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Original Article

Epidemiology of extended-spectrum β-lactamase producing *Escherichia coli* from hospital settings in Yemen

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Abstract

Introduction: Infection with Extended spectrum β-lactamases (ESBLs) producing bacteria is considered as serious health problem worldwide. The aim of this cross-sectional study was to investigate the prevalence of ESBL producing *Escherichia coli* in hospitalized patients and the risk factors contributed for its nosocomial infections in addition to the antibiotics susceptibility patterns of isolates from 130 inpatients collected in Al Thawra General Hospital and Al-Kuwait University Hospital in Sana'a city.

Methodology: Antibiotic susceptibility testing and confirmation of ESBL production were performed according to the Clinical and Laboratory Standards Institute guidelines.

Results: Out of 130 *E. coli* isolates, 44 (33.8%) were ESBLs producers, the majority of ESBLs producers were in wound exudates samples (52.2%). The highest significant rates were among the elderly, patients with previous hospitalization, patients who have stayed in hospital more than 22 days, patients who have taken third generation cephalosporins as treatment and diabetic patients. All ESBL-producing isolates were resistant to amoxicillin, trimethoprim-sulfamethoxazole and the third generation cephalosporins (100%). Resistance to other antimicrobial agents among these isolates was: amoxicillin-clavulanic acid (90.9%), nalidixic acid (95.5%), ciprofloxacin (90.9%), ofloxacin (88.6%) and tetracycline (54.5%). The most effective antibiotics in vitro for both types of isolates (ESBL producing and non ESBL producing *E. coli*) were Imipenem (100%), Amikacin (75%) and (93.0%), respectively, and Pipracillin-tazobactam (68.2%) and (88.4%), respectively.

Conclusion: ESBLs detection tests must be performed as routine work in all hospitals and laboratories. Furthermore, a strict adherence of infection control policies and procedures with continuous antibiotics resistance surveillance are important to prevent nosocomial infections.

Key words: *Escherichia coli*; extended spectrum β-lactamases; Yemen.

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Introduction

Worldwide, antibiotic-resistant microorganisms are major public health threat, particularly in hospitals and other health care settings [1]. Antibiotic-resistant bacteria are able to cause serious and severe infections, posing a great challenge for the management of different infectious diseases [2]. Antimicrobial resistance may emerge in susceptible bacteria as a response to selective antibiotic pressure invoked by random or misuse of antibiotics.

Hospitals deal with a high number of patients (many of them are immunocompromised patients), who are relatively close to each other. A resistant bacterium may spread from person to person or from a contaminated equipment (especially indwelling devices) or environment. Health care providers also can contribute in the dissemination of infection, when failing to practice simple control measures or a combination of these factors can occur and stimulate the emergence of multidrug resistance in hospitals [2].

Significantly, antimicrobial resistance among Gram-negative bacilli expressing Extended-spectrum β-lactamases (ESBLs) is a problematic in nosocomial and community acquired infections. ESBL are enzymes produced by many Gram-negative bacteria that are able to change the susceptibility of different antimicrobial agents [3] and are plasmid-mediated enzymes that can hydrolyze a broad spectrum of β-Lactam antimicrobials and make them inactive, including third-generation cephalosporins, penicillins and aztreonam; but are inhibited by clavulanic acid [4,5].

The most common β-Lactamases are the TEM, SHV and CTX families, mainly expressed in *Escherichia coli* and *Klebsiella pneumoniae*. ESBL-expressing bacteria can also reduce the susceptibility to other non β-lactamases antibiotics and consequently, the treatment of theses infection becomes more difficult [6].

The emergence of ESBL resistant bacteria is responsible of treatment failure and enormous cost due to long hospital stay and additional supportive therapy [7]. The expression of ESBLs in *E.coli* strains is a serious threat because these bacteria are able to induce several human illnesses ranging from simple urinary tract infection to severe bloodstream infections [8].

The Clinical Laboratory Standards Institute (CLSI) has published guidelines for ESBLs detection in Enterobacteriaceae. These guidelines are based on phenotypic microbiological tests. The principle is that most ESBLs hydrolyze 3rd generation cephalosporins such as ceftazidime, cefotaxime and ceftriaxone, but they are inhibited by clavulanate [8]. The phenotypic methods show high sensitivity of up to 94% and specificity of 98% specifically for *E. coli*, *Klebsiella* spp, and *Proteus* spp [9].

