academicJournals

Vol. 6(1), pp. 13-20, January 2014
DOI: 10.5897/JMA2013.0280
ISSN 2141-2308 ©2014 Academic Journals
http://www.academicjournals.org/JMA

Journal of Microbiology and Antimicrobials

Full Length Research Paper

AmpC beta-lactamase production among *Pseudomonas aeruginosa* and *Proteus mirabilis* isolates at the Komfo Anokye Teaching Hospital, Kumasi, Ghana

Feglo, P.* and Opoku, S.

Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana.

Accepted 16 January, 2014

Extended spectrum β-lactamases (ESBLs) are frequently reported in Ghana, but AmpC bacteria reports are scanty. This study determines the prevalence of AmpC *Pseudomonas aeruginosa* and *Proteus mirabilis* clinical isolates at the Komfo Anokye Teaching Hospital (KATH), Kumasi, where ESBL and AmpC are not routinely tested. Non-duplicate 245 isolates comprising 187 (76.3%) *P. aeruginosa* and 58 (23.7%) *P. mirabilis* were tested for AmpC and ESBL production using the modified three-dimensional test method and the double disc synergy test (DDST) methods, respectively. The proportion of the 245 isolates producing AmpC β-lactamase was 93 (38.0%) and 79 (32.2%) produced ESBL. AmpC producers confirmed 49 (52.7%) as inducible and 44 (47.3%) non-inducible AmpC producers. *P. aeruginosa* and *P. mirabilis* that produced AmpC were 84 (44.9%) and 9 (15.5%), respectively. ESBL production was 41 (21.9%) in *P. aeruginosa* and 38 (65.6%) in *P. mirabilis.* Co-producers of AmpC together with ESBL were 7.3%. Both enzyme were detectable in 13 (7.0%) of *P. aeruginosa* and 5 (8.6%) of *P. mirabilis*. AmpC and ESBL are detectable in high proportions among *P. aeruginosa* and *P. mirabilis* isolates at KATH and the Kumasi community. This emphasizes the need to start testing for both enzymes to guide therapeutics.

Key words: AmpC beta-lactamase, extended spectrum beta-lactamase, inducible AmpC beta-lactamase, non-inducible beta-lactamase.

INTRODUCTION

Extended spectrum β-lactamases and AmpC β-lactamases are enzymes encoded by the chromosomal and plasmid genes of many bacteria (Jacoby, 2009). These ESBL and AmpC enzymes are produced as metabolic by-products which have the ability to hydrolyze beta-lactam antibiotics often leading to multiple antibiotic resistances (Black et al., 2005). The enzymes mediate resistance to cephalosporins, penicillins including even β-lactamase inhibitor-β-lactam combinations (Jacoby, 2009). According to Bell et al. (2007), many organisms that produce ESBL sometimes also produce AmpC β-lactamase (Bell). Some bacteria co-produce ESBL and AmpC β-lactamases making the organisms non-suceptible to β-lactam antibiotics including β-lactam and beta-lactam-inhibitor combinations.

ESBL-producer prevalence at the Komfo Anokye Teaching Hospital was reported to be 44.37% for *Escherichia coli* and 55.67% for *Klebsiella pneumoniae* and 42.86% for *Klebsiella* spp. (Adu-Sarkodie, 2010). The only documented study on ESBLs was by Sarkodie (2010) when the ESBL phenotypes of *Klebsiella* and *E. coli* were studied. That study found out that 44% of *E. coli* and 55% of *Klebsiella* sp. (57.8% *K. pneumoniae*) produced ESBL. This ESBL prevalence was found to be high among these enterobacteria and as a result the antibiotic spectrum

*Corresponding author. E-mail: pfeglo.sms@knust.edu.gh or pfeglo@gmail.com. Tel: 233 208807693.

from which to choose to treat severe infections was reduced, creating difficulties with therapeutic options. AmpC β-lactamases are frequency reported worldwide (Coudron et al., 2000) and yet in Ghana, reports on AmpC β-lactamase producing bacteria are scanty. Very few studies have reported on the problem of ESBL in Africa (Ndugulile et al., 2005). There have been reports of ESBL on *K. pneumoniae* in Kenya (Kariuki et al., 2001) South Africa (Essack et al., 2001) and Tanzania (Blomberg et al., 2005). ESBLs have been reported in *Salmonella enterica* and *Salmonella enteritidis* from Mali (Weill et al., 2004) and *S. typhimurium* in Morocco (AitMhand et al., 2002) and in Nigeria (Adeniji and Okesola 2010; Romanus et al., 2009) but in Ghana information on ESBLs and AmpC β-lactamase phenotypes began emerging (Hackman et al., 2013), but information on *Pseudomonas aeruginosa* and *Proteus mirabilis* are still very rare.

