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Antimicrobial Resistance Patterns of Extended Spectrum B-Lactamase Producing *Klebsiellae* and *E. coli* Isolates from a Tertiary Hospital in Ghana

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Abstract

Introduction: High proportions of *E. coli* and *Klebsiellae* isolates at Komfo Anokye Teaching Hospital (KATH) have developed resistance to the commonly prescribed antimicrobial drugs, but the cause of which is unknown. Detailed data upon which to advocate control interventions are scanty. This study determined the prevalence of ESBL *Klebsiellae* and *E. coli* at KATH, so as to establish the linkage (if any) between ESBL production and drug resistance to antimicrobials drugs at the Komfo Anokye Teaching Hospital.

Method: 405 isolates consisting of 156 *E. coli* strains and 234 *Klebsiella pneimoniae* and 15 *Klebsiella oxytoca* were collected and tested for their antimicrobial susceptibility, ESBL production and the ESBL genotypes were determined by PCR.

Results: High proportions of isolates were resistant to the β-lactam antibiotics with ampicillin recordeing 391 (91.7%) resistance followed by cefpodoxime 299 (73.8%), cefuroxime 286 (70.6%), ceftriaxone 224 (55.3%) and then cefotaxime 195 (48.1%). Proportion of isolates resistant non β-lactams tested ranged from 61% - 79%. ESBL producers had higher resistance proportions than non-ESBL producers. ESBL prevalence range from 49.4% in E coli, 61.5% in *Klebsiella kpeumoniae* to 86.7% in *Klebsiella oxytoca*. ESBL genotypes TEM, CTX-M were found in 151(64.5) isolates while 70(29.9) acquired the three ESBL genotypes.

Conclusion: The widespread prevalence of ESBL producing *E. coli* and *Klebsiellae* call for immediate intervention strategies to prevent further spread. Training of laboratory personnel on phenotypic testing of ESBLs in addition to training clinical staff and prescribers on ESBL issues are advocated.

Keywords: Antibiotic resistance, ESBL, ESBL genotype

Introduction

Extended Spectrum B-lactamases (ESBLs) are enzymes that evolved with the capacity to degrade β-lactam antibiotics. They also have extended action against non-β-lactam antibiotics such as the aminoglycosides, tetracyclines, chloramphenicol and quinolones (Shaikh et al., 2015). ESBLs have been found originally among the *Klebsiella pneumoniae,* and *Escherichia coli*, but have now been detected among various members of the family *Enterobacteriaceae* (Lahlaoui et al., 2014) because they are able to spread from one organism to another (Souverein et al., 2016). ESBLs have evolved from plasmid-encoded enzyme families of Temoniera (TEM), Sufhydryl variable (SHV) and Oxacillin (OXA) (Kuster et al., 2010; Søraas et al., 2013). CTX-M evolved directly with the introduction of cefotaxime into clinical use. These families and their variants have remained the most prevalent ESBL types (Sarma and Ahmed, 2010). High proportions of *E. coli* and *Klebsiellae* isolates at Komfo Anokye Teaching Hospital (KATH) have developed resistance to the commonly prescribed antimicrobial drugs, but the cause of this high resistance is not known. So detailed data upon which antimicrobial resistance control or intervention strategies are to be instituted at KATH are scanty. This study therefore investigated the prevalence of ESBL *Klebsiellae* and *E. coli* at KATH. It also established the linkage between ESBL production and the resistance phenotypes of the isolates to antimicrobials drugs.

Materials and methods

Non-repeat isolates of 156 *E. coli,* 234 *Klebsiella pneumoniae* and 15 *Klebsiella oxytoca* were collected from a variety of clinical specimens namely, blood, urine, sputum and miscellaneous samples (body fluid aspirates, wound and pus). The bacteria were identified on single colony taken from agar plates. The identification involved pre-screening for typical *E. coli* growth and lactose fermenting characteristic on MacConkey agar and followed by Gram stain (Gram negative rods), and indole and methyl red positivity, citrate negative, and Voges-Proskauer test negative. The *Klebsiellae* characteristics included Gram negative reaction, indole negativity and also methyl red, citrate test and Voges-Proskauer test positivity in addition to other protocols for identification of enterobacteria. Isolates so identified were then confirmed using the API 20E identification systems (bioMerieux SA, Marcy l'Etoile, France).