Existing phenotypic methods are based on double-disk synergy test (DDST) and double disk diffusion test (DDDT) by the investigation of cefotaxime and ceftazidime hydrolysis with and without the addition of clavulanic acid [10].

The available data on ESBL- producing *Enterobacteriaceae* in the Middle East countries are alarmingly drawing attention because this region becomes a major epicenter of the worldwide pandemic ESBL [11].

In Yemen, the only report about ESBLs was done in 2014 by Gharout-Sait et al, in which the authors demonstrated the presence of *Enterobacteriaceae* (eight *Klebsiella pneumoniae* isolates and two *Enterobacter cloacae*) isolates carrying the New Delhi metallo-β-lactamase gene [12].

The prevalence of ESBLs in *E.coli* strains is not investigated yet in Yemen and we aim to carry out an epidemiological study of ESBLs in *E. coli* strains and to determine the antimicrobial susceptibility patterns of isolates from hospital setting of Yemen.

Methodology

Study design and population

We performed an analytical cross-sectional study involving inpatients of two selected public hospitals in Sana'a city-Yemen (Al-Kuwait University Hospital and Al-Thawra General Hospital), for the isolation of extended spectrum β-lactamase (ESBL) producing *E. coli* from different clinical specimens, except stool specimens. All patient`s age were enrolled in this study except specimens of neonates and children under 18 years old. Data were obtained from each patient in a predesigned questionnaire, which included demographic information and relevant predisposing factors associated with nosocomial infection of ESBL producing *E. coli*. Then, the data was analyzed by Statistical Package for Social Sciences (SPSS) version 20 computer statistical program for testing: frequencies, percentage, chi-square (χ^2) that are used for comparison between two variables to determine the P values and Odd ratio (OR). P value < 0.05 considered statistically significant.

Sample size

The sample size was calculated in Epi info version 6, taking into consideration the following: nearly a number of isolated *E. coli* from clinical specimens in Sana'a city was 10000 isolates /year, the expected frequency of ESBL *E. coli* is ~ 20%, and the worst acceptable result is 4% with a confidence level of 95%. Therefore, a total of 130 samples was included in this study at a confidence level of 95%.

Specimen types and sampling

Various clinical specimens (Urine, sputum and other body fluids) were collected for routine investigation of significant pathogens in National Center of Public Health Laboratories (NCPHL). Blood specimens were obtained under aseptic conditions and transferred immediately into sterile bottles containing Tryptone Soya broth (Oxoid, Basingstoke, Hampshire, England). Wounds specimens were collected by swabs, then placed in transport media (Amise transport medium) and immediately examined. All specimens that were not collected under adequate amounts or conditions were excluded from the study.

Bacterial Culturing and identification

The media used for isolation and identification (biochemical tests) as well as reagents were supplied by Oxoid (Basingstoke, Hampshire, England). All types of media were prepared according to manufacturers and standard procedures. Specimens were inoculated directly on MacConkey, Blood and nutrient agar plates. Sterile plastic loop was used for cross-streaking to spread the sample over the surface of the plate to obtain pure and separate colonies. All cultured plates were incubated aerobically for 18-24 hours at 37°C and examined for suspected *E. coli* growth. Only pure growth of *E. coli* was included in this study. The culture plate that yielded organism other than *E. coli* or yielded more than one type of organism per specimen were excluded from the study. Each isolate of pathogenic *E. coli* was identified by the use of gram stain and then confirmed by API 20 E (bioMerieux, Marcy-I, Etoile, France).

Antibiotics susceptibility testing (AST)

The antibiotics disks that were used in antimicrobial susceptibility test were supplied from (Oxoid, Basingstoke, England), including: Amikacin (30 µg), Amoxicillin (10 µg), Amoxicillin-clavulanic acid (20/10 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (30 µg), Imipenem (10 µg), Nalidixic acid (30 µg), Ofloxacin (5 µg), Pipracillin/Tazobactum (110/10 µg), Tetracycline (30UI) and Trimethoprim-sulfamethoxazole (25 µg).

