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Extended-Spectrum-Beta-Lactamase (ESBL) production among *Escherichia coli* and *Klebsiella species* in Kumasi, Ghana.

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Abstract

In recent times *Enterobacteriaceae* including *Escherichia coli* and *Klebsiella spp.*, isolated in Kumasi at the Komfo Anokye Teaching Hospital (KATH) have shown significant resistance to 2nd and 3rd generation cephalosporins. Microbial resistance to these antimicrobials if due to the production of Extended Spectrum-Beta-Lactamases (ESBLs) may also indicate resistance to the aminoglycosides and other antibiotics. A total of 300 non-selected, non-duplicate isolates were collected and ESBL production was phenotypically determined using the Combined Disc Method. 149 (49.67%) of the isolates were ESBL- producing *E. coli* (44.37%) or *Klebsiella spp.* (55.03%). ESBL-producing organisms were more common among in-patients (54.60%) than they were among out-patients (46.27%). Meropenem emerged as the antimicrobial agent of choice for the treatment of serious infections associated with ESBL-producers on the basis of *in vitro* testing. The high level of ESBL production found in these *Enterobacteriaceae* with the resultant microbial resistance to the available cephalosporins and other agents may pose difficulties with the choice of therapeutic options for the treatment of severe infections. Efforts to prevent and/or control outbreaks of infections with ESBL-producing organisms must emphasize on the judicious use of all antibiotics.

Keywords: Extended Spectrum-Beta-Lactamases (ESBLs), Cephalosporins, Enterobacteriaceae.

1. Introduction

Extended-Spectrum-Beta-Lactamases (ESBLs) are enzymes which confer antibiotic resistance on certain bacteria in the family *Enterobacteriaceae*[1].

Microorganisms which produce ESBLs could be of great health concern in Kumasi at the Komfo Anokye Teaching Hospital (KATH) not just because they have been reported worldwide but also because in 2006, 25.02% of all *Enterobacteriaceae* isolated from urine and blood, from KATH, were resistant to cephalosporins (cefuroxime, ceftriaxone, cefotaxime and ceftazidime) (KATH Lab records, 2006). Again, records from the KATH Microbiology Laboratory in 2006 indicated that, of the total *Enterobacteriaceae* isolated from urine and blood 31.19% were resistant to gentamicin (an aminoglycoside), 23.00% to ciprofloxacin (a fluoroquinolone) and 75.34% to tetracycline. Some of these multi-resistant isolates could probably harbour ESBLs thus limiting therapeutic options. The delay in detecting and reporting ESBL-producers may lead to prolonged hospitalization of patients, increased morbidity and mortality as well as increased cost of health care[2].

The study investigated ESBL production among *E. coli and Klebsiella spp.* isolated from mainly blood and urine, at the Komfo Anokye Teaching Hospital, Kumasi.

2. Materials and Methods

2.1 Materials

Blood and urine samples were collected and cultures performed to isolate *E. coli and Klebsiella spp.* used in this work.

2.2 Study Site

This study was undertaken in Kumasi at the Microbiology laboratory of the Komfo Anokye Teaching Hospital (KATH).

2.3 Sample Size

300 non duplicate *E. coli and Klebsiella spp.* used in the study were isolated mainly from blood and urine in this study.

2.4 Methods

2.4.1 Sample Collection and Culture

The blood specimens collected were inoculated into a 25 ml brain-heart infusion broth. The bottles were then incubated overnight and subsequently subcultured on blood and MacConkey agar plates to isolate the microorganisms. The urine isolates were obtained after inoculating 20ml of mid-stream-urine onto Cysteine Lactose Electrolytes Deficient (CLED) agar.

2.4.2 Data Collection

The age, gender and location (i.e. in- or out-patient) of patients from whom the microorganisms were isolated were recorded.

2.4.3 Identification of Isolates

The isolates were identified using the API 20E (bioMérieux, France).