Ethical considerations

The culture collection was done at KATH between 2008 and 2010 after obtaining ethical clearance from joint Committee on Human Research Publications and Ethics of the School of Medical Sciences and the Komfo Anokye Teaching Hospital.

Antimicrobial susceptibility testing of the isolates

The antimicrobial sensitivity of each of the isolates was determined using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2007).

ESBL Phenotype detection

The isolates were first screened for ESBL production and then confirmed.

Screening

ESBL production by the isolates was determined by inoculating Muller-Hinton agar with overnight culture with a turbidity of 0.5 Macfarland standard (Dade Microscan turbidity meter, CA, USA) using cefotaxime (30µg) and cefpodoxime (10µl). An isolate obviously resistant to cefpodoxime (zone diameter ≤25mm) and/or to cefotaxime (zone diameter ≤29mm) was reported as suspect in the screening test for ESBL production.

Confirmatory test for ESBL production

Isolates that were positive for ESBL production in the screening tests were selected for confirmation of ESBL production by the double disc synergy method. Isolates that showed key-hole patterns formed by the zones of inhibition between the co-amoxiclave (10µg/10µg) and a cefpdoxime disc confirmed ESBL production (CLSI, 2009). Quality control was performed on each batch of the antibiotic discs using the following positive ESBL control strains: *Escherichia coli* (TEM3) NCTC 13351, *Escherichia coli* (CTX-M15) NCTC13353 and *Klebsiella pneumoniae* (SHV-3) NCTC 165032. *Escherichia coli* NCTC 10418 was used as ESBL negative control organism.

Detection of ESBL genes by PCR

Duplex PCR was performed to determine Bla_{TEM} and $Bla_{CTX\text{-}M}$ using illustra PuReTaq Ready-To-Go™ PCR beads (GE Health Biosciences, Pittsburg, PA, USA) in a 25µl reaction volume. The cycling conditions employed following the protocol of Monstein and LE (2007) were: denaturation at 94^{o}C for 10 min, 30 cycles of denaturation at 94 ^{o} C for 1 min, followed by annealing at 60 ^{o}C for 1 min, extension at 72 ^{o} C for 1 min

and a final extension step at 72^{o}C for 7 min. The *Bla*$_{SHV}$ failed to amplify with the other primer pairs so was determined alone with cycling conditions as follows: denaturation at 94 ^{o}C for 10 min, 30 cycles of denaturation at 94 ^{o}C for 1 min, followed by annealing at 59 ^{o}C for 1 min, extension at 72 ^{o}C for 1 min and a final extension step at 72 ^{o}C for 7 min. The primer pairs sequences, sizes and melting temperatures are as provided in Table 1.

Table 1 *Bla*$_{CTX-M}$ and *Bla*$_{TEM}$ and SHV primers employed to determine ESBL genotypes of the isolates

Primer Name	Primer sequence	Size bp	T_M ^{o}C
Bla-CTX-M forward	5'ATGTGCAGYACCAGTAARGTKATGGC'3	593	53
Bla-CTX-M reverse	5'TGGGTRAARTARGTSACCAGAAYCAGCGG'3	593	52
Bla-TEM forward	5'-TCGCCGCATACACTATTCTCAGAATGA-3'	445	54
Bla-TEM reverse	5'-ACGCTCACCGGCTCCAGATTTAT-3'	445	58
Bla-SHV forward	5'-ATGCGTTATATTCGCCTGTG-3'	747	45
Bla-SHV reverse	5'-TGCTTTGTTATTCGGGCCAA-3'	747	45

T_M =Melting Temperature. Source: (Monstein and LE, 2007) Key for Standard Mixed Base Symbols: R-A,G;
Y-C,T; M-A,C; K-G,T; S-C,G; W-A,T; H-A,C,T; B-C,G,T; V-A,C,G; D-A,G,T(Integrated DNA Technologies, Inc , USA)

Results

General characteristics of the study population

A total of 405 isolates of *Klebsiella* and *Escherichia coli* were obtained during the sampling period between 2008 and 2010. The *Klebsiellae* were 249 being 61.5% of the isolates, out of which 234 (94.0%) were *Klebsiella pneumoniae* and 15(6.0%) were *K. oxytoca* and there were 56 (38.0%) *E. coli*.