AST for bacterial strains was done by the standard Kirby Bauer Disk Diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations and guidelines. Each isolate was suspended and standardized to 0.5 McFarland concentration and then inoculated on Muller Hinton agar plates and their susceptibility was tested against 13 antibiotic disks. All the quality measures regarding the distance between antibiotics and incubation conditions were performed as recommended by the CLSI guidelines. The inhibition zones for antibiotic was measured by sliding calipers.

All *E. coli* isolates were screened by phenotypic disk diffusion method for ESBL production, then confirmed by phenotypic double disk synergy test (DDST), we simultaneously tested the reference strains: non ESBL-producing bacteria (E. *coli* ATCC 25922) and the ESBL-producing bacteria (*K. pneumoniae* ATCC 700603) as recommended by CLSI 2012 [13].

Phenotypic screening method

The CLSI has recommended the use of any of the following antibiotic disks for screening of ESBLs producing. Antibiotic disks of ceftazidime, cefotaxime and ceftriaxone were used. More than one of these agents were used for screening to improve the sensitivity of ESBLs detection. Each *E. coli* isolates that gave diameter zone < 22 mm with Ceftazidime (CAZ), < 25 mm with Ceftriaxone (CRO) and < 27 mm with Cefotaxime (CTX) were confirmed by phenotypic confirmatory method (DDST) for ESBLs production [13].

Phenotypic confirmation method by Double Disk Synergy (DDST)

As recommended by the CLSI, the DDST was carried out for all the bacterial strains to investigate about the ESBL expression. A disk of Amoxicillin-clavulanic acid was placed (AMC 30 µg) at the center of a plate and different disks (CAZ, CTX and CRO) were placed 25-30 mm from the Amoxicillin-clavulanic acid and then the palates were incubated at 37ºC for 18 hours.

The clear inhibition zones of cephalosporins toward the disk of Amoxicillin-clavulanic acid was an indicator of ESBL production [13].

Results

Percentage of ESBL producing E. coli

A total of 130 clinical specimens were collected from inpatients of Al-Thawra General hospital and Al-Kuwait University hospital from November 2014 to April 2015 to study the prevalence of extended spectrum β-lactamase producing *E. coli.* Out of 130 inpatients, 68 (52.3%) were males while 62 (47.7%) were females.

A total of 130 clinical specimens were included: 73 (56.2%) urine, 23 (17.7%) wound exudates, 12 (9.2%) sputum samples, 10 (7.7%) blood, 6 (4.6%) peritoneal fluids, 4 (3.1%) vaginal swabs and two (1.5%) CSF.

Out of the 130 *E.coli* isolates tested for ESBL production by phenotypic method (DDS), 44 (33.8 %) isolates were ESBL-producers, while 86 (66.2 %) isolates were non-ESBL producers. The highest rate of ESBL-producer was obtained from wound exudates 12 (52.2 %), but none were found in body fluids, swabs or CSF specimens (Table 1).

Predisposing factors associated with the contraction of ESBL-producing E. coli

Different predisposing factors associated with contracting ESBL producing *E. coli* infections were tested: sex, age, previous hospitalization, length of stay in hospital, the use of antibiotics, underlying diseases and medical devices (Table 2).

The ESBL rate was higher in females than males with a percentage of 38.7% versus 29.4%. This result was not statistically significant (P>0.05). Additionally, the highest representation of ESBLs was 62.5% for the age group of 40–49 years old with an estimated risk of

Table 1. Distribution of ESBLs producing E. coli according to the type of specimens.

Type of specimens (n = 130)	ESBL Positive (n = 44)
Urine (n = 73)	27 (37.0%)
Wound exudates (n = 23)	12 (52.2%)
Sputum (n = 12)	3 (25%)
Blood (n = 10)	2 (20.0%)
Peritoneal fluid (n = 6)	0 (0.0%)
Vaginal swabs (n = 4)	0 (0.0%)
Cerebrospinal spinal fluid (n = 2)	0 (0.0%)

2.50, followed by (40.0%) for the age group of ≥ 50 years old then, (31.3%) for the age group of 29–39 years old (P =.0.02).

Higher significant rate (45.7%) was found among patients who had previous hospitalization (P = 0.002) with an estimated risk of 3.37. Additionally, the highest significant rate was found among the patients who stayed in hospitals more than 22 days (47.7%) (P =.0.04), with an estimated risk of 2.80.

