Occurrence Of Beta- Lactamase Resistance Among Isolates From Cancer Patients in Lagos, Nigeria

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Running title: Beta-lactamase resistance from isolates of cancer patients

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ABSTRACT: Background: Bacteria infections associated with multidrug resistance have been implicated in the high mortality and morbidity reported among cancer patients. In recent years gram negative organisms isolates from patients with neoplasia have been found to produce beta-lactamases (β-lactamase) and this is of interest in developing countries where it is unreported or underreported. This study determined beta-lactamase mediated resistance in gram negative bacteria isolates from patients attending the radiology and Oncology clinic of Lagos University Teaching Hospital between April and November 2006. **Methods**: One hundred and nineteen samples were analyzed and sixty one gram negative isolates were recovered and were characterized with the analytical profile index(API) tests .Antimicrobial susceptibility testing was determined using the disk diffusion method according to CLSI standard. Production of beta-lactamase and Extended spectrum beta-lactamase (ESBