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Antimicrobial resistance patterns of phenotype Extended Spectrum Beta-Lactamase producing bacterial isolates in a referral hospital in northern Tanzania

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Abstract

Background: Production of Extended Spectrum Beta-Lactamase (ESBL) by bacteria is a chronic problem in a health care set up. In order to have adequate information for treatment of bacterial infections especially ESBL producing isolates, it is crucial to understand the trends in the antibiotic-resistance pattern, occurrence and their geographical spread. The objective of this study was to determine the antimicrobial resistance pattern among phenotype ESBL producing isolates in northern Tanzania.

Methods: From July 2013 to January 2014, urine, pus and blood samples were collected from patients suspected to have bacterial infections at Kilimanjaro Christian Medical Centre in Moshi, Tanzania. The isolates were identified based on standard laboratory procedures. Antimicrobial susceptibility tests were carried out using various antimicrobial discs as per the recommendations of Clinical Laboratory Standard Institute.

Results: A total of 330 specimens were collected. They consisted of 46 urine, 264 pus (from wound) and 20 blood samples. Among isolated bacteria, ESBL producers were 29.7% (98) and non-producers were 70.5% (232). *Escherichia coli* and *Klebsiella pneumoniae* were the most isolated bacteria and dominant ESBL producers. ESBL production was highly associated with moderate condition at discharge and longer periods of admission. More than 60% of the ESBL producing *E. coli* were resistant to ceftazidime, cefpodoxime, cefotaxime, amoxycilin, ciprofloxacin, and gentamycin. More than 80% of ESBL producing *K. pneumonia* and *Proteus mirabilis* were resistant to ceftazidime and cefotaxime. Fifty four percent of ESBL producing *K. pneumonia* were resistant to gentamycin.

Conclusion: This study shows that ESLB phenotypes among Gram-negative bacteria are common among patients attending a tertiary hospital in northern in Tanzania. The findings suggest that clinical microbiology laboratories should take into account the diagnosis of ESBL producers in order to define the degree of the problem so as to establish a proper treatment protocol.

Keywords: Antimicrobial resistance, Extended spectrum beta lactamase, Tanzania

Introduction

Production of β-lactamase is the most commonly encountered mechanism of resistance of bacterial pathogens to β-lactam antibiotics. Extended-spectrum β-lactamases (ESBLs) are diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of patients (Rawat & Nair, 2010). ESBL-producing gram-negative bacteria have posed a significant threat to patients due to their hydrolyzing activity against extended spectrum cephalosporin (Paterson *et al.*, 2001; Paterson & Bonomo, 2005) Resistance to third generation cephalosporin by possession and expression of ESBL enzymes among gram-negative bacilli is on a rise (Rawat & Nair, 2010). ESBLs confer resistance not only to penicillin, aztreonam, and cephalosporin but could also be resistant to other antimicrobial classes including aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones (Paterson *et al.*, 2000; Paterson & Bonomo, 2005).

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ESBLs are encoded by genes located on large plasmids, and these also carry genes for resistance to other antimicrobial agents (Paterson & Bonomo, 2005). It has been documented that majority of ESBL producing bacteria are *Klebsiella pneumoniae, K. oxytoca and Escherichia coli* (Babini & Livermore, 2000). The presence of ESBL producing bacteria significantly affects the course and outcome of an infection and poses a challenge to infection management worldwide (Tsering *et al*., 2009).

Patients may be affected with resistant bacteria or acquire resistance during/ prior antimicrobial exposure during treatment or in food residues (Food insight, 2012). Patients infected with ESBL-producing bacteria may have a higher mortality rate and may require longer hospital stays because they are generally sicker and have received more antibiotics than patients who are not infected with ESBL-producing bacteria (Ramphal & Ambrose, 2006). It has been shown that infection caused by ESBL producing gram-negative bacteria complicate therapy and limit treatment options because such bacteria are resistant to a number of broad-spectrum antibiotics (Paterson, 2000). The efficacy of antibiotic treatment is rapidly decreasing as a result of the continual spread of antibiotic resistance genes in pathogen populations. However, extent of ESBL-producing bacteria remains unclear in most parts of the world. This study aimed to provide baseline information on antibiotic resistance patterns mediated by ESBLs of bacteria isolated from urine, blood and pus samples in northern Tanzania. The objective of the study was to isolate ESBL producing bacterial isolates and to determine the clinical outcome of the patients infected with ESBL bacteria at a tertiary hospital in Tanzania.

Materials and Methods

Study site

Kilimanjaro Christian Medical Centre (KCMC) is a consultant referral hospital with 500-bed capacity. KCMC is located in Moshi town in northern Tanzania. It has a 500-bed capacity and the second largest consultant referral teaching hospital serving over 11 million people from northern and central regions of Tanzania (http://www.kcmc.ac.tz/).