Work has been done on most strains of the enterobacteriaceae (*Klebsiellae and Escherichia coli*) in Ghana but not *P. aeruginosa* and the *P. mirabilis*. Meanwhile the hospital laboratory has been recording multidrug resistant *P. aeruginosa* and *P. mirabilis* more recently than the previous years. This study therefore determines the prevalence of AmpC β-lactamase and ESBL production among *P. aeruginosa* and *P. mirabilis* clinical isolates *vis-a-vis* the high antimicrobial resistance being observed at the Komfo Anokye Teaching Hospital.

METHODOLOGY

A total of 5, 859 samples comprising 3,012 blood, 1,794 urine and 1,053 wound samples were taken and analyzed from both in-patients and out-patients from November 2011 to 31 January 2012. The blood cultures were performed using BACTEC 9240 (Becton, Dickinson and company, USA). Clean-catch mid-stream urine samples were cultured on cystein lactose electrolyte deficient (CLED) agar (Oxoid limited, Basingstoke, UK) using a calibrated 2 mm diameter bacteriological loop. Wound samples were collected with sterile cotton wool swabs and transported to the laboratory in Stuart Transport medium. After overnight incubation, a single colony was picked from the growths that occurred and sub-cultured onto nutrient agar slants in test-tubes and stored in the refrigerator. These stored isolates were later identified with biochemical tests and confirmed with API 20E (bioMérieux, Marcy l'Etoile, France) identification system. The biochemical tests employed for the identification of the isolates included oxidase test, citrate utilization test, urease test, indole test and Kligler iron agar test. Antimicrobial susceptibility test of the isolates was performed using the disc diffusion (Kirby-Bauer) test method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2010). The antimicrobial discs tested against the isolates were obtained from Becton, Dickinson and Company, USA.

Screening for AmpC production

The *P. aeruginosa* and *P. mirabilis* isolates were tested for AmpC production using the cefoxitin disc (30 µg) (Becton, Dickinson and Company, USA) as recommended by Upadhyay et al. (2010). In this test, bacterial suspension of 0.5 McFarland density of the test isolate was prepared. Using a sterile cotton-tipped swab the bacterial suspension was inoculated onto the surface of the Mueller-Hinton agar (Cypress Diagnostics, Langdorp, Belgium). The cefoxitin antibiotic disc (Becton, Dickinson and Company, USA) was placed on the inoculated agar using a pair of sterile forceps, and incubated overnight at 37°C. Isolates that yielded zone diameters of less than 18 mm or frank resistance were considered positive for AmpC beta-lactamase production in the screening test (Upadhyay et al., 2010). A previously known AmpC producing *P. aeruginosa* isolate and *E. coli* ATCC 25922 were used as controls.

AmpC confirmatory test

The modified three-dimensional test was used to confirm the production of AmpC beta-lactamase. This test was useful in detecting both inducible and non-inducible AmpC beta-lactamases (Upadhyay et al., 2010). Overnight culture on Mueller-Hinton agar (Cypress Diagnostics, Langdorp, Belgium) was transferred to sterile micro-centrifuge tube containing peptone water and then pelleted by centrifugation at 3000 rpm for 15 min. The supernatant was decanted and fresh peptone water was added and the bacterial suspensions were repeatedly frozen and thawed about 10 times to obtain crude AmpC enzyme extract. Mueller-Hinton agar surface was inoculated with *E. coli* ATCC 25922 to produce a lawn culture and cefoxitin (30 µg) disc was placed at the centre of the plate. On the agar, three linear slits about three centimeters (3 cm) long were made three millimeters (3mm) away from the cefoxitin disc using a sterile surgical blade. A small circular well was made on each slits 5 mm from the cefoxitin disc by stabbing the agar with the tip of a sterile Pasteur pipette. One of the wells was filled with the enzyme extracted from the test organism. Each of the remaining wells was filled with extracts from the positive control (previously known *P. aeruginosa* isolate) and the third well filled with the negative control (*E. coli* ATCC 25922). The plates were kept for about 10 min for the surface moisture to dry. They were then incubated at 37°C overnight. After incubation, isolates that showed clear distortion of zone of inhibition around cefoxitin discs were confirmed as AmpC producers. The isolates that produced no zones of distortion were AmpC non-producers (Shoorashetty et al., 2011).

Testing for inducible AmpC

Isolates that were positive in the AmpC test were further tested to determine whether the enzyme was inducible or non-inducible type. The inducible AmpC beta-lactamase was tested for by the disc antagonism test method (Upadhyay et al., 2010). A sterile cotton-tipped swab was used to seed Mueller-Hinton agar (Cypress Diagnostics, Langdorp, Belgium) with the 0.5 McFarland bacterial suspension of the test organism. A disc of cefotaxime (30 µg) and cefoxitin (30 µg) (Becton, Dickinson and Company, USA) were placed 20 mm apart on the seeded Mueller-Hinton agar surface and incubated overnight at 37°C. Isolates that produced blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin discs were taken as inducible AmpC beta-lactamase producers (Upadhyay et al., 2010). A previously known AmpC producing *P. aeruginosa* isolate and *E. coli* ATCC 25922 were used as controls.