2.4.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of each isolate was determined by the Kirby-Bauer disc diffusion method, and interpreted using the British Society of Antimicrobial Chemotherapy (BSAC) standards[4].

The antibiotic discs used in this study were cefotaxime (CTX:30µg), ceftazidime (CAZ:30µg), ceftriaxone (CTR:30µg), cefuroxime (CXM:30µg), ampicillin (AMP:10µg), amikacin (AMK:30µg), gentamicin (GEN:10µg), nitrofurantoin (NIT:300µg), nalidixic acid (NAL:30µg), norfloxacin (NOR:10µg), ciprofloxacin (CIP:5µg), cotrimoxazole (COT:1.25/23.75µg), tetracycline (TET:30µg), chloramphenicol (CHL:30µg), pipemidic acid (PPA:20µg), meropenem (MEM:10µg), imipenem (IMP:10µg) and levofloxacin (LVX:5µg).

2.4.5 Determination of ESBL-producers

All isolates were tested for ESBL production by the Combined Disc Method (CDM) using Cefotaxime (30 µg) and ceftazidime discs (30µg) or cefpodoxime (30µg) disc with and without clavulanate (10 µg) on Mueller-Hinton agar plate.[5,6] The CDM was selected for the phenotypic detection of ESBL-producers because it has a good sensitivity and specificity (i.e. 94.4% and 97.0% respectively) for the detection ESBL producing *E. coli* and *Klebsiella spp*.[6]

2.4.6 Statistical Analysis

SPSS 14 Evolution (SPSS Inc.) software was used to analyse the epidemiological data and risk factors for the isolation of an ESBL-producer.

3. Results

149 out of the 300 isolates studied were ESBL producers. Of the ESBL-producers, 115 were from urine (n=72) and blood (n=43). 37 of the ESBL-producers from urine were *Klebsiella spp*., and 35 *E.coli*. Of the 43 ESBL-producers isolated from blood, 23 and 20 were *Klebsiella spp.* and *E. coli* respectively.

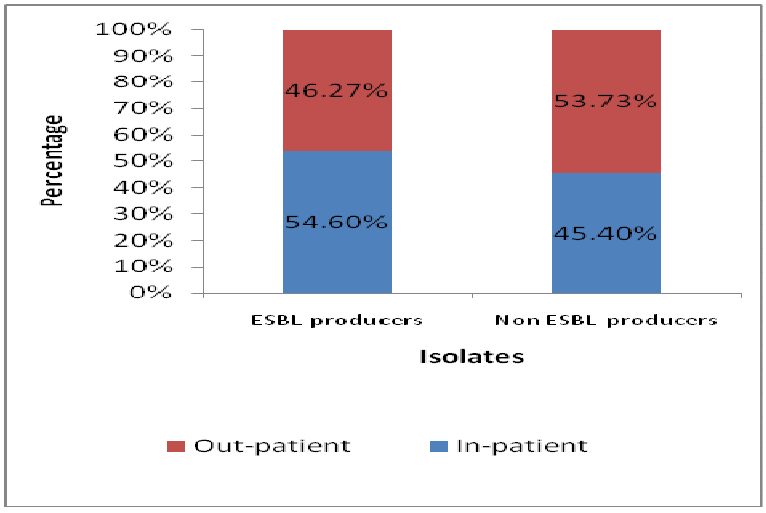

Figure 1: ESBL-producers from in-patients and out-patients

ESBL-producers were more common among in-patients (54.60%) than out-patients (46.27%), although the difference was not statistically significant (OR = 1.40, 95% CI = 0.79-2.46, *p*= 0.30). 95 out of the 174 organisms isolated from in-patients were ESBL-producers whilst 31out of 67 from out-patients were ESBL-producers. (Figure 1)

Table 1: Antimicrobial susceptibility profile of all isolates, non ESBL-producers and ESBL-producers

