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Prevalence and risk factors for extended-spectrum β-lactamase-producing Gram-negative bacterial infections in hospitalized patients at a tertiary care hospital, southwest Nigeria

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Abstract:

Background: Clinical infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria constitute great burden to healthcare delivery with these resistant pathogens contributing largely to the magnitude and spread of antimicrobial resistance globally. Hence, knowledge of the risk factors for acquisition of infection caused by ESBL-producing bacteria is crucial to instituting prompt and appropriate treatment as well as prevention and control measures**.** This study investigated the risk factors associated with the prevalence of ESBL-producing Gram-negative bacteria (GNB) infections among hospitalized patients in Uniosun Teaching Hospital (UTH), Osogbo, Nigeria.
Methodology: A total of 359 hospitalized patients with clinical infections from whose clinical samples we isolated non-duplicate GNB were consecutively recruited. GNB were isolated following aerobic cultures of appropriate clinical samples and Microbact™GNB 24E kit was used for species identification. All isolates were screened for ESBL production by the combination disc method. Relevant clinical and demographic information was obtained using a designed data collection form, and multivariate logistic regression analysis was used to identify associated risk factors.
Results: Ninety-four (26.2%) of the 359 patients had ESBL-producing GNB isolated from their clinical samples, with a preponderance of *Escherichia coli* (26.6%, n=25/94), although the most frequent ESBL-producer was *Stenotrophomonas maltophilia* (100%, n=2/2) and least frequent was *Pseudomonas aeruginosa* (2.6%, n=1/39). The study indicated that male gender, age group >60 years and farming were socio-demographic factors associated with significantly higher prevalence of ESBL-producing GNB infection. Other independent risk factors significantly associated with high prevalence of ESBL GNB infections were; (i) admission into intensive care unit and male surgical ward, (ii) presence of invasive devices such as intravenous line, endotracheal tube and urinary catheter, (iii) underlying conditions such as diabetes mellitus and benign prostatic hyperplasia, and (iv) immunocompromised state.
Conclusion: The information obtained from this study can serve as baseline data for designing strategy to prevent drug-resistant infections and transmission in our hospital.

Keywords*:* Prevalence, risk factors, extended-spectrum β-lactamase, Gram-negative bacilli

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Facteurs de prévalence et de risque pour les infections de bactéries gram-négatives de la β-lactamase prolongées de la β-lactamase chez les patients hospitalisés dans un hôpital de soins tertiaires, au sud-ouest du Nigéria

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Résumé:

Contexte: Les infections cliniques causées par des bactéries de la β-lactamase de spectre prolongée (ESBL) constituent une grande charge à la livraison des soins de santé avec ces agents pathogènes résistants contribuant en grande partie à la magnitude et à la propagation de la résistance antimicrobienne mondiale. Par conséquent, la connaissance des facteurs de risque d'acquisition d'une infection causée par les bactéries produisant des ESBL est essentielle à l'institution de traitement rapide et approprié, ainsi que des mesures de prévention et de contrôle. Cette étude a enquêté sur les facteurs de risque associés à la prévalence des bactéries gram-négatives de l'ESBL (GNB) parmi les patients hospitalisés dans l'hôpital d'enseignement Uniosun (Uth), Osogbo, Nigéria.
Méthodologie: Un total de 359 patients hospitalisés avec des infections cliniques de laquelle les échantillons cliniques de laquelle nous avons isolé le GNB non dupliqué ont été recrutés consécutivement. GNB ont été isolés à la suite de cultures aérobies d'échantillons cliniques appropriés et de kit MicroBact™ GNB 24E a été utilisé pour l'identification des espèces. Tous les isolats ont été criblés pour la production ESBL par la méthode des disques combinées. Des informations cliniques et démographiques pertinentes ont été obtenues à l'aide d'un formulaire de collecte de données conçu et une analyse de régression logistique multivariée a été utilisée pour identifier les facteurs de risque associés.
Résultats: Quatre-vingt-quatorze (26,2%) des 359 patients avaient des GNB producteurs de BLSE isolés de leurs échantillons cliniques, avec une prépondérance d'*Escherichia coli* (26,6%, n=25/94), bien que le producteur de BLSE le plus fréquent soit *Stenotrophomonas maltophilia* (100.0%, n=2/2) et la moins fréquente était *Pseudomonas aeruginosa* (2,6%, n=1/39). L'étude a indiqué que le sexe masculin, le groupe d'âge > 60 ans et l'agriculture étaient des facteurs sociodémographiques associés à une prévalence significativement plus élevée d'infections à GNB productrices de BLSE. D'autres facteurs de risque indépendants significativement associés à une prévalence élevée d'infections à BLSE GNB étaient; (i) admission en unité de soins intensifs et en salle de chirurgie pour hommes, (ii) présence de dispositifs invasifs tels qu'une ligne intraveineuse, un tube endotrachéal et un cathéter urinaire, (iii) conditions sous-jacentes telles que le diabète sucré et l'hyperplasie bénigne de la prostate, et (iv) immunodéprimé Etat.
Conclusion: les informations obtenues à partir de cette étude peuvent servir de données de base pour la conception de la stratégie visant à prévenir les infections et la transmission résistantes à la drogue dans notre hôpital.