Test for ESBLs

The isolates were first screened for ESBL production and those positive in the screening test were later confirmed by the double synergy test. In the screening test, outright resistance of the isolate to third generation cephalosporins namely ceftazidime (30 µg) with < 22 mm zone size, cefotaxime (30 µg) with < 27 mm zone size and

Table 1. General characteristics of the study population stratified by the *P. aeruginosa* and *P. mirabilis* isolates.

Variable	Total (n= 245)	*Pseudomonas aeruginosa* (n=187)	*Proteus mirabilis* (n=58)	P value
Patient demographic data				
Age (years)	36	47	26	
Patient				
Outpatient	99 (40.4%)	71 (38.0%)	28 (48.3%)	0.16
Inpatient	146 (59.6%)	116 (62.0%)	30 (51.7%)	0.16
Sample type				
Urine	70 (28.6%)	43 (23.0%)	27 (46.6%)	< 0.01
Wound	144 (58.7%)	122 (65.2%)	22 (37.9%)	< 0.01
Blood	31 (12.7%)	22 (11.8%)	9 (15.5%)	0.45

Continuous data are presented as mean ± SD and categorical data presented as proportions. Continuous data are compared with each other using unpaired t-test while categorical data are compared with each other using Chi-square analysis. P = comparison between prevalence of *P. aeruginosa* and *P. mirabilis* isolates, P< 0.05 means significant.

ceftriaxone (30 µg) with zone size < 25 mm, as recommended by the CLSI (CLSI 2010) made an isolate an ESBL suspect.

Confirmatory test for ESBL production

ESBL detection was done using the double disc synergy test (DDST). In this test, the test organism (0.5 McFarland's turbidity) was inoculated onto Mueller-Hinton agar surface (Cypress Diagnostics, Langdorp, Belgium) by using a sterile cotton swab. A disc of co-amoxiclav (20 µg amoxycillin + 10 µg clavulanate) was placed at the centre of the inoculated Mueller-Hinton agar. A disc of cefotaxime (30 µg) and another of ceftriaxone (30 µg) were placed at both sides to the co-amoxiclav disc (Metri et al., 2011). The set-up was incubated at 37°C overnight. The enhancement of the zones of inhibition of any of the cephalosporin disc towards the clavulanic acid disc confirms an isolate as an ESBL producer (Metri et al., 2011). The positive and negative control strains used in this test were *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922, respectively.

Pearson X^2 test (P-value < 0.05) was used for data analysis to assess the level of significance of assumed hypothesis using STATA (STATA^{TM} 10, StataCorp., 4905 Lakeway Drive, College Station, Texas 77845 USA).

RESULTS

A total of 245 non-select and non-duplicate isolates were collected for the study. They comprise 187 *P. aeruginosa* and 58 *P. mirabilis*, recording 76.3 and 23.7%, respectively. The ages of patients from whom the isolates were obtained ranged from one day to 82 years with the median age of 36 years. The isolates were obtained from 131 (53.5%) males and 114 (46.5%) females in the study. *P. aeruginosa* was isolated from 95/187 (50.8%) and *P. mirabilis* isolates were obtained from 36/58 (62.1%) male patients. Female patients with *P. aeruginosa* infection were 92/187 (49.2%) and those with *P. mirabilis* infection were obtained from female patients. These were obtained from both in- and out-patients and also from the three sample types studied. Among the sample types there were *P. aeruginosa* isolates of 144 (58.7%), 43 (23.0%) and 31 (12.7) obtained from wound, urine and blood, respect-tively. There were 27/58 (46.6%) *P. mirabilis* obtained from urine, 122 (65.2%) obtained from wound and 22 (11.8%) were obtained from blood as shown in Table 1.

Out of the 245 isolates tested, 93 (38.0%) tested positive for AmpC β-lactamase production. Among the AmpC β-lactamase producers 49 (52.7%) were confirmed inducible AmpC β-lactamase producers while 44 (47.3%) were non-inducible AmpC producers. There were 84/187 (44.9%) *P. aeruginasa* isolates that produced AmpC β-lactamase out of which 49/187 (26.2%) were inducible AmpC β-lactamase producers. There were 9 (15.5%) *P. mirabilis* isolates that produced AmpC β-lactamase, but the enzyme they produced were non-inducible AmpC β-lactamase. Overall results of ESBL prevalence was 79 (32.2%) for both *P. aeruginosa* and *P. mirabilis*. Of the *P. aeruginosa* isolates, 41 (21.9%) were ESBL producers and the 38 (65.5%) *P. mirabilis* isolates were ESBL producers (Table 2). The proportion of *P. aeruginosa* isolates producing only AmpC β-lactamse was 71/187 (38.0%) and 28/187 (15.0%) produced only ESBL while 13 (7.0%) co-produced both AmpC β-lactamse and ESBL. Proportion of the *P. mirabilis* isolates that produced AmpC β-lactamase only was 4 (6.9%), and 33 (56.9%) produced only ESBL, whilst 5 (8.6%) co-produced AmpC β-lactamse and ESBL.