Socio-demographic characteristics of the study population.

Age

The mean age of the patients infected with *E. coli* alone was 32.1±26.8 years, *Klebsiella pneumoniae* alone was 29.4±27.5 years and for *Klebsiella oxytoca* alone it was 47.9±18.8 years. The mean age (±SD) of all patients was 30.8±27.2 years. There was significant difference between ages of those affected with *Klebsiella oxytoca* when compared with those with *Klebsiella pneumoniae* (p=0.02), so also there was a significant age difference between *E. coli* patients and *Klebsiella oxytoca* infected patients (P=0.04)

Gender

More females 212 (52.3%) than males 193 (47.7%) participated in this study. Of the 156 patients infected with *E. coli* 83 (53.2%) were females and 73 (46.8) were males. *Klebsiella pneumoniae* were isolated from 119/234 (50.9 %) females and 10/15 (66.7%) *K. oxytoca* isolates were obtained from females. There were no significant differences when number of patients infected with *E. coli*, *K. pneumoniae,* and *K. oxytoca* were compared.

Place (in/out-patient)

Samples analyzed came from many places in the hospital with the majority 161(39.8%) coming from the Out-Patients Department (OPD). There were no significant differences when isolate types and the places (MBU, PEU, OPD and others) were compared.

Variables	Total (n=405)	*E coli* (n=156)	*K. pneumoniae* (n=234)	*K. oxytoca* (n=15)	P value[a]	P value[b]	P value[c]
Socio-demographic data							
Age (yrs)	30.8 ± 27.2	32.1 ± 26.8	29.4 ± 27.5	47.9 ± 18.8	0.3648	0.0467	0.0224
Female	212 (52.3%)	83 (53.2%)	119 (50.9%)	10 (66.7%)	0.6490	0.3174	0.2348
Ward							
MBU	47 (11.6%)	15 (9.6%)	32 (13.7%)	0 (0.0%)	0.2276	0.2086	0.1250
PEU	58 (14.3%)	18 (11.5%)	38 (16.2%)	2 (13.3%)	0.1947	0.8363	0.7664
OPD	161 (39.8%)	65 (41.2%)	85 (36.3%)	11 (73.3%)	0.2881	0.0184	0.0043
Others	139 (34.3%)	58 (37.2%)	79 (33.8%)	2 (13.3%)	0.4884	0.0645	0.1016
Sample type							
Blood	109 (26.9%)	37 (23.7%)	71 (30.3%)	1 (6.7%)	0.1521	0.1292	0.0499
Urine	155 (38.3%)	75 (48.1%)	72 (30.8%)	8 (53.3%)	0.0005	0.6972	0.0655
Wound	80 (19.8%)	33 (21.2%)	42 (17.9%)	5 (33.3%)	0.4314	0.2785	0.1399
Sputum	47 (11.6%)	8 (5.1%)	39 (16.2%)	0 (0.0%)	0.0006	0.3690	0.0851
Others*	14 (3.5%)	3 (1.9%)	10 (4.3%)	1 (6.7%)	0.2052	0.2456	0.6619

Table 2 General characteristic of the studied population stratified by enterobacteria isolate

Sample types

There were more proportions of *E. coli* than *K. pneumoniae* isolates obtained from urine (p=0.01). So also the difference between *K. pneumoniae* and *E. coli* proportions obtained from sputumwas found to be significant. No significant differences were observed amongst the isolates obtained from wound when *E.coli* and *K. pneumoniae* were compared (p=0.4314), and also when *E.coli* and *K.oxytoca* were compared. The demographic characteristic data are shown in Table 2.