Antimicrobial agents	% resistant to antimicrobial agents		Total number
	Non ESBL-producers	ESBL-producers	
CTX	11.36	72.72	154
CAZ	40.00	71.43	12
CTR	11.96	70.58	160
CXM	28.35	92.42	259
AMP	93.18	96.92	262
AMK	10.00	20.00	30
GEN	31.94	81.43	284
NIT	28.86	19.70	118
NAL	60.38	80.88	121
NOR	44.68	79.41	115
CIP	32.35	56.73	206
COT	80.15	96.30	271
TET	86.27	94.23	103
CHL	75.00	90.00	154
PPA	50.00	73.68	31
MEM	0.00	2.12	82
LVX	0.00	66.67	9
IMP	0.00	0.00	1

There was a very high antimicrobial resistance to ampicillin, tetracycline, cotrimoxazole and chloramphenicol in both non ESBL-producers and ESBL-producers but a marked increase in the prevalence of drug resistance amongst ESBL-producers to the cephalosporins, fluoroquinolones and aminoglycosides. (Table 1)

Table 2: Predictors of ESBL production

Antimicrobial agents	Odds ratio	*p* Value	Total number (*n*)
CTX	**20.8**	**0.001**	**154**
CAZ	3.75	0.636	12
CTR	**17.67**	**0.001**	**160**
CRX	**30.84**	**0.001**	**259**
AMP	2.3	0.268	262
AMK	2.25	0.865	30
GEN	**9.34**	**0.001**	**284**
NIT	0.61	0.348	118
NAL	**2.78**	**0.023**	**121**
NOR	**4.78**	**0.001**	**115**
CIP	**2.74**	**0.001**	**206**
COT	**6.44**	**0.001**	**271**
TET	2.6	0.305	103
CHL	**3**	**0.029**	**154**
PPA	2.8	0.346	31

From logistic regression analysis, resistance to any of the following antimicrobial agents, namely cefotaxime (OR=20.80), ceftriaxone (OR=17.67), cefuroxime (OR=30.84), gentamicin (OR=9.34), nalidixic acid (OR=2.27), norfloxacin (OR=4.78), ciprofloxacin (OR=2.74), cotrimoxazole (OR=6.44) and Chloramphenicol (OR=3) is closely associated with the production of ESBL. (Table 2)

4. Discussion

Several studies have shown that ESBL- production amongst *Enterobacteriaceae* is a worldwide problem[7] and its prevalence varies from country to country. The prevalence levels of ESBL-producers obtained in this study (44.37% *E. coli,* 55.03% *Klebsiella spp.*) are higher than those reported for *E. coli* and *K. pneumoniae* in most parts of the world including, USA[8], India[9], China[10] and Nigeria[11]. The high prevalence of ESBL producing *K. pneumoniae* at KATH was however comparable to the situation in Turkey which was 57.1%[12].

More than 50% of the isolates from blood (58.11%) and urine (57.14%) were ESBL-producers with blood accounting for the highest incidence of ESBL-producers. This observation is disturbing because of the serious nature of blood stream infections. Patients who were treated with any of the cephalosporins may not have benefited from its use as a result of treatment failures which may have been avoided if ESBL testing had been done.

In contrast to findings in an earlier report[13], hospitalization was not a risk factor associated with infection caused by ESBL-producers in this study (OR = 1.40, 95% CI = 0.79-2.46, p= 0.30). This is because ESBL producing *E. coli* or *Klebsiella spp.* were wide spread among both in-patients and out-patients. This observation therefore confirms that ESBL-producers are indeed as much a problem in the communities as in the hospitals[14].

ESBL-producers may have spread through colonized hospital equipment such as thermometers or bronchoscopes, and contaminated hands of health-care-givers as was reported elsewhere[15].

Most patients with ESBL- producing *Klebsiella pneumoniae* have their gastrointestinal tracts colonized[3,15] and so ESBL-producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through faecal contamination of soil and water.

Cockroaches, which were reported to be vectors of ESBL-producers[16], are common insects in many homes in Ghana. They could have also contributed to the spread of ESBL-producers in the community by contaminating

water and food.