The *P. aeruginosa* isolates had no resistant strains against meropenem but susceptibility levels to aminoglycosides (amikacin and gentamicin) were 94.7 and 79.7%, respectively and to ciprofloxacin was 90.4%. Susceptibility levels to other antibiotics recorded 92.0% to ceftazidme and 60.4% to cefotaxime. The isolates had low susceptibility levels (45.5%) to ceftriaxone and 24.1%

Table 2. Proportion of isolates stratified by susceptibility to antibiotics, ESBL, AmpC and inducible AmpC production.

Variable	Total (n = 245)	*Pseudomonas aeruginosa* (n =187)	*Proteus mirabilis* (n = 58)	P value
Beta-lactam antibiotic susceptibility				
Meropenem	245 (100%)	187 (100%)	58 (100%)	0.379
Cefotaxime	146 (59.6%)	113 (60.4%)	33 (56.9%)	0.632
Ceftazidime	216 (88.2%)	172 (92.0%)	44 (75.9%)	**< 0.001**
Ceftriaxone	108 (44.1%)	85 (45.5%)	23 (39.7%)	0.437
Cefuroxime	69 (28.2%)	45 (24.1%)	24 (41.4%)	**0.0104**
Ampicillin	0 (0.0%)	N/A	0 (0.0%)	N/A
Non-beta-lactam antibiotic susceptibility				
Ciprofloxacin	204 (83.3%)	169 (90.4%)	35 (60.23%)	**< 0.001**
Amikacin	228 (93.1%)	177 (94.7%)	51 (87.9%)	0.078
Gentamicin	193 (78.8%)	149 (79.7%)	44 (75.9%)	0.580
Co-trimoxazole	1 (0.4%)	N/A	1 (1.7%)	N/A
Chloramphenicol	0 (0.0%)	N/A	0 (0.0%)	N/A
ESBL	79 (32.2%)	41 (21.9%)	38 (65.5%)	**< 0.001**
AmpC	93 (38.0%)	84 (44.9%)	9 (15.5%)	**< 0.001**
Inducible AmpC	49 (20.0%)	49 (26.2%)	0 (0.0%)	N/A
Non-inducible AmpC	44 (18.0%)	35 (18.7%)	9 (15.5%)	0.580
ESBL only	61 (24.9%)	28 (15.0%)	33 (56.9%)	**< 0.001**
AmpC only	75 (30.6%)	71 (38.0%)	4 (6.9)	**< 0.001**
Both ESBL and AmpC	18 (7.3%)	13 (7.0%)	5 (8.6%)	0.670

Data are presented as proportions. ESBL = Extended spectrum beta-lactamase, AmpC = AmpC beta-lactamase, N/A = Not-applicable, P = Comparison between *P. aeruginosa* and *P. mirabilis* isolates. P< 0.05 means significant (bolded).

to cefuroxime. The *P. mirabilis* isolates had no resistant strains to meropenem but had susceptibility proportion of 87.9% to amikacin, 75.9% to gentamicin and 60.2% to ciprofloxacin and other antimicrobials registering lower susceptibility values as shown in Table 2.

AmpC producing *P. aeruginosa* and *P. mirabilis* were obtained from 93 patients with the mean age of 35.41 years (SD = 23.89) and males yielding 51 (54.8%) females 42 (45.2%). Among the AmpC producing isolates, 49 (52.7%) were inducible AmpC and 44 (47.3%) were non-inducible AmpC producers. There were 61 (24.9%) isolates that produced only ESBLs while 18 (7.3%) of the isolates co-produced AmpC and ESBLs. There were 58 *P. mirabilis* isolates, of which 15.52% produced AmpC and 56.89% produced ESBL. There were 187 *P. aeruginosa* isolates involved in the study and 84 (44.92%) of them tested positive for AmpC. Among the 84 AmpC producing *P. aeruginosa* isolates 49 (58.33%) of them were positive for inducible AmpC. There were no *P. mirabilis* isolates that yielded inducible AmpC enzymes.

By sample type, most of the AmpC producers 66 (71.0%) were found in wound followed by 19 (20.4%) obtained from urine and then 8 (8.6%) obtained from blood. Most of the non-AmpC-producing isolates were obtained from wound 78 (51.3%), followed by urine 51 (33.6%) and 23 (15.1%) were obtained from blood. The details of these results are presented in Table 3.

Antimicrobial susceptibility of the isolates in relation to β-lactamase production

The AmpC producing isolates had no resistant strains to meropenem. Susceptibility to amikacin was 92.5 and 77.4% to gentamicin. Similar results were obtained for the non-AmpC producing isolates where there were no resistant strains against meropenem but susceptibility to amikacin was 92.8%. Proportions of isolates susceptible to other antibiotics, though high, were varied recording 86.0% for ciprofloxacin. Susceptible proportions of the isolates to cephalosporins were generally low with only 32.3% being susceptible to cefotaxime and 10.8% to ceftriaxone but were high to ceftazidme (83.9%). All the AmpC producing isolates were resistant to cefuroxime, ampicillin, co-trimoxazole and chloramphenicol.