Continuous data are presented as mean ± Sd and categorical data presented as proportion. Continuous data were compared to each other using unpaired t-test whilst categorical data compared to each other using Chi-square analysis. P value[a] = E coli vrs K. pneumoniae, P value[b] = E coli vrs K. oxytoca and P value[c] = K. pneumoniae vrs K. oxytoca, MBU = Mother-Baby Unit, PEU = Peadiatric-Emergency Unit and OPD = Out patient

department, Others = Other wards, and Others* = other samples (ear, pus and aspirates).

Antibiotic susceptibility profiles of the isolates.

A total of 405 enterobacteria were tested against 11 different antibiotics namely cefotaxime, ceftriaxone, cefuroxime, ampicillin, cefpodoxime, co-amoxiclav, imipenem, gentamicin, nalidixic acid, ciprofloxacin, co-trimoxazole and chloramphenicol. High proportions of isolates were resistant to the β-lactam antibiotics with ampicillin recording 391 (91.7%) followed by cefpodoxime 299 (73.8%), cefuroxime 286 (70.6%), ceftriaxone 224 (55.3%) and then cefotaxime 195 (48.1%). The proportion of isolates resistant to other antibiotics (non β-lactams) tested ranged from 61% - 79% (Table 3).

Variables	Total (n=405)	E. coli (n=156)	K. pneumoniae (n=234)	K. oxytoca (n=15)	P value[a]	P value[b]	P value[c]
β-lactam antibiotic resistance							
Cefotaxime	195 (48.1%)	61 (39.1%)	125 (53.4%)	9 (60.0%)	0.01	0.11	0.62
Cefriaxone	224 (55.3%)	66 (42.3%)	146 (62.4%)	12 (80.0%)	< 0.01	0.01	0.17
Cefuroxime	286 (70.6%)	91 (58.3%)	180 (76.9%)	15 (100.0%)	< 0.01	0.01	0.04
Ampicillin	391 (96.5%)	143 (91.7%)	233 (99.6%)	15 (100.0%)	< 0.01	0.21	0.78
Cefpodoxime	299 (73.8%)	101 (64.7%)	183 (78.2%)	15 (100.0%)	0.01	0.01	0.04
Co-amoxiclav	90 (57.7%)	272 (67.2%)	169 (72.2%)	13 (86.7%)	0.01	0.01	0.22
Imipenem	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	NA	NA
Non- β-lactam antibiotic resistance							
Gentamicin	248 (61.2%)	87 (55.8%)	149 (63.7%)	12 (80.0%)	0.11	0.06	0.19
Nalidixic acid	266 (65.7%)	97 (62.2%)	156 (66.7%)	15 (100.0%)	0.36	0.01	0.01
Ciprofloxacin	77 (19.0%)	29 (18.6%)	43 (18.4%)	7 (46.7%)	0.95	0.01	0.01
Cotrimoxazole	249 (61.5%)	140 (89.7%)	195 (83.3%)	14 (93.3%)	0.07	0.65	0.31
Chloramphenicol	321 (79.3%)	119 (76.3%)	188 (80.3%)	14 (93.3%)	0.33	0.12	0.21
ESBL phenotype							
ESBL	234 (57.8%)	77 (49.4%)	144 (61.5%)	13 (86.7%)	0.01	0.01	0.05
ESBL genotype							
SHV	76 (32.5%)	32 (41.6%)	36 (25.0%)	8 (61.5%)	0.01	0.17	0.01
CTX-M	221 (94.4%)	72 (93.5%)	136 (94.4%)	13 (100.0%)	0.77	0.34	0.38
TEM	225 (96.2%)	74 (96.1%)	138 (95.8%)	13 (100.0%)	0.92	0.46	0.45

Table 3 Proportion of Enterobacteria isolates stratified by resistance to antibiotics and ESBL genotype

Data were presented as proportions. P value[a] = E. coli vrs K. pneumoniae, P value[b] = E coli vrs K. oxytoca and P value[c] = K. pneumoniae vrs K. oxytoca, ESBL = Extended spectrum beta-lactamase.ESBL phenotypes.

ESBL phenotypes were detected in high proportions in all the isolates tested. Generally ESBL production was detected at 234 (57.8%) for the isolates. ESBL production was found in 77(49.4%) *E. coli*, 144(61.5) *K. pneumoniae* and 13(86.7%) *K. oxytoca*. Similarly the proportion of *E. coli* producing ESBL were significantly lower than that of K. oxytoca (see Table 3).