Specimen collection and identification of isolates

Between July 2013 and January 2014, 330 consecutive and non-duplicate specimens (urine, blood and pus) were collected aseptically from all patients aged 1 to 80 years who attended outpatient and inpatient departments of the hospital. Specimens were transported to the Department of Microbiology laboratory and processed immediately. The specimens were cultured on to Mac-Conkey agar and blood agar plates. Culture plates were incubated aerobically at 37°C for 18-24 hours and the colonies identified based on morphology. All patients with positive cultures were included in the study. Gram-negative bacilli isolated were characterized by performing gram's staining, motility and standard biochemical tests as described by Collee *et al*. (1996).

Antimicrobial susceptibility testing

Bacterial isolates were selected to determine their susceptibility patterns against selected antimicrobial agents, by the disc diffusion method of Kirby- Bauer as described by Clinical Laboratory Standards Institute (CLSI) (CLSI, 2011). The antimicrobial agents used were: gentamicin (10 µg), Ciprofloxacin (5 µg), amoxicillin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg) and cefpodoxime (10 µg). Bacteria showing zone of inhibition of ≤ 25 mm for ceftriaxone and ceftazidime were selected for confirmation test of ESBL, according to guidelines of CLSI (CLSI, 2011).

The potential ESBL producing bacteria were confirmed for ESBL production by phenotypic test. Briefly, a lawn culture of the isolated bacteria on Mueller Hinton agar (MHA)

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was made and ceftazidime (30 µg) and the combination disc ceftazidime + clavulanic acid (30 µg + 10 µg) was placed with 25 mm apart. An increase of ≥ 5 mm in zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime alone was confirmed as ESBL producers, as per recommendations of CLSI (CLSI, 2011). ESBL producing *Klebsiella pneumonia* ATCC 700603 and non-ESBL producing *E. coli* ATCC 25922 were used as positive and negative control respectively.

Clinical outcome determination

Clinical outcome was evaluated as length of hospital stay (in days) after diagnosis of infection. Death was considered attributable to infection if occurred within two weeks from the diagnosis of infection, in the presence of clinical and laboratory evidence of active infection/ and or if it occurs during the admission period, and could not be strictly related to other fatal conditions. Death was considered to be unrelated to infection when it occurred more than two weeks after the diagnosis of infection or will be strictly related to other fatal conditions (Bellíssimo-Rodrigues et al., 2006). A condition at discharge was scored as severe, moderate and mild. Patients were followed until hospital discharge.

Data analysis

Analysis of data was carried out using Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, USA).

Ethical considerations

During this study interviewers introduced themselves and explained the objectives and all procedures to all potential interviewees. A written consent form was obtained from adults or guardians of those individuals aged less than 18 years prior to inclusion. If the legal guardian was illiterate, signed consent was sought from another literate witness chosen by the guardian. This study received ethical approval from Kilimanjaro Christian Medical University College Research and Ethics Review Committee with Research Ethical clearance number 550.

Results

A total of 330 participants were recruited, more males were recruited than females. Adults (≥ 18 years) were more recruited than children (<18 years) (Table 1). The specimens collected consisted of 46 urine, 264 pus (from wound) and 20 blood samples. Out of 330 bacteria isolates 214 were Gram-negative and 116 were Gram-positive bacteria. *Escherichia coli* and *Klebsiella pneumonia* were most isolated bacteria. Some 44.4% and 40.7% of *E.coli* and *K. pneumonia* were ESBL producers, respectively. We analyzed the association of ESBL producing bacteria and the clinical outcome of patients who were followed up. ESBL production was highly associated with moderate condition at discharge; X^2=10.89. p=0.001. ESBL production was also highly significantly associated with longer periods of admission (X^2=19.83, p<0.001) (Table 1).

ESBL producers were tested for their susceptibility to 3rd generation cephalosporin. Generally, most of isolates were resistant to cephalosporin. More than 60% of the ESBL producing *E. coli* were resistant to ceftazidime, cefpodoxime and cefotaxime (p<0.05). More than 90% and 80% of ESBL producing *K. pneumonia* and *P. mirabilis* were resistant *to* ceftazidime and cefotaxime, respectively (p<0.05). Surprisingly all ESBL producing *P. aureginosa* were resistant to all 3rd generation cephalosporin tested (p<0.05) (Table 2).