There were however high proportions, about 90.8% being susceptible to ceftazidime, 81.6% to ciprofloxacin, gentamicin 79.6%, cefotaxime 76.3%, ceftriaxone 64.5% and cefuroxime 45.4%. The non-AmpC producing isolates had very low proportions susceptible to co-trimoxazole, cefuroxime, ampicillin and chloramphenicol.

Table 3. Patient socio-demographic characteristic, sample type and antibiotic susceptibility level of the isolates stratified by AmpC and ESBL phenotypes.

Variable	AmpC (n= 93)	Non-AmpC (n= 152)	Inducible AmpC (n= 49)	Non-inducible AmpC (n= 44)	ESBL Only (n= 61)	ESBL and AmpC (n= 18)	P value[a]	P value[b]	P value[c]
Socio-demographic data									
Age (years)	35.41 ± 23.89	32.9 6 ± 2 3.50	16.48 ±10.84	56.50 ± 15.03	33.89 ± 22.36	43.83 ± 22.69	0.432	**< 0.001**	**< 0.001**
Male	51 (54.8%)	83 (54.6%)	21 (42.9%)	30 (68.2%)	37 (60.7%)	9 (50.0%)	0.972	**0.014**	0.421
Female	42 (45.2%)	69 (45.4%)	28 (57.1%)	14 (31.8%)	24 (39.3%)	9 (50.0%)	0.972	**0.014**	0.421
Patient									
Outpatient	31 (33.3%)	58 (38.2%)	14 (28.6%)	17 (38.6%)	18 (29.5%)	6 (33.3%)	0.446	0.304	0.757
Inpatient	62 (66.7%)	94 (61.8%)	35 (71.4%)	27 (61.4%)	43 (70.5%)	12 (66,.7%)	0.446	0.304	0.757
Sample type									
Urine	19 (20.4%)	51 (33.6%)	11 (22.4%)	8 (18.2%)	23 (37.7%)	7 (38.9%)	**0.027**	0.610	0.928
Wound	66 (71.0%)	78 (51.3%)	38 (77.6%)	28 (63.6%)	27 (44.3%)	9 (50.0%)	**0.002**	0.140	0.668
Blood	8 (8.6%)	23 (15.1%)	0 (0.0%)	8 (18.2%)	11 (18.0%)	2 (11.1%)	0.136	N/A	0.487
Beta-lactam antibiotic susceptibility									
Meropenem	93(100%)	152 (100%)	49 (100%)	44 (100%)	61 (100%)	18 (100%)	0.725	0.939	0.353
Cefotaxime	30 (32.3%)	116 (76.3%)	12 (24.5%)	18 (40.9%)	41 (67.2%)	6 (33.3%)	**< 0.001**	0.091	**0.010**
Ceftazidime	78 (83.9%)	138 (90.8%)	38 (77.6%)	39 (88.6%)	50 (82.0%)	14 (77.8%)	0.104	0.157	0.691
Ceftriaxone	10 (10.8%)	98 (64.5%)	1 (2.0%)	9 (20.5%)	32 (52.5%)	3 (16.7%)	**< 0.001**	**0.004**	0.070
Cefuroxime	0 (0.0%)	69 (45.4%)	0 (0.0%)	0 (0.0%)	26 (42.6%)	0 (0.0%)	N/A	N/A	N/A
Ampicillin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A	N/A	N/A
Non-beta-lactam antibiotic susceptibility									
Ciprofloxacin	80 (86.0%)	124 (81.6%)	41 (83.7%)	38 (86.4%)	41 (67.2%)	13 (72.2%)	0.366	0.717	0.688
Amikacin	86 (92.5%)	141 (92.8%)	45 (91.8%)	41 (93.2%)	56 (91.8%)	16 (88.9%)	0.933	0.806	0.702
Gentamicin	72 (77.4%)	121 (79.6%)	39 (79.6%)	33 (75.0%)	45 (73.8%)	10 (55.6%)	0.802	0.597	0.140
Co-trimoxazole	0 (0.0%)	1 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A	N/A	N/A
Chloramphenicol	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A	N/A	N/A

Continuous data are presented as mean ± SD and categorical data presented as proportions. Continuous data are compared to each other using unpaired t-test while categorical data are compared with each other using Chi-square analysis. AmpC = AmpC beta-lactamase, ESBL = extended spectrum beta-lactamase, N/A = non-applicable. P value[a] = AmpC vrs Non- AmpC, P value[b] = Inducible AmpC vrs non- inducible AmpC, P value[c] = ESBL only vs. ESBL and AmpC. P = Comparison between two phenotypes. P< 0.05; means significant, P> 0.05; means not significant.