ESBL Genotypes:

Bla$_{CTX-M}$ and *Bla*$_{TEM}$ genotypes were more prevalent among isolates than the *Bla*$_{SHV}$. *Bla*$_{CTX-M}$ prevalence was 94.4% and for *Bla*$_{TEM}$ was 96.2% amongst the isolates. *Bla*$_{SHV}$ genotypes prevalence was 32.5%, much lower than for *Bla*$_{CTX-M}$ and *Bla*$_{TEM}$. *Bla*$_{SHV}$ prevalence was significantly higher in *E. coli* (32 (41.6%)) than in *K. pneumoniae* (36 (25.0%)) (p=0.0050). The *Bla*$_{SHV}$ prevalence in *E. coli* was again significantly higher than among *K. oxytoca* and *K. pneumoniae* (p=0.0050). The prevalences of both *Bla*$_{CTX-M}$ and *Bla*$_{TEM}$ genes were high (above 90%) in *E .coli* 72 (93.5%).

Socio-demographic characteristics of the study population and ESBL production

The socio-demographic characteristics of the patients from whom samples were taken and antibiotic resistance prevalence were stratified by ESBL phenotype (Table 4). The mean age of the patients infected with ESBL was 31.1±27.4 whilst the non-ESBL infected patients had mean age of 30.4±26.9. There was no significant difference between proportion of female patients infected with ESBL and the male patients. ESBL prevalence levels among the wards were also determined. The Out-Patient Department (OPD) with prevalence 39.7% was the highest. The prevalence of ESBL phenotypes in all the wards ranged from 12.3% to 39.7%. The differences were all not significant when compared with prevalence of non-ESBL isolates (Table 4).

ESBL production of the isolates as a predictor of antimicrobial resistance

Antimicrobial resistance for ESBL positive isolates and the non-ESBL phenotypes were compared to determine whether ESBL production influenced the antimicrobial resistance prevalence. With exception of imipenem, to which all isolates were sensitive, significantly higher proportions of ESBL-producers were resistant to the antibiotics tested (P=0.0001) with details shown in Table 4.

Table 4 Socio-demographic characteristic, sample type, ward and antibiotic resistance in relation to ESBL phenotype.

Variable	ESBL (n=234)	Non-ESBL (n=171)	P value
Socio-demographic data			
Age (yrs)	31.1 ± 27.4	30.4 ± 26.9	0.81
Female	120 (51.3%)	92 (53.8%)	0.61
Ward			
MBU	84 (35.9%)	55 (32.2%)	0.43
PEU	29 (12.3%)	18 (10.5%)	0.56
OPD	93 (39.7%)	78 (45.6%)	0.23
Others	38 (16.2%)	20 (11.7%)	0.19
Sample type			
Blood	64 (27.4%)	45 (26.3%)	0.81
Urine	101 (43.2%)	54 (31.6%)	0.01
Wound	41 (17.5%)	39 (22.8%)	0.18
Sputum	24 (10.3%)	23 (13.5%)	0.32
Others*	4 (1.7%)	10 (5.8%)	0.02
β-lactam antibiotic resistance			
Cefotaxime	170 (72.6%)	25 (14.6%)	< 0.01
Ceftriaxone	185 (79.1%)	39 (22.8%)	< 0.01
Cefuroxime	212 (90.6%)	74 (43.3%)	< 0.01
Cefpodoxime	214 (91.5%)	85 (49.7%)	< 0.01
Ampicillin	231 (98.7%)	160 (93.6%)	0.01
Co-amoxiclav	190 (81.2%)	83 (48.5%)	< 0.01
Imipenem	0 (0.0%)	0 (0.0%)	NA
Non-β-lactam antibiotic resistance			
Gentamicin	176(75.2%)	72 (42.1)	< 0.01
Nalidixic acid	183(78.2%)	85 (49.7%)	< 0.01
Ciprofloxacin	69(29.5%)	8 (4.7%)	< 0.01
Co-trimoxazole	218(93.2%)	131 (76.6%)	< 0.01
Chloramphenicol	206(88.0%)	116 (67.8%)	< 0.01

Continuous data were presented as mean ± Sd and categorical data presented as proportion. Continuous data were compared to each other using unpaired t-test whilst categorical data compared to each other using Chi-square analysis. MBU = Mother-Baby Unit, PEU = Peadiatric-Emergency Unit and OPD = Out-patient department, Others = Other wards, and Others* = other samples (ear, pus and aspirates).