The association between the underlying diseases and contracting of ESBL producing *E. coli* showed a significant rate of (60.0%) in diabetic patients, P = 0.001 and the estimated risk was 4.27.

The highest rate was 42.1% was observed in patients who used intravenous devices whereas the lowest rate (35.9%) was observed in patients who used urinary catheter. The results were not statistically significant (P > 0.05).

The association between the use of antibiotics and of ESBL producing *E. coli* demonstrated that the highest significant rate was 56.4% among patients who used 3rd generation cephalosporin as treatment, the estimated risk was 4.1 (Table 3).

Susceptibility patterns of ESBL producing and non-ESBL producing E. coli

The results of the susceptibility patterns of ESBL producing & non-ESBL producing *E. coli* showed that Imipenem was the most effective antibiotic in vitro for both types of isolates (ESBL producing & non ESBL producing *E. coli*) with the percentage (100.0%), followed by Amikacin (75.0% and 93.0%) respectively, then Pipracillin-tazobactam (68.2% and 88.4%), respectively with a significant correlation (P value < 0.05).

Table 2. Demographic data of patients.

Characters	ESBL positive	OR	CI	χ^2	P
Gender					
Female (n = 62)	24 (38.7%)	1.23	0.86-1.77	1.25	0.263
Male (n = 68)	20 (29.4%)				
Age					
18-28 (n = 27)	2 (7.4%)	0.24	0.13-1.19	15.32	0.02
29-39 (n = 32)	10 (31.3%)	0.68	0.24-1.94		
40-49 (n = 16)	10 (62.5%)	2.50	0.72-8.71		
≥ 50 (n = 55)	22 (40.0%)	1.45	0.17-5.19		
Recent hospitalization					
Yes (n = 70)	32 (45.7%)	3.37	0.53-7.41	9.54	0.002
No (n = 60)	12 (20.0%)				
Length of hospital stay (days)					
7–14 (n = 33)	6.0(18.2%)	1.71	0.27-4.70	4.63	0.04
15–21 (n = 53)	17(32.1%)	2.24	0.34-3.76		
≥ 22 (n = 44)	21(47.7%)	2.80	0.36-5.45		
Underlying disease					
Recurrent UTI (Yes, n = 38)	14 (36.8%)	1.21	0.55-2.66	0.22	0.643
Recurrent UTI (No, n = 92)	30 (32.6%)				
Diabetes (Yes, n = 30)	18 (60.0%)	4.27	1.81-10.05	11.9	0.001
Diabetes (No, n = 100)	26 (26.0%)				
Malignancies (Yes, n = 12)	5 (41.7%)	1.45	0.43-4.82	0.36	0.548
Malignancies (No, n = 118)	39 (33.1%)				
Surgery site infection (Yes, n = 16)	7 (43.8%)	1.62	0.56-4.69	0.79	0.371
Surgery site infection (No, n = 114)	37 (32.5%)				
Medical devices					
Urinary Catheter (Yes, n = 64)	23 (35.9%)	1.20	0.58-2.49	0.25	0.620
Urinary Catheter (No, n = 66)	21 (31.8%)				
Mechanical ventilation (Yes, n = 12)	5 (41.7%)	1.45	0.43-4.85	0.36	0.548
Mechanical ventilation (No, n = 118)	39 (33.1%)				
Intravenous devices (Yes, n = 38)	16 (42.1%)	1.66	0.76-3.64	1.64	0.201
Intravenous devices (No, n = 92)	28 (30.4%)				

OR: Odds ratio > 1 (at risk); CI: Confidence intervals 95%; χ^2: Chi-square ≥ 3.84; *p*-value < 0.05 (significant).