The proportions of non-AmpC producing isolates that were susceptible to cefotaxime was significantly higher than the AmpC producing isolates that were susceptible to cefotaxime ($p < 0.001$). There was also a significantly higher proportion of ceftria-xone- susceptible strains among non-AmpC producing isolates than AmpC producing isolates ($p < 0.001$). The detailed results are shown in Table 3.

Antimicrobial susceptibility patterns of AmpC- and non-AmpC- producing isolates

The inducible AmpC-producing isolates had no resistant strains to meropenem but proportions susceptible to amikacin, ciprofloxacin, gentamicin and ceftazidime were 91.8, 83.7, 79.6 and 77.6%, respectively. The isolates had very low proportions (24.5 and 2.0%) susceptible to cefotaxime and ceftriaxone. All the isolates were resistant to cefuroxime, ampicillin, co-trimoxazole and chloramphenicol, where no susceptible strains were recorded as shown in Table 3.

The isolates that produced only ESBL had no resistant strains to meropenem but their susceptibility levels varied recording 91.8, 82.0 and 73.8% to amikacin, ceftazidime and gentamicin, respectively. Their susceptibility levels to ciprofloxacin, ceftriaxone and cefotaxime were 67.2, 52.5 and 42.6%, respectively, whilst only 27.3% were susceptible to cefuroxime.

DISCUSSION

This was a prospective study involving the AmpC β-lactamase production among *P. aeruginosa* and *P. mirabilis* clinical isolates at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. There was a high prevalence of AmpC production among the isolates (38.0%) as compared to other studies in which AmpC prevalence of about 20.7% was recorded in Delhi, India (Manchanda and Singh, 2003). Another study undertaken among hospitalized patients in Kolkata, India reported 17.3% (Arora and Bal, 2005) but among Gram negative isolates obtained from ICU patients in Bombay again in India showed only 5.4% prevalence (Oberoi et al., 2012). These differences in prevalence may be due to differences in the geographical distribution as a result of degree of antibiotic usage in the different localities (Canton et al., 2008). AmpC β-lactamase-producing isolates were obtained from male and female patients (Rand et al., 2011) of all ages, but there were no significant differences between gender and age of affected patients with AmpC enzymes.

AmpC beta-lactamase phenotypes were isolated from all the sample types tested in this study (urine, wound and blood), just as was reported that urine, blood, wounds, sputum, and stool were reported as sources of positive cultures for AmpC phenotypes (Jacoby, 2009). Wound was the commonest source of the isolates obtained in the study producing majority (65.2%) of the *P. aeruginosa* and 37.9% of the *P. mirabilis* isolates whilst blood sample produced the least proportion (12.7%) of the isolates.

The differences in prevalence may also be due to the different methods of selection of isolates, the variation in an ability to produce AmpC beta-lactamases among different Gram-negative bacteria (Arora and Bal, 2005). The high prevalence of AmpC producing isolates may be indicative of the threatening trends of more isolates acquiring resistance and mechanisms to render antibiotic therapy ineffective (Bell et al., 2007).

AmpC phenotypes were significantly higher among the *P. aeruginosa* isolates than the *P. mirabilis* isolates. *Pseudomonas aeruginosa* produces both inducible chromosomal AmpC and non-inducible plasmid mediated AmpC (Lister et al., 2009) whilst *P. mirabilis* produces only non-inducible plasmid mediated AmpC (Parveen et al., 2012). The *P. aeruginosa* isolates obtained in the study produced both inducible and non-inducible AmpC whereas the *P. mirabilis* isolates produced only inducible AmpC (Upadhyay et al., 2010), the very reason for which treatment of *P. aeruginosa* infections are more challenging than *P. mirabilis* infections (Lister et al., 2009).

In this study, AmpC phenotypes were obtained from both outpatients and inpatients but with the majority (66.7%) of the AmpC phenotypes isolated from inpatients, an indication that the enzymes have spread from the hospitals where antimicrobials are heavily used in the Kumasi community, as observed in England by the Infection Control Committee of Royal Devon and Exeter in England (2010). A similar study reported that AmpC beta-lactamases have been isolated from patients after several days of hospitalization (Jacoby, 2009), confirming the association of antibiotics intake of antibiotics. Co-production of AmpC and ESBL was detected in 7.3% of the isolates. This finding is comparable to the 8.0 and 6.59% reported in separate studies India by Sinha et al. (2008) and Oberoi et al. (2012), respectively. The existence of AmpC and ESBL co-production isolate especially *P. aeruginosa* is of serious concern because the organisms already has intrinsic resistance to wide range of clinically important antimicrobials (Lister et al., 2009) and acquisition of ESBLs make the organisms become resistance not only to β-lactam antibiotics but also to other non-β-lactam drugs such as the quinolones and the aminoglycosides. These drugs are also the drugs of choice for the treatment of infections caused by the *P. aeruginosa* and *P. mirabilis* (Hackman et al., 2013). Besides, co-production of both AmpC and ESBL by an organism makes detection of ESBL difficult by laboratory methods. So such strains often give false negative tests results in the detection of ESBLs (Sinha et al., 2008). The resistance proportions of ESBL-producing *P. mirabilis* strains to cefotaxime and ceftazidime were 43.1 and 24.1%, respectively, values considerably lower than the 56.9% ESBL *P. mirabilis* isolates recorded. This observation may be as a result of differences in β-lactamase expression or outer membrane permeability. For example, decreased expression of outer membrane porins that are channels for β-lactam entry can considerably add to or increase β-lactamase action to cause more resistance. In addition, *P. mirabilis* lacks intrinsic chromosomal β-lactamase genes, so, are dependent upon acquisition of different β-lactamase mechanisms to express a β-lacta-

