI, namely, travel to Asia, Middle East or Africa either during the past six weeks to 2 years, recent use of fluoro-quinolones or beta lactam antibiotics, diabetes mellitus, and freshwater swimming in the past year. They further concluded that increasing number of fish meals will reduce the risk of ESBL producing UTI. In the present study we could not find statistically significant relationship between those same risk factors and some others and ESBL UTI.

Conclusions

ESBL prevalence in CA-UTI in this community is 40.44%. Only nitrofurantoin, mecillinam and fosfomycin can be used as empirical oral antibiotics according to sensitivity rates. Most prevalent ESBL gene in this community is CTX-M gene. Haematuria and structural abnormalities of the urinary tract are having statistically significant relationship with ESBL CA-UTI.

Acknowledgements

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