Table 3. The association between the use of antibiotics and contracting of ESBL producing *E. coli.*

Antibiotic			ESBL Positive (n = 44)	OR	CI	χ^2	P
2nd generation cephalosporin		Yes (n = 2)	0.0 (0.0%)	0.79	-	-	NA
		No (n = 128)	44 (34.4%)				
3rd generation cephalosporin		Yes (n = 39)	22 (56.4%)	4.1	1.83-8.98	12.7	0.000
		No (n = 91)	22 (24.2%)				
Quinolones		Yes (n = 15)	7 (46.7%)	1.85	0.62-5.47	1.25	0.265
		No (n = 115)	37 (32.2%)				
Aminoglycosides		Yes (n = 12)	5 (41.7%)	1.45	0.34-4.85	0.36	0.548
		No (n = 118)	39 (33.1%)				
Carbapenems		Yes (n = 24)	10 (41.7%)	1.51	0.61-3.75	0.80	0.370
		No (n = 106)	34 (32.1%)				

OR: Odds ratio > 1 (at risk); CI: Confidence intervals 95%; χ^2: Chi-square ≥ 3.84; *p*-value < 0.05 (significant); NA not applicable.

On the other hand, the results showed that there is a high resistance rate in ESBL producing isolates against Amoxicillin, Trimethoprim-sulfamethoxazole and all 3rd generations of cephalosporins with the percentage (100.0%) for all, followed by Nalidixic acid (95.5%), Amoxicillin-clavulanic acid (90.9%), Ciprofloxacin (90.9%) and Ofloxacin (88.6%.)

Likewise in non-producers isolates, a high resistance rate was observed in Amoxicillin and Cefotaxime with the percentage (100.0%) followed by Nalidixic acid (93.0%), Ceftriaxone and Ceftazidime (91.9%) for both, Amoxacillin clavulanic acid (86.0%), Trimethoprim-sulfamethoxazole (74.4%), Ofloxacin (67.4%) and Ciprofloxacin (69.8%).

Intermediate resistance was noticed with Tetracycline in both ESBLs producing and non ESBLs producing isolates (54.5% and 51.2%), respectively, while the significant resistance rates between ESBL producing and non ESBL producing strains of *E. coli* was with Trimethoprim-sulfamethoxazole, Ofloxacin, Ciprofloxacin, Ceftriaxone and Ceftazidime (P value< 0.05) (Table 4).

Discussion

Extended spectrum β-lactamases (ESBLs) are known to cause problems in patients who are especially hospitalized with an increase of prevalence during the exposure to different antibiotics [14]. Consequently, this may result in treatment failure and death due to a delay of an adequate antimicrobial therapy. Worldwide, the incidence of ESBL *E. coli* in hospitals is dramatically increasing and the therapeutic option are very limited [15].

No published data are available on the prevalence of ESBL producing *E. coli* and its antimicrobial susceptibility patterns in Yemen. So this study is considered the first study that investigated the prevalence and antibiotics susceptibility patterns of ESBL producing *E. coli* among inpatients of two hospitals in Sana`a city-Yemen.

Table 4. Susceptibility patterns of ESBL producing and non-ESBL producing *E. coli.*

Antibiotics	ESBL positive				ESBL negative				P
	Sensitive		Resistant		Sensitive		Resistant		
	No	%	No	%	No	%	No	%	
Imipenem	44	100	0.0	0.0	86	100	0.0	0.0	NA
Amikacin	33	75.0	11.0	25.0	80	93.0	6.0	7.0	0.004
Pipracillin-Tazobactam	30	68.2	14	31.8	76	88.4	10	11.6	0.005
Tetracycline	20	45.5	24	54.5	42	48.8	44	51.2	0.72
Ofloxacin	5	11.4	39	88.6	28	32.6	58	67.4	0.009
Ciprofloxacin	4	9.1	40	90.9	26	30.2	60	69.8	0.007
Nalidixic acid	2	4.5	42	95.5	6	7.0	80	93.0	0.59
Trimethoprim-Sulfamethoxazole	0.0	0.0	44	100	22	25.6	64	74.4	0.000
Ceftriaxone	0.0	0.0	44	100	7	8.1	79	91.9	0.04
Ceftazidime	0.0	0.0	44	100	7	8.1	79	91.9	0.04
Amoxicillin-Clavulanic acid	4	9.1	40	90.9	12	14.0	74	86.0	0.43
Cefotaxime	0.0	0.0	44	100	0.0	0.0	86	100.0	NA
Amoxicillin	0.0	0.0	44	100	0.0	0.0	86	100.0	NA

In the present study, the prevalence of ESBL producing *E. coli* was 33.8% among enrolled inpatients and this was nearly similar to studies performed in Sudan and Saudi Arabia (30.2 % and 35.8%, respectively) [16,17] and was lower than that was reported in Egypt (60%) [18].