mase- mediated resistance phenotype (Livermore, 1995). It has been reported in a study that about 85 to 97% of ESBL-producing *P. mirabilis* strains appeared to be susceptible to ceftazi-dime, cefepime and aztreonam (Wang et al., 2011). An ESBL producing *P. mirabilis* strain may be susceptible to higher derivatives of a β-lactam antibiotic because its production may have been induced by lower one, eg ESBL induced by cefuroxime would tend to be resistant to ceftriaxone and/or cefotaxime (Jacoby, 2009). It is gratifying to observe that all the isolates, including AmpC and ESBL producing isolates were susceptible to meropenem which recorded no resistant strains against it. This observation supports the report that carbapenems are usually the drugs of choice against AmpC- and ESBL-producing bacteria (Lister et al., 2009). The AmpC producing isolates also had high proportions susceptible to aminoglycosides (amikacin 92.5% and gentamicin 77.4%), quinolone (ciprofloxacin, 86.0%) cephalosporin (ceftazidime, 83.9%), but care must be taken not to abuse these antimicrobials. The isolates recorded low susceptible proportions to some of the cephalosporins where cefotaxime had 32.3%, ceftriaxone had 10.8% and none of the isolates at all tested sensitive to cefuroxime a situation attributed to inducible AmpC. Introduction of cephalosporins antibiotic into a hospital triggers production of inducible AmpC in bacteria causing them to hyperproduce the inducible AmpC enzyme in that hospital (Thomson, 2001). β-Lactam antibiotic exposure confers resistance to the β-lactam antibiotics (Cruz et al., 2013).

It has been found that the organisms tested have the ability to co-produce ESBL and AmpC. Also, the drugs intended to treat infections caused by these *P. aeruginosa* induce the production of inducible AmpC. These mechanisms reduces the very narrow already treatment options available for treating the AmpC-producing bacteria (Rodriguez-Martinez et al., 2003). The approach now is to adopt good diagnostic laboratory procedures and institute good prevention strategies as a matter of priority in controlling the development and spread of these strains.

Conclusion

This study has demonstrated that there is high prevalence of AmpC- and ESBL- producing *P. aeruginosa* and *P. mirabilis* strains circulating in the Komfo Anokye Teaching Hospital and in the community with higher antimicrobial resistance than the non AmpC and ESBL strains. Proportion of isolates that co-produced both enzymes was more resistant than those that possess either ESBL or AmpC only.

REFERENCES

Adeniji AO, Okesola TW (2010). Pattern of extended spectrum beta lactamase production among clinical isolates of *Proteus* species in Western Nigeria. World J. Med. Sci. 5(4):94-97.

Adu-Sarkodie Y (2010). Extended-Spectrum-Beta-Lactamase production among *Escherichia coli* and *Klebsiella* species at the Komfo Anokye Teachin Hospital in Kumasi, Ghana. Int. J. Infect. Dis. 23(14S):1-23.

AitMhand RA, Soukri N, Moustaoui H, Amarouch N, ElMdaghri DS, Benbachir M (2002). Plasmid mediated TEM - 3 extended spectrum beta lactamase production in Salmonella typhimurium in Casablanca. J. Antimicrob. Chemother. 49:169-172.

Arora S, Bal M (2005). The AmpC β-lactamase producing bacterial isolates at a Kolkata hospital. Indian J. Med. Res. 122:224-233.

Bell JMM, Chitsaz JD, Turnidge M, Barton LJW, Jones RN (2007). Prevalence and significance of a negative extended-spectrum β-lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of Escherichia coli and Klebsiella pneumoniae: results from the SENTRY Asia-Pacific surveillance program. J. Clin. Microbiol. 45:1478-1482.

Black JA, Moland ES, Thomson KS (2005). AmpC disc test for detection of plasmid-mediated AmpC beta-lactamases in Enterobacteriaceae lacking chromosomal AmpC- blactamases. J. Clin. Microbiol. 43:3110-3113.

Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DS, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY, Langeland N (2005). High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J. Clin. Microbiol. 43:745-749.

Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM (2008). Prevalence and spread of extended spectrum beta-lactamase- producing Enterobacteriaceae in Europe. Clin. Microbiol. Infect. 14(1):144-153.