In vitro susceptibility studies of ESBL- producers showed that meropenem was the most effective against these isolates. However carbapenems are not easily accessible because they are too expensive for the average Ghanaian to purchase and they are also not covered by the National Health Insurance Scheme in Ghana.

The carbapenems should be reserved and used to treat only serious or life threatening infections in order to minimize cases of carbapenem resistance though rare[15]. Worryingly, one ESBL producing *Klebsiella pneumoniae* was resistant to meropenem. This is of great concern given that Klebsiellae have a high propensity to host plasmids[15]. Appropriate prevention and infection control measures must be put in place to prevent the spread of these strains within the hospital and the community.

Amikacin was also very effective against ESBL-producers isolated from blood and urine.

Interestingly ciprofloxacin was very effect against isolates from blood but not effective against those from urine. ESBL-producers isolated from urine were generally susceptible to Nitrofurantoin.

It has been reported that the increasing use of ciprofloxacin in the community in many countries has resulted in quinolone resistance in *Enterobacteriaceae*[17]. In Ghana ciprofloxacin is recommended for the treatment of many infections including urinary tract infections, typhoid fever, acute gonorrhoea and osteomylitis[18]. Unfortunately many individuals abuse ciprofloxacin, when they have recurrent malaria, on the assumption that they have typhoid fever.

Routine antimicrobial suseptibility testing may not identify ESBL-producing organisms and so, if the ESBL test had been performed, all the ESBL- producing strains would have been reported as resistant to all penicillins, cephalosporins and aztreonam as recommended by CLSI.[5] The false sensitives (23.21%) recorded especially for the cephalosporins which may have resulted in treatment failure and prolonged hospital stay may have been avoided.

The high odds ratio for ESBL-producers being reported as resistant to cefotaxime (OR=20.80), ceftriaxone (OR=17.67), cefuroxime (OR=30.84), gentamicin (OR=9.34), nalidixic acid (OR=2.27), norfloxacin (OR=4.78), ciprofloxacin (OR=2.74) and cotrimoxazole (OR=6.44) show that there is a close association between the high levels of resistance observed in Kumasi at KATH and the production of ESBL by *E. coli* and *Klebsiella spp.* It also confirms reports that ESBL production confers resistance to several of the commonly used antimicrobials such as gentamicin[10], cotrimoxazole, and fluoroquinolones.[9,15] Chloramphenicol, a phenicol, was also co-resistant with cephalosporins in the presence of an ESBL producing *E. coli* or *Klebsiella spp.* (OR= 3.00, 95% CI 1.19-7.56, $p = 0.029$).

Any *E. coli* or *Klebsiella spp.* isolated in Kumasi at KATH which is resistant to cefotaxime, ceftriaxone, cefuroxime, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, cotrimoxazole and chloramphenicol should be regarded as a potential ESBL-producer, and hence it should undergo ESBL testing. This is because there was a close association between ESBL production and resistance to any of the above mentioned antimicrobial agents by these organisms.

5. Conclusion

This study has shown that there is a high prevalence of ESBL producing *E. coli* and *Klebsiella spp.* Kumasi. This was partly responsible for the high levels of resistance of *E. coli* and *Klebsiella spp.* to the fluoroquinolones and cephalosporins. ESBL-producers were generally resistant to the quinolones, aminoglycosides, tetracyclines, trimethoprim-sulfamethoxazole and phenicol but susceptible to the carbapenems. There was however no independent risk factor associated with the isolation of an ESBL producing *E. coli* or *Klebsiella spp.* in this study in terms of age, gender and location (i.e. In-/ Out-patient) of a patient.

Based on this study ESBL detection should be performed routinely on all isolates of *E. coli* and *Klebsiella spp.* Amikacin and Ciprofloxacin should be recommended for treatment whenever ESBL-producers are susceptible to them and Nitrofurantoin should also be considered as the drug of choice whenever an ESBL-producer isolated from urine is susceptible to it. Meropenem should be considered for treatment of only serious or life threatening infections.

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