ESBL distribution in sample types

ESBL prevalence was determined for the sample types tested for the 234 ESBL phenotypes. Urine isolates recorded a significant (p= 0.01)

prevalence of 101 (43.2%), compared to non-ESBL isolates (54 (31.6%) (Table 3).

Some of the isolates had multiple genes, where both $Bla_{CTX\text{-}M}$ and Bla_{TEM} were found in 151(64.5%) of the isolates, but there were no isolates with Bla_{SHV} and Bla_{CTX_M}. All the three genes (Bla_{SHV}, $Bla_{CTX\text{-}M}$ and Bla_{TEM}) were found in 70 (29.9%) of the isolates (Table 5).

Table 5 Prevalence of multiple ESBL genotypes and their distribution among the isolates.

No (%) of isolates with multiple ESBL genes (n=234)							
None	Single gene			Two genes			All three genes present
All genes absent	CTX-M only	SHV only	TEM only	SHV + CTX-M	SHV + TEM	CTX-M + TEM	SHV + CTX-M + TEM
4 (1.7)	0	5 (2.1)	3 (1.3)	0	1 (0.4)	151 (64.5)	70 (29.9)

Discussions

This study has demonstrated high prevalence of ESBLs among *Klebsiellae* and *E. coli* isolates obtained from clinical samples of both in- and out-patients at Komfo Anokye Teaching Hospital (KATH). With the exception of imipenem to which no resistant isolates were detected, high proportions isolates were resistant to all the other antimicrobials tested. The resistance proportions ranged from 48.1% to cefotaxime to 91.7% to ampicillin. *Klebsiella* resistance to ampicillin was 100% because of the intrinsic resistance characteristics of *Klebsiellae (Zhang et al., 2016)*. In the present study it was found that *K. oxytoca* isolates were more resistant to the antibiotics tested than *K. pneumoniae* and *E. coli*. Also the *Klebsiellae* (*K. oxytoca* and *K. pneumoniae*) were more resistant than *E. coli* to the antimicrobials tested. These high proportions of isolates to antibiotics have been reported and attributed to indiscriminate sale and misuse of antibiotics in Ghana over the years by patients (Newman et al., 2006) and by farmers who employ the antibiotics in animal production (Azanu et al., 2016; Tajick, 2006). Manufacture and sale of substandard antibiotics in many developing countries including Ghana (Osei-Safo et al., 2016; Shakoor et al., 1977) makes it difficult to prescribe the correct dosage of medicines by health professionals. In hospitals most of such drugs may not be identified as substandard, so the patient may not be given the correct dosage (Osei-Safo et al., 2016; Shakoor et al., 1977), leading to treatment failures and hence repeated treatments resulting in sensitizing the bacteria and then causing them to become resistant (Blomberg, 2008).

Another important factor include poor prescribing practices (Newman et al., 2006) where physicians prescribe antibiotics without asking