Mots-clés: Prévalence, facteurs de risque, β-lactamase de spectre prolongé, bacille Gram-négatif

Introduction:

Extended-spectrum β-lactamases (ES BL) are enzymes that confer on many Gram-negative bacilli (GNB) of the family Entero-bacteriaceae the ability to hydrolyze β-lactam ring, thereby inactivating β-lactam antibiotics such as penicillins and cephalosporins including oxyimino-cephalosporins and monobactams which are common agents for treatment of clinical infections (1). ESBL-producing bacteria are particularly more worrisome especially in healthcare settings because of their high adaptability and efficient dissemination. The rising incidence of ESBL-producing GNB infections has raised serious health concerns globally. Prevalence of infections by ESBL-producers varies considerably and is largely dependent on local epidemiology as well as prevailing antimicrobial prescribing policies and patterns. Previous reports have shown the value to vary from 0-80% across different African regions which is essentially due to differences in levels of antibiotic use (2-5).

Increasing occurrence of clinical infections caused by ESBL-producing bacteria reduces therapeutic options for patients, and this situation has been made worse by inherent problems militating against medical care in the low-and-middle-income-countries (LMICs) including poor drug supply chain, ineffective health insurance, financial burden of out-of-pocket (OOP) drug procurement by patients, rudimentary laboratory diagnostics and under-developed hospital infection prevention and control. Infections by these "super bugs" have increased the usage of the few available 'last resort' reserved antibiotics like the carbapenems, leading to increasing resistance to these agents, with associated huge mortality.

Factors predisposing patients to infections by ESBL-producing pathogens are numerous, diverse and commonly related to infection sites and interventions. Recent hospitalization and prolonged exposure to antibiotics were among the most commonly documented risks for acquisition of clinical infections caused by ESBL-producing bacteria (6-10). A systematic review of 51 studies showed that previous hospitalization and prolonged use of cephalosporins are the independent risk factors for infections by hospital-acquired ESBL-producing Entero bacteriaceae among hospitalised paediatrics patients (11). Utilization of invasive devices is another risk for infections by the drug-resistant pathogens (12). Furthermore, it has also been

widely reported that chronic and immunosuppressive illnesses including diabetes mellitus (DM) and malignancies are predisposing factors harbouring infection by ESBL-producing bacteria (7, 10, 11).

Despite the increasing prevalence of ESBL-producing bacterial infections locally and globally with many reports on the epidemiology of these infections, risk factors for the acquisition of such infections have not been well defined in our hospital settings. Early identification of patients at risk of infection with ESBL-producing bacteria will enhance prescribing of most effective empirical therapy and applying appropriate infection prevention and control (IPC) measures to limit the spread of these multidrug-resistant infections. This can reduce the complications associated with ESBL infections, cost of treatment and emergence of antibiotic resistance, and improve patients' survival. In this study, we investigated the risk factors for ESBL-GNB infections from a previous study that determined the prevalence and molecular characteristics of ESBL-producing GNB for a more proactive approach in patient case management and effective IPC in our hospital.

Materials and method:

Study setting

This study was conducted between January and December 2016 at the Ladoke Akintola university of Technology (LAUTECH) Teaching Hospital, now Uniosun Teaching Hospital (UTH), Osogbo, Nigeria. UTH is a 600-bed hospital which provides healthcare services in various specialties for the people of Osogbo and its environs.

Ethical approval

Ethical approval for this study was obtained from Ethics Committee of LAUTECH Teaching Hospital, Osogbo, Nigeria (Protocol Number- LTH/REC/2015/06/05/210).