In addition, the majority of ESBL-producing *E. coli* was obtained from wound exudates (52.2%), which can be attributed to prolonged hospital stay, treatment with antibiotics in different combinations that causes acquisition of multiple resistant organisms from medical devices and hospital environment [19]. Several factors were studied and the predisposing factors associated with contracting ESBL producing *E. coli* infections in this study were: age, previous hospitalization, length of stay in hospital, the use of antibiotics and underlying diseases.

ESBL producing *E. coli* was higher among females than males with the percentage of (38.7%) without a statistical significance. Several studies on multi-drug resistance infections such as UTIs showed that the infections occurred among females more than males due to different factors such as: menopause, hormonal imbalance and short urethra close to anus which increases the rate of UTI infection as well as the frequency of ESBL producing *E. coli*, whereby *E. coli* is responsible of ~60% of UTIs in females [20].

The statistical correlation between the age groups of patients was significant (P value = 0.02) and the highest prevalence was noticed in elder patients. However, the significant high risk was among the age group of 40-49 years old. The age group 40-49 has a relatively smaller sample number than other groups, but most of patients in this group are diabetic females having different health problems. In general, elder patients are immune-compromised and more subjected to be infected by multidrug resistance microorganisms.

High percentage (45.7%) was among inpatients who had previously admitted to hospitals and stayed in hospital more than 3 weeks.

Significantly, the prevalence of ESBL producing *E. coli* was higher among patients who had undergone 3rd generation Cephalosporins treatment with a percentage of (56.4%) $P = 0.000$ and this confirms the fact that ESBL emerges as a result of the excessive Cephalosporin use. Third generation Cephalosporins are the most commonly used antibiotics in hospitals, which can lead to a predominant selective pressure for the resistance development [21].

It is noteworthy to mention that all the hospital inpatients who had undergone a medical intervention by the use of different medical devices (intravenous devices, mechanical ventilation and urinary catheter) were infected by ESBL producing *E.coli* without significant correlations. The rise in the incidence of devices-associated colonization and infection with ESBL-producing organisms had been observed in different studies, but with different degrees [22,23].

In general, a high risk of developing colonization or infection with ESBL-producing organisms is common in seriously ill patients who used invasive medical devices for a prolonged duration.

The prevalence of ESBL *E. coli* infections was among patients who were diabetic with the percentage (60.0%), followed by surgery site infections (43.8%), malignancies (41.7%) and recurrent UTI (36.8%). This might be due to immune suppression and diabetic complications, which make patients, have more antibiotic treatments which can increase the antibiotic resistance rates [24].

The comparison of antibiotic susceptibility patterns between ESBL producing and non-ESBL *E.coli* strains showed that ESBL producers were more resistant than non-ESBL producers *E.coli* with significant correlation ($P < 0.05$).

A high resistance rate in ESBL-producing *E. coli* isolates was seen against the first line antimicrobial therapy such as Amoxicillin, Trimethoprim/sulfamethoxazole and all 3rd generations of cephalosporins with the percentage (100%), followed by Nalidixic acid (95.5%), Amoxacillin-clavulanic acid (90.9%), Ciprofloxacin (90.9%) and Ofloxacin (88.6%).

Interestingly, the antibiotic susceptibility patterns of the isolates revealed that the highest antimicrobial activities against ESBL-producing *E. coli* was observed with Imipenem (100.0%), followed by Amikacin (75.0%) then Piperacillin-tazobactam (68.2%).

Carbapenem antibiotics are the first line of therapy choice against ESBL-producing *Enterobacteriaceae*. However, there is a continuous emergence of carbapenem-resistant ESBL-producing *Enterobacteriaceae* [25].

Conclusions

ESBLs detection tests must be performed as routine task in all hospitals and laboratories. Phenotypic method using double disk synergy (DDS) test is cost-effective and easily to perform and can be used to diagnose ESBLs efficiently.

Furthermore, a strict adherence of infection control policies and procedures with continuous antibiotics resistance surveillance and vigilant use of antibiotics,

all are important to prevent the ESBLs producing *E. coli* nosocomial infections.

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