ESBL producers were also tested for their susceptibility to cephalosporin (amoxycilin, ciprofloxacin and gentamycin). More than 62% of ESBL producing *E. coli* were resistant to Amoxycilin, Ciprofloxacin and Gentamycin, (p<0.05). Fifty four (54.4)% of ESBL producing *K. pneumonia* were resistant to Gentamycin, (p<0.006). All *K. oxytoka* were resistant to

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Ciprofloxacin (p=0.002), and all *P. aureginosa* were susceptible to Gentamycin (p=0.04) (Table 3).

Table 1: Demographic characteristics and clinical outcome of ESBL producers and Non-ESBL producers in bacteraemic patients

Characteristic	Variable	No.	No. (%) ESBL producer	No. (%) Non-ESBL producer	P-value
All		330	98 (29.7)	232 (70.5)	
Age (years)	<18	70	26 (37.1)	44 (62.9)	0.1
	≥18	260	72 (27.7)	188 (72.3)	
Sex	Male	212	66 (31,1)	146 (68,9)	0.4
	Female	118	32 (27.1)	86 (72.9)	
Department	Inpatient	280	10 (20.0)	40 (80.0)	0.1
	Outpatient	50	88 (31.4)	192 (68.8)	
Sample	Urine	46	10 (27.7)	26 (78.3)	0.4
	Pus	264	82 (31.1)	182 (68.9)	
	Blood	20	6 (30.0)	14 (70.0)	
Ward/Clinic	Medical	20	8 (40.0)	12 (60.0)	0.7
	Surgical	242	70 (28.9)	172 (71.1)	
	Urology	58	18 (31.0)	40 (69.0)	
	OG	8	2 (25.0)	6 (75.0)	
	Paediatric	2	0 (0.0)	2 (100.0)	
Bacteria isolate	*E. coli*	54	24 (44.4)	30 (55.6)	0.005
	K. pneumonia	54	22 (40.7)	32 (59.3)	
	K. oxytoka	10	2 (20.0)	8 (80.0)	
	P. aeruginosa	32	8 (25.0)	24 (75.0)	
	P. mirabilis	44	10 (22.7)	34 (77.3)	
	Other Proteus species	20	8 (40.0)	12 (60.0)	
Condition at discharge	Moderate	16	12 (75.0)	4 (25.0)	0.001
	Mild	28	6 (21.4)	22 (78.6)	
Period of admission (days)	2				
	3-7	34	10 (29.4)	24 (70.6)	<0.001
	8-14	16	8 (50.0)	8 (50.0)	
	15-21	14	12 (85.7)	2 (14.3)	
	>21	16	16 (100.0)	0 (0.0)	

OG= Obstetrics & Gynecology

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Table 2: Antibiotic resistance pattern of ESBL producing bacteria to 3rd generation cephalosporin

Bacteria	No.	Ceftazidime			Cefpodoxime			Cefotaxime		
		S	R	p-value	S	R	p-value	S	R	p-value
E. coli	24	8(33.3%)	16(66.7%)	<0.001	2(9.1%)	20(90.9%)	0.01	2(8.3%)	22(91.7%)	<0.001
K. pneumoniae	22	2(9.1%)	20(90.9%)	<0.001	6(27.3%)	16(72.7%)	0.2	2(9.1%)	20(90.9%)	<0.001
K.oxytoka	2	0(0%)	2(100%)	0.002	2(100%)	0(0%)	0.4	0(0%)	2(100%)	0.197
P.aeroginosa	8	0(0%)	8(100%)	0.001	0(0%)	8(100%)	0.007	0(0%)	8(100%)	0.02
P.mirabilis	10	2(20%)	8(80%)	<0.001	6(60%)	4(40%)	0.7	2(20%)	8(80%)	0.001
Other Proteus	8	2(25%)	6(75%)	0.06	4(50%)	4(50%)	0.4	0(0%)	8(100%)	0.003

R=Resistant, S=Susceptible

Table 3: Antibiotic resistance pattern of ESBL producing bacteria to Non- 3rd generation cephalosporin

Bacteria	No.	Amoxycilin			Ciprofloxacin			Gentamycin		
		S	R	p-value	S	R	p-value	S	R	p-value
E. coli	24	2(8.3%)	22(91.7%)	0.005	9(37.5%)	15(62.5%)	<0.001	6(25.0%)	18(75.0%)	<0.001
K. pneumoniae	22	4(18.2%)	18(81.8%)	0.05	14(63.6%)	8(36.4%)	0.1	10(45.5%)	12(54.5%)	0.006
K.oxytoka	2	0(0%)	2(100%)	0.1	0(0%)	2(100%)	0.002	0(0%)	2(100%)	0.05
P.aeroginosa	8	0(0%)	8(100%)	0.1	7(87.5%)	1(12.5%)	0.07	8(100%)	0(0%)	0.04
P.mirabilis	10	4(40%)	6(60%)	0.4	8(80.0%)	2(20%)	0.2	4(40%)	6(60%)	0.5
Other Proteus	8	0(0%)	8(100%)	0.06	6(75.0%)	2(25.0%)	0.06	4(50%)	4(50%)	0.1