CLSI (2010). Clinical Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing. Twentieth informational supplemented. CLSI document Wayne, PA, USA M100-S20.

Coudron PE, Moland ES, Thomson KS (2000). Occurrence and detection of AmpC β-lactamases among Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis isolates at a veterans medical Center, J. Clin. Microbiol. 38:1791-1796.

Cruz GR, Radice M, Samantha S, Pallecchi L, Rossolini GM, Gutkind G, and Di Conza JA (2013). Prevalence of plasmid-mediated quinolone resistance determinants among oxyiminocephalosporin-resistant Enterobacteriaceae in Argentina. *Mem Inst Oswaldo Cruz*, Rio de Janeiro 108(6):1-4.

Essack SY, Hall LM, Pillay D, McFadyen ML, Livermore DM (2001). Complexity and diversity of Klebsiella pneumoniae strains with extended-spectrum beta-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. Antimicrob. Agents Chemother. 45:88-95.

Hackman HK, Osei-Adjei G, Gordon A, Laryea E, Quaye S, Anison L, Brown CA, Twum-Danso K (2013). Phenotypic Characterization of AmpC beta-lactamase among Cefoxitin Resistant Escherichia coli and Klebsiella pneumoniae Isolates in Accra, Ghana. J. Biol. Agric. Healthcare 3(16):102-105.

Jacoby GA (2009). AmpC β-Lactamases. Clin. Microbiol. Rev. 22(1):161-182.

Kariuki S, Corkill J, Revathi GRM, Hart C (2001). Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical Klebsiella pneumoniae isolates from Kenya. Antimicrob. Agents Chemother. 45:2141-2143.

Lister PD, Wolter DJ, D HN (2009). Antibacterial-Resistant Pseudomonas aeruginosa: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin. Microbiol. Rev. 22(22):582-610.

Livermore DM (1995). β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. 8:557-584.

Manchanda V, Singh NP (2003). Occurrence and detection of AmpC beta-lactamases among Gram negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi. Indian J. Antimicrob. Chemother. 51:415-418.

Metri BC, Jyothi PP, Basavaraj V (2011). The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India. J. Clin. Diagn. Res. 5(3):470-475.

Oberoi L, Singh N, Sharma P, Aggarwal A (2012). ESBL, MBL and Ampc β Lactamases Producing Superbugs - Havoc in the Intensive Care Units of Punjab India. J. Clin. Diagn. Res. 5016:2497-2499.

Parveen R, Mohamudha BN, Parija SC (2012). Molecular description of plasmid-mediated AmpC β-lactamases among nosocomial isolates of Escherichia coli & Klebsiella pneumoniae from six different hospitals in India. Indian J. Med. Res. 135(1):114-119.

Rand KH, Turner B, Seifert H, Hansen C, Johnson JA, Zimmer A (2011). Clinical Laboratory Detection of AmpC β-Lactamase Am. J. Clin. Pathol. 135(4):572-576.

Rodriguez-Martinez JM, Pascual A, Garcia I, Martinez-Martinez L (2003). Detection of the plasmid-mediated quinolone resistance determinant qnr among clinical isolates of Klebsiella pneumoniae producing AmpC-type β-lactamase. J. Antimicrob. Chemother. 52:703-706.

Romanus II, Egwu OA, Ngozi AT, Chidiebube NA, Chika EP (2009). Extended spectrum beta - lactamase (ESBL) mediated resistance to antibiotics among Klebsiella pneumoniae in Enugu Metropolis. Macedonian J. Med. Sci. 2(3):1-4.

Shoorashetty RM, Nagarathnamma T, Prathibha J (2011). Comparison of the boronic acid disc potentiation test and cefepime-clavulanic acid method for the detection of ESBL among AmpC-producing Enterobacteriaceae. Indian J. Med. Microbiol. 29(3):297-301.

Sinha P, RS, Rishi S, Sharma R, Sood S, Pathak D (2008). Prevalence of extended spectrum beta lactamase and AmpC beta lactamase producers among Escherichia coli isolates in a tertiary care hospital in Jaipur. Indian J. Pathol. Microbiol. 51:367-369.

Thomson KS (2001). Controversies about extended-spectrum and AMP C beta-lactamases. Emerg. Infect. Dis. 7:333-336.

Upadhyay S, Sen MR, Bhattacharjee A (2010). Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. J. Infect. Dev. Ctries. 4(4):239-242.

Wang P, Fupin H, Xiong Z, Ye X, Zhu D, Wang YF, Wang M (2011). Susceptibility of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae According to the New CLSI Breakpoints. J. Clin. Microbiol. 49(9):3127-3131.

Weill FX, Demartin M, Tande D, Espie E, Rakotoarivony I, Grimont PA (2004). SHV-12-like extended-spectrum-beta-lactamase-producing strains of Salmonella enterica serotypes Babelsberg and Enteritidis isolated in France among infants adopted from Mali. J. Clin. Microbiol. 42:2432-2437.