Study design and sampling method

This was a descriptive cross-sectional study in which 359 hospitalized patients with symptoms and signs of clinical infections were consecutively recruited over a period of one year. Consecutive non-duplicate Gram-negative bacilli (GNB) were isolated from the patients' clinical samples at the diagnostic microbiology laboratory of the hospital. Clinical and demographic information were obtained from patients' clinical folders into a designed data collection form.

Collection of samples and laboratory analysis

All urine, faecal, blood, cerebrospinal fluids, sputum, wound and aspirates samples were collected by managing physicians and surgeons from in-patients with clinical symptoms and signs of infection. The samples were processed using standard methods by inoculating all the clinical samples except stool on Blood and MacConkey agar (Oxoid, Basingstoke, United Kingdom). Stool samples were cultured on Deoxycholate Citrate agar (Oxoid, Basingstoke, United Kingdom).

Identification of Gram-negative bacilli

Isolates were identified by colonial morphology, Gram staining and standard biochemical tests including the use of Microbact™ GNB 24E (Oxoid, Basingstoke, United Kingdom) which is a standardised microsubstrate system for the identification of Enterobacteriaceae and miscellaneous GNB based on colour changes in the test due to pH change and/or substrate utilization. The test results generated an octal code which was entered into the Microbact computer aided identification package to give an identification profile for the organism.

Antimicrobial susceptibility test

All Gram-negative bacilli were tested against gentamicin (10µg), amoxicillin-clavulanate (20/10µg), amikacin (10µg), meropenem (10µg), ceftazidime (30µg), cefotaxime (30µg), ciprofloxacin (5µg), cefepime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cotrimoxazole (1.25/23.75µg), and piperacillin-tazo-bactam (100/10µg) (Oxoid, England, UK) using the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (13). Zones of inhibition diameters were measured and interpreted using the guidelines. Isolates which were not sensitive to one or more of the tested third generation cephalosporins and/or aztreonam were regarded to be screening positive for ESBL-production and were subjected to the confirmatory testing.

Phenotypic detection of extended spectrum beta-lactamase (ESBL)

Phenotypic confirmatory test for ESBL production was carried out by combination disc method on GNB that showed resistance to one or more of the tested third generation cephalosporins. This test utilised both single discs of cefotaxime and ceftazidime and their respective clavulanic acid-augmented (13, 14). An increase of 5 mm or more in zones of inhibition of combination discs (cephalosporins with clavulanate) when compared with their respective single discs was taken as phenotypic confirmatory evidence of ESBL production. *Klebsiella pneumoniae* ATCC 700 603 and *Escherichia coli* ATCC 25922 respectively served as positive and negative controls.

Data analysis

The R statistical software package (version 3.3.0) was used for data analysis (15). Univariate analysis was performed to determine the variables associated with acquisition of ESBL-producing GNB. Continuous and categorical variables were compared using Wilcoxon rank-sum and Fisher exact tests respectively, and statistical testing performed using 2-tailed tests. Multivariate logistic regression was used to measure the association between ESBL infection and the prior identified risk factors adjusting for potential confounding variables, with all variables associated with acquisition of ESBLs in the bivariate analysis were included in the initial (full) model. Adjusted odds ratio (OR) and 95% confidence intervals were calculated on the basis of the final multivariate regression model.

Results:

A total of 359 consecutively recruited hospitalized patients from whom GNB were isolated from their different clinical samples over a period of one year (January–December 2016) were studied. One hundred and sixty-eight (46.8%) were males while 191 (53.2%) were females, with a male to female ratio of 0.88 (Table 1). The age range of the patients is 5 days to 83 years while the mean age is 38.9 ± 21.16 years. Patient age > 60 years were the most frequent (n=72; 20.1%) followed by those within age range 51-60 years (n=66; 18.4%) and 31-40 years (n= 58; 16.4%). The patients were predomi nantly traders (n=83; 23.1%), artisans (n= 54; 15%), students (n=51; 14.2%) and civil servants (n=40; 11.1%).

Table 1: Socio-demographic characteristics of hospitalized patients at Uniosun Teaching Hospital, Osogbo (Jan – Dec, 2016)

Variables	Frequency	Percentage
Age group (years)		
≤10	54	15.0
11-20	33	9.2
21-30	24	6.7
31-40	59	16.4
41-50	51	14.2
51-60	66	18.4
>60	72	20.1
Mean age (± SD)	38.91±21.16	
Gender		
Male	168	46.8
Female	191	53.2
Occupation		
Artisan	54	15.0
Civil Servant	40	11.1
Clergy	4	1.1
Farming	20	5.6
Professional	12	3.3
Retiree	27	7.5
Student	51	14.2
Teaching	4	1.1
Trading	83	23.1
Unemployed	34	9.5
Unspecified	30	8.4
Ward/Clinic		
Female Medical Ward	67	18.7
Male Medical Ward	66	18.4
Male Surgical Ward	44	12.3
Gynaecology	24	6.7
Female Surgical Ward	21	5.8
Neonatal ward	19	5.3
Children ward	18	5.0
Children emergency unit	17	4.7
Antenatal ward	16	4.5
Intensive care unit	15	4.2
Accident and Emergency	12	3.3
Orthopaedics ward	11	3.1
Postnatal ward	9	2.5
Burns	8	2.2
Renal	7	1.9
Psychiatric	4	1.1
Ear, nose and throat ward	1	0.3

Table 2: Clinical characteristics of hospitalized patients at Uniosun Teaching Hospital, Osogbo, Nigeria (Jan – Dec 2016)

Clinical characteristics	Number	Percentage
Clinical Diagnoses		
Urinary tract infection	146	40.7
Sepsis	72	20.1
Chronic wound infection	55	15.3
Surgical site infection	32	8.9
Pneumonia	28	7.8
Meningitis	10	2.8
Gastroenteritis	7	1.9
Chronic osteomyelitis	5	1.4
Ear infection	2	0.6
Pyomyositis	2	0.6
Invasive devices		
IVL only	282	78.6
IVL, urocatheter	43	12.0
IVL, endotracheal tube, urocatheter	9	2.5
IVL, urocatheter, wound drains	4	1.1
IVL, abdominal drains	3	0.8
IVL, endotracheal tube	2	0.6
Urocatheter only	2	0.6
None	14	3.9
Co-morbidities		
Hypertension	46	12.8
Malaria	38	10.6
Inguino-scrotal hernia	34	9.5
Diabetes	17	4.7
Head injury	5	1.4
Mania/Psychosis/Schizophrenia	4	1.1
Vaso-occlusive crisis	4	1.1
Malignancy	3	0.8
RTA/Fracture of bones	3	0.8
Ectopic Pregnancy	2	0.6
Cerebrovascular disease	2	0.6
None	201	56.0
Length of admission		
Less than 1 month	332	92.5
More than 1 month	25	7.0
Not available	2	0.6
Length of admission (days) Mean ± SD	11.8 ± 9.1	
Previous admission in the last 1 year	105	29.2
Previous ESBL culture	12	3.3
Previous antibiotic use in the last 3 months	93	25.9
Immuno-compromised state	85	23.7

IVL = Intravenous line; SD = Standard deviation; RTA = Road traffic accident

Of the 359 patients, the most frequent infection type is urinary tract infection, (n=146, 40.7%), followed by sepsis (n=72; 20.1%) and chronic wound infections (n=55; 13.3%). More than 95% of the patients used intravenous catheters either alone (78.6%) or with other invasive devices (17.0%). The major co-morbidities in the patients were hypertension (n=46; 12.8%), malaria (n=38; 10.6%), inguino-scrotal hernia (n=34; 9.5%) and diabetes mellitus (n=17; 4.7%). However, there was no co-morbidity in 56% of the patients. Eighty-five patients (23.7%) had at least one immunocompromised condition and ESBL bacteria were previously isolated in 3.3% of the patients. Other clinical details of the patients are shown in Table 2.

Of the total 359 non-duplicate GNB isolates recovered from different clinical specimens screened for ESBL production, 94 (26. 2%) were ESBL producers, with *Escherichia coli* (n=25; 26.6%) as the commonest ESBL producer followed by *Citrobacter freundii* (n= 23; 24.5%) and *Klebsiella pneumoniae* (n= 13; 13.8%) (Table 3) with the most frequent ESBL producing GNB being *Stenotrophomonas maltophilia* 100% (2/2), followed by *Shigella dysenteriae* 66.7% (2/3), *Yersinia enterocolitica* 50% (2/4), *Enterobacter* sp 35.3% (12/34), *Klebsiella* sp 34.1% (14/41), *Citrobacter* sp 32.5% (27/83) and *Escherichia coli* 27.8% (25/84) while the least frequent ESBL producers are *Proteus* sp 11.1% (5/45), *Acinetobacter baumannii* 10% (1/10), *Pseudomonas aeruginosa* 2.6% (1/39) and *Hafnia alvei* 0%. The prevalence of ESBL-production was significantly lower among *Proteus* sp (OR = 0.3160, 95% CI = 0.1208-0.8268, p=0.0172) and *Pseudomonas* sp (OR=0.06423, 95%CI=0.0087-0.4749, p< 0.0001) compared to other GNB isolates (Table 3).

Table 3: Prevalence of ESBL-producing Gram-negative bacilli infection and univariate analysis

GNB isolates	No of isolates	No of isolates producing ESBL (%)	Crude OR	95% CI	p value
Escherichia coli	84	25 (29.8)	1.265	0.7361 – 2.174	0.3978
Citrobacter sp	83	27 (32.5)	1.1504	0.8804 - 2.569	0.1545
Proteus sp	45	5 (11.1)	0.3160	0.1208 - 0.8268	0.0172*
Klebsiella sp	41	14 (34.1)	1.543	0.7710 - 3.087	0.2569
Pseudomonas aeruginosa	39	1 (2.6)	0.06423	0.0087 - 0.4749	<0.0001*
Enterobacter sp	34	12 (35.3)	1.616	0.7660 - 3.411	0.2205
Morganella morganii	11	3 (27.3)	1.059	0.2749 - 4.080	1.000
Acinetobacter baumannii	10	1 (10.0)	0.3059	0.0382 - 2.448	0.4646
Yersinia enterocolitica	4	2 (50.0)	2.859	0.3968 - 20.597	0.2813
Shigella dysenteriae	3	2 (66.7)	5.739	0.5140 - 64.075	0.1690
Stenotrophomonas maltophilia	2	2 (100)	14.351	0.6822 - 301.92	0.0680
Hafnia alvei	3	0	NA	NA	NA
Total	**359**	**94 (26.2)**			

OR=Odds Ratio; CI=Confidence Interval; NA = Not Applicable; ESBL=Extended Spectrum Beta Lactamase; *=statistically significant; NA=Not applicable

Table 4: Prevalence of ESBL-producing Gram-negative bacilli infection and univariate analysis in relation to specimen types

Clinical samples	No of GNB isolates	No of ESBL producing isolates (%)	Crude OR	95% CI	P value
Urine	159	35 (22.0)	0.6745	0.4162 - 1.093	0.1174
Wound	105	29 (27.6)	1.110	0.6647 - 1.852	0.6939
Blood	46	11 (23.9)	0.8709	0.4228 - 1.794	0.8577
Sputum	28	10 (35.7)	1.634	0.7253 - 3.679	0.2633
CSF	10	3 (30.0)	1.215	0.3076 - 4.800	0.7256
Stool	7	4 (57.1)	3.881	0.8520 - 17.682	0.08
Joint aspirate	1	0	NA	NA	NA
Endocervical swab	1	1 (100.0)	8.519	0.3438 - 211.10	0.2618
Ear swab	1	0	NA	NA	NA
Sequestrum	1	1 (100.0)	8.519	0.3438 - 211.10	0.2618
Total	**359**	**94 (26.2)**			

GNB=Gram-negative bacilli; ESBL=Extended Spectrum Beta-Lactamase; OR=Odds Ratio; NA=Not Applicable

Most of the ESBL producing isolates were obtained from urine (n=35; 37.2%), wound (n=29; 27.6%) and blood (n=11; 11.7%), however in descending order, the frequency of ESBL isolation from the clinical samples is as follows; endocervical swab 100% (1/1), sequestrum 100% (1/1), stool 57.1% (4/7), sputum 35.7% (10/28), CSF 30% (3/10), wound 27.6% (29/105), blood 23.9% (11/46), urine 22% (35/159), joint aspirate 0% and ear swab 0% (Table 4). Comparing the frequency of ESBL producing GNB isolates with respect to age group of patients, gender, ward from where organisms were isolated, diagnosis of infection/disease and other clinical parameters, showed that there is no significant differences in ESBL production with respect to type of specimen, diagnosis, site of infection, admission in previous year, previous use and type of antibiotics, duration of antibiotic treatment, and length of admission (p>0.05) (Tables 4 & 5).

However, from univariate analysis shown in Table 5, the prevalence of clinical infection by ESBL-producing bacteria was significantly higher (OR=1.90, 95% CI=1.18-3.06, p=0.0114) in male (32.7%, 55/168) compared with the female patients (20.4%, 39/191). The prevalence was also significantly higher (OR=2.13, 95% CI=1.23-3.69, p=0.0103) in patients' age above 60 years (38.9%, 28/72) than other age groups. In the same vein, significantly higher prevalence rates were seen in patients admitted into intensive care unit (p<0.001), male surgical ward (p<0.001), farmers (p=0.0191) and retirees (p=0.0385). Significantly higher prevalence of ESBL were also reported in patients who used intravenous line (IVL) alone, or IVL with urocatheter, or IVL with endotracheal tube and urocatheter (p<0.001). In addition, presence of co-morbidities such as head injury, hypertension, benign prostatic hyperplasia (BPH), diabetes mellitus (DM) and immunocompromised states were all significantly associated with prevalence of ESBL-producing organisms (p<0.05) in the univariate analysis.

The results of multivariate logistic regression analysis are shown in Table 6. Apart from hypertension (OR=1.57, 95% CI=0.685-3.637, p=0.2846) and head injury (OR = 2.226, 95% CI = 0.9006 - 5.499, p= 0.084) which were not significantly associated with the prevalence of ESBL in the logistic regression analysis, all other variables such as male gender, admission to intensive care unit or male surgical ward, patients' occupation (farming, retired), presence of multiple invasive devices (IVL, endotracheal tube

and urocatheter, or IVL and urocatheter), underlying co-morbidities such as diabetes mellitus and benign prostatic hyperplasia (BPH), and immunocompromised state, were independent risk factors associated with the prevalence of ESBL GNB in this study.

Table 5: Predictors of ESBL producing Gram-negative bacilli infection by univariate analysis

Variables	**ESBL (%) n=94 (26.2)**	**Non-ESBL (%) n=265 (73.8)**	**Crude OR**	**95% CI**	**p value†**
Male gender	55 (32.7)	113 (67.3)	1.897	1.18 - 3.06	0.0114*
Age >60 years	28 (38.9)	44 (61.1)	2.131	1.232-3.686	0.0103*
Farming occupation	10 (50.0)	10 (50.0)	3.036	1.221-7.549	0.0191*
Retiree	12 (44.4)	15 (55.6)	2.439	1.10-5.42	0.0385*
Clinical diagnosis					
Chronic osteomyelitis	3 (60.0)	2 (40.0)	4.34	0.49-52.41	0.115
Gastroenteritis	3 (42.9)	4 (57.1)	2.15	0.31-12.95	0.384
Meningitis	3 (30.0)	7 (70.0)	1.22	0.20-5.46	0.726
Pneumonia	10 (35.7)	18 (64.3)	1.63	0.65-3.90	0.263
Sepsis	20 (27.8)	52 (72.2)	1.11	0.59-2.03	0.765
Surgical site infection	10 (31.3)	22 (68.7)	1.31	0.53-3.03	0.529
UTI	31 (21.2)	115 (78.8)	1.07	0.64-1.77	0.804
Chronic wound infection	13 (23.6)	42 (76.4)	0.85	0.40-1.72	0.740
Site of infection					
Abdomen	11 (33.3)	22 (66.7)	1.46	0.78-2.20	0.405
Blood stream	20 (27.8)	52 (72.2)	1.07	0.58-1.92	0.886
Extremities	12 (22.6)	41 (77.4)	0.80	0.49-1.38	0.613
Respiratory tract	9 (36.0)	16 (64.0)	1.65	0.62-4.13	0.245
Urinary tract	32 (21.5)	117 (78.5)	1.09	0.65-1.81	0.713
Previous admission in the last 1 year	25 (23.8)	80 (76.2)	0.84	0.47-1.46	0.598
Previous ESBL culture in last 1 year	3 (25.0)	9 (75.0)	0.94	0.16-3.87	1.000
Previous antibiotic use in last 3 mths	20 (21.5)	73 (78.5)	0.71	0.42-1.17	0.274
Admission for longer than 1 month	7 (28.0)	18 (72.0)	1.10	0.38-2.89	0.816
Antibiotic treatment for > 1 week	22 (22.7)	75 (77.3)	0.77	0.43-1.37	0.418
Admission into ICU	11 (73.3)	4 (26.7)	8.65	1.23-3.69	0.0001*
Admission into MSW	24 (54.5)	20 (45.5)	4.2	2.19 - 8.05	0.0001*
IVL	56 (19.9)	226 (80.1)	0.254	0.15-0.43	0.0001*
IVL, endotracheal tube, urocatheter	8 (88.9)	1 (11.1)	24.558	3.03-199.26	0.0001*
IVL, urocatheter	25 (58.1)	18 (41.9)	4.972	2.564-9.641	0.0001*
Head Injury	4 (80.0)	1 (20.0)	11.73	1.294-106.41	0.0179*
Hypertension	21 (45.7)	25 (54.3)	2.762	1.46 – 5.22	0.0021*
BPH	6 (54.5)	5 45.5)	3.545	1.056-11.907	0.0401*
Diabetes Mellitus	6 (60.0)	4 (40.0)	4.449	1.227-16.135	0.0231*
Immunocompromised states	30 (35.3)	55 (64.7)	1.790	1.058-3.028	0.0342*

†Fisher exact test for dichotomous predictors, MSW= male surgical ward, ICU =intensive care unit, IVL = intravenous line, BPH = benign prostatic hyperplasia; OR = Odds Ratio; CI = Confidence Interval; ESBL= Extended Spectrum Beta Lactamase, UTI = urinary tract infection, *statistically significant

Table 6: Independent predictors of ESBL-producing Gram-negative bacilli infection in multivariate logistic regression model

Predictor	Adjusted OR	95% CI	p value
Male gender	1.131102	1.033234 - 1.238240	0.00798*
Age >60years	3.264896	2.315363 - 4.603835	0.0225*
ICU	1.7623826	1.2886753 - 2.410221	0.000443*
MSW	1.4605132	1.1224911 - 1.900326	0.005078*
Farming	1.3448686	1.0755175 - 1.681676	0.009800*
Retiree	1.2721912	1.0402855 - 1.555794	0.019660*
IVL, Endotracheal Tube, Urocatheter	1.9943284	1.5203033 - 2.616153	$9.74e^{-7*}$
IVL/Urocatheter	1.4664049	1.2860782 - 1.672016	$2.31e^{-8*}$
Head Injury	2.225541	0.9006394 - 5.499462	0.0840
Hypertension	1.578574	0.6850692 - 3.637435	0.2846
BPH	1.725392	1.2017102 - 2.477285	0.00335*
Diabetes Mellitus	1.822119	1.2583221 - 2.638527	0.00164*
Immunocompromised states	1.126781	1.012884 - 1.253484	0.0288*

ICU- intensive care unit, MSW- male surgical ward, IVL- intravenous line, BPH- benign prostatic hyperplasia; OR = Odds Ratio; CI = Confidence Interval; *=statistically significant

Discussion:

In this study, we identified some risk factors for ESBL-producing bacterial infection by univariate and multivariate logistic regression analyses. Age greater than 60 years, male gender, admission to the intensive care unit (ICU) and male surgical ward, patients' occupation (farmers and retirees), use of IVL, endotracheal tube, and urocatheter, underlying illnesses such as DM, BPH, immunocompromised state of the patients were identified as independent risk factors for ESBL infection. Only a few studies have investigated the risk factors for ESBL-GNB infections in a tertiary care hospital in this environment. Therefore, this study adds new information to the knowledge gap that exists in this area of clinical care.

High prevalence of ESBL-producing bacteria among retirees and patients aged 60 years and above noted in our study has previously been reported by Musikatavorna et al., (16) among bloodstream infection cases in Thailand. This can be explained by the increase in frequency and duration of hospital admissions usually associated with this advanced age category as well as the immune system fragility of these elderly patients, making them more prone to clinical infection, leading to increase antimicrobial use (17).

Uropathogens contributed the largest number of uropathogens while prevalence for ESBL-producing bacteria is highest in stool (57.1%), followed by sputum (35.7%) and wound (27.6%). This study also showed that BPH and admission into surgical wards are predictors of infection by ESBL-producing bacteria. Complicated UTI in men is associated with structural or functional abnormality in the urinary tract, often requiring prolonged antibiotic treatment (18). All these factors make male patients with BPH admitted to surgical wards more prone to acquiring multidrug resistant bacteria (19). These findings had been well noted in an Asian study which reported high prevalence of ESBL-producing pathogens as agents of UTI especially among surgical patients (9). Similar to our findings, a study in Mexico also reported urological abnormalities (OR=3.88, 95% CI 1.31-11.47, p=0.005), and urinary catheterization (OR = 3.90, 95% CI = 1.13 - 14.08, p = 0.008) as factors significantly associated with acquisition of ESBL-producing uropathogens (20). Likewise, high prevalence of ESBL-producing bacterial infection in surgical wards was also established in a hospital-based case control study among Swedish population (9). Though a higher prevalence of ESBL-producing bacteria was found in male patients compared to female patients in our study, other researchers have reported significantly higher rate in the female gender (21), hence it is expedient that clinicians look out for factors predisposing factors to infection by ESBL-producing bacteria in individual patients irrespective of their age and gender.

It has been shown in this study and those of others (22,23) that patients admitted to ICU were more likely to have infections by ESBL-producing bacteria compared to patients in the other wards. It is a common knowledge that ICU is a hotspot for antibiotic-resistance and this is attributed to excessive use of broad-spectrum antimicrobial agents such as third-generation cephalosporins, vancomycin and imipenem, which have a higher propensity for selecting antimicrobial-resistant bacteria (24). Also, the specific risk profiles of patients coupled with multiple procedures and use of invasive devices (such as intubation, mechanical ventilation, vascular access) makes ICU the epicentre of resistance development. Among critically ill patients, invasive devices/procedures like central venous line, mechanical ventilation and stomach tube catheterization constituted significant risks for infections by the drug-resistant pathogens (6,25). Worse still for these patients, commencement of appropriate antibiotic therapy is often delayed with grave consequences. Delay in effective treatment of patients with systemic infe-

ction caused by ESBL-producing bacteria has far reaching prognostic implications, patients often become clinically critical necessitating ICU care in many cases which further reduces their chances of survival. A study done in the UK revealed a significant delay in instituting appropriate therapy for cases of ESBL-producing bacterial infections (OR 9.17, 95% CI 2.00 - 42.20, p=0.0005) with survival estimates demonstrating a significantly increased early (<25 days after infection) mortality (OR for death 3.93, 95% CI 1.05-14.63, p=0.03) (35). Admission into ICU is a documented risk for death from bacteraemia caused by ESBL-producing pathogens (26).

Our study further revealed immunocompromised states including DM as risks for ESBL-producing bacterial infections. Silva et al., (7) had reported similar findings in Brazil in which malignancy and DM independently predisposed patients to nosocomial infections caused by ESBL-producing *Klebsiella pneumonia*. Similarly, in a study conducted in a paediatric tertiary hospital in Bangkok Thailand, prevalence of ESBL infection was reported to be higher among patients with immuno compromised conditions, especially haematologic malignancies than among patients without underlying disease. Although the development of antimicrobial resistance is a natural phenomenon, immuno-compromised conditions such as DM predispose patients to repeated and multiple infections making excessive use of antimicrobials inevitable, and thus contributing to emergence and spread MDR organisms including ESBL-producing bacteria (27). Immunocompromised conditions such as AIDS make it possible for patients to become reservoirs of MDR opportunistic pathogenic organisms with vast abilities for horizontal dissemination (28).

Our study also identified an association between farming occupation and infection by ESBL-producing pathogens. Farmers in this environment are generally peasant who are mostly down in the socioeconomic ladder, and lack of money to facilitate appropriate antimicrobial treatment is one of the major poverty-driven factors contributing to AMR among them (29). These patients may only complete a truncated course of therapy because of their inability to pay for the full course of medications. Widespread neglects of health financing is responsible for persistence of user fees as mainstay of health financing manifesting in increasing out-of-pocket expenditures which further aggravates poverty in-country (30).

The limitations of our study include being a cross-sectional design, it did not allow determination of cause-effect relationship, and also data were collected from case folders of participating patients with the possibility of incomplete record.

Conclusion:

In conclusion, our study revealed that elderly, male gender and farming as well as admission into male surgical ward and intensive care unit are predisposing factors for acquisition of ESBL-producing bacteria. Other identified risks are use of invasive devices, benign prostatic hyperplasia and immunocompromised states including DM. Identifying these factors provides the basis for infection prevention and control interventions as well as protocols for improved antibiotic use to strengthen antimicrobial stewardship in our hospital setting.

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