R=Resistant, S=Susceptible

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Discussion

In present study ESBL prevalence is high and comparable to findings from other studies in referral hospitals in Tanzania (Ndugulile *et al.,* 2005; Kariuki *et al.,* 2007; Mshana *et al.*, 2011). In clinical settings in Tanzania, the prevalence of extended spectrum beta-lactamases (ESBL)-producing bacteria ranges from 25% to 50% (Mshana *et al*.,2009, 2013a). Other studies in sub-Saharan Africa have shown similarly high levels (20-90%) of antibiotic resistance for common isolates clones of ESBL producing *Escherichia coli* ST131 and *Klebsiella pneumonia* from humans and animals (Laxminarayan et al. 2013; Mshana et al., 2013a). ESBL producers were commonly found in *E. coli* and *K. pneumonia* with most of the bacteria isolated from pus samples. ESBL producing isolates were more common among the *E. coli* as compared to the *K. pneumoniae* isolates which is in agreement with the findings of a study by Umadevi *et al.* (2011). However, in a study by Mshana *et al*. (2009), *K. pneumonia* was reported as the most common ESBL producers.

Most ESBL producing isolates showed resistance to advance generation cephalosporins (cefotaxime, ceftazidime, and cefpodoxime). This could happen due to the incidental presence of various other mechanisms of resistance in a given bacterium. These findings are in agreement with previously published studies (Chander & Shrestha, 2013). We have noted that ESBL producers were significantly resistant to ciprofloxacin and gentamicin, indicating co-transfer of aminoglycoside resistance on a resistance plasmid, thus posing clinical challenges. This phenomenon has been reported by other studies (Jocoby & Sutton, 1991; Paterson, 2000; Spanu *et al.*, 2002). The transfer of resistance from one bacteria species is possible and this is likely due to the fact that ESBLs are plasmid mediated. Plasmids carrying ESBLs are transferable from one bacteria to the next and between different bacterial species (Rupp & Fey, 2003).

Patients infected with ESBL-producing bacteria may have a higher mortality rate and may require longer hospital stays because they are generally sicker and have received more antibiotics than patients who are not infected with ESBL-producing bacteria (Ramphal & Ambrose, 2006). In this study we associated ESBL producers with longer hospital stay and condition at discharge, and we found significant association. Most patients infected with ESBL bacteria were hospitalized for more than three weeks, and this can cause high cost. Apart from high cost of care and treatment among hospitalized patients, continual exposure to antibiotics during hospitalization eventually can lead to development of resistance to bacteria. Moderate conditions during discharge were more pronounced among ESBL producers as compared to non-ESBL producers.

Most isolated ESBL producing bacteria were resistant to third generation cephasporins. Antimicrobial resistance has become a public health problem in Sub-Saharan Africa (Mshana *et al.*, 2013b). Our data indicate high proportion of ESBL resistant isolates suggesting cepharosporins use should be limited in order to control the increasing treatment challenge of ESBL. In our settings screening for ESBL production among bacteria isolates is not routinely done in many clinical laboratories and the evidence of treatment failure of most antibiotics is not well documented. Thus, the detection of ESBLs indicates the need for the use of appropriate antibacterial agents. ESBL detection has become a diagnostic challenge in clinical laboratories. In most cases, the standard disk diffusion tests are effective, and they are still recommended for ESBL detection in routine laboratories (Giriyapur *et al*., 2011).

There are limited data from surveillance studies on antimicrobial resistance in northern Tanzania. To the best of our knowledge this is the first report from the region with an exclusive focus on investigating the prevalence and antimicrobial resistant pattern of ESBL producing isolates. Therefore, our study presents the baseline information on the ESBL prevalence and resistance to antimicrobial agents and its association with clinical outcome. This present study suggests that clinical microbiology laboratories should take into account the diagnosis of ESBL

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producers in order to define the degree of the problem so as to establish a proper patient management and treatment protocol. We recommend more studies to investigate the magnitude of ESBL production in bacteria isolated from food of animal origins so that to have a comparison and envisage antibiotic usage in animal and human.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

DK, PN, JC, and BN were involved in the conception and study design as well as data analysis. Microbiological analyses were done by NK, CM, FK and BK. VM and RK drafted the manuscript. All authors read and approved the final version of the manuscript.

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