for microbiological tests, which are compounded by lack of competent personnel to perform antimicrobial sensitivity testing even in teaching hospitals where the testing facilities exist. Major antibiotics to which resistant bacteria were found include the ampicillin, tetracycline and co-trimoxazole which are capsules or tablets because they are easy to dispense, and administer, so they are the most abused than the injectable antibiotics like the gentamicin and the third generation cephalosporins. Meanwhile, these antibiotics are the major choices for the treatment of severe infections at KATH (Saana et al., 2014) *sine qua non* for the development of multidrug resistance caused by the selection for ESBL genotypes (Barogui et al., 2013). Prevalence of ESBL production was detected among the isolates at a significantly high level of 57.8%, meaning that more than half of the enterobacteria isolates tested produce ESBL. Ciprofloxacin resistance was 29.5% in ESBL positive and 4.7% in ESBL-negative isolates and this difference was significant (p= <0.01), an indication that ciprofloxacin resistance in the isolates was closely associated with ESBLs (Shaikh et al., 2015). Common co-resistance has been found in ESBL-producing organisms to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim (Bajaj et al., 2016). This is a demonstration of the emergence of epidemic clones harbouring several beta-lactamases simultaneously (*Bla*$_{\text{CTX-M}}$, *Bla*$_{\text{SHV}}$ and *Bla*$_{\text{TEM}}$) and perhaps of other mechanisms of resistance. Treatment outcomes for these patients, especially with β-lactams, would likely be less satisfactory if the organisms produced ESBL (Shaikh et al., 2015). For example, *in-vitro* susceptible-cefepime treatment of ESBL producing *E. coli* and *Klebsiella pneumoniae* was associated with a failure rate of 23-83% (Projan, 2008). Aminoglycosides, sulphonamides and quinolones have been suggested as drugs which can be used when in-vitro results show that they are susceptible (Cerceo et al., 2016). Unfortunately epidemiological studies revealed a strong link between fluoroquinolone resistance and ESBL production (Cremet et al., 2011).

Kumasi strains have ESBL prevalence of 86.7% in *K. oxytoca* followed by *K. pneumoniae* (61.5%) and then *E. coli* (49.4%). The high prevalence in *K. oxytoca* probably explains why ESBLs were first detected among the *Klebsiellae* (Knothe, 1983). More importantly in this present study about 30 of the isolates had all genes (SHV, CTX-M and TEM) suggesting that other ESBL genotypes might also be present in addition to those tested for similar to the studies in Erbil, Iraq (Ahmad and Ali, 2014). All the ESBL types were found in the hospital and in the community isolates with the Bla$_{\text{CTX-M}}$ dominating. It is unclear why *Bla*$_{\text{CTX-M}}$ type enzymes have spread much more in Kumasi community and in the hospital than the *Bla*$_{\text{SHV}}$ enzyme. Though the risk factors for ESBL acquisition are many e.g. cephalosporin administration, faecal carriage etc (Hijazi et al., 2016) it can

emerge in the community as a result of cephalosporin intake in the community and spread by the unhygienic conditions that prevail in the communities. In the Hospital, the spread of an ESBL variant can be facilitated by a referral system, where the presence of a single ESBL variant in a different centre may be imported by a patient on referral to another centre (Rodriguez et al., 2010). This situation can hold for KATH which is a tertiary referral centre, receiving patients from many parts of the country.

In many parts of Ghana including Kumasi it is common to see humans share the same compound with animals such as cattle, sheep and goats and fowls. Therefore microbes from humans and animals get into contact with one another and may exchange genetic material by conjugation (Leverstein-van Hall et al., 2011; Rodriguez-Villalobos et al., 2010), resulting in some isolates acquiring more than one gene type (Rodriguez-Villalobos *et al.,* 2010) a situation found at KATH. This warrants future surveillance studies at KATH, because treatment of these multi-drug-resistant organisms is a therapeutic challenge but so far there are no clear data in the Hospital morbidity and mortality resulting from treatment failures.

Conclusion

This study detected high antimicrobial resistance amongst *E. coli* and *Klebsiella* sp to the commonly prescribed antimicrobial drugs at KATH. These resistance levels, perhaps are attributed to ESBL production by these isolates obtained from both the community and the hospital wards. Ciprofloxacin and imipenem had excellent performance against the isolates tested. All three ESBL genes ($Bla_{\text{CTX-M}}$, Bla_{TEM} and Bla_{SHV}) tested for were prevalent among the isolates. The high levels of antimicrobial resistance and the widespread prevalence of ESBL producing *E. coli* and *Klebsiellae* emphasize the necessity to adopt immediate intervention strategies to prevent severe nosocomial infections in the hospital and to continuously monitor ESBL spread into the community. Training of laboratory staff on phenotypic testing of ESBLs and also training clinical staff and prescribers on ESBL issues are warranted and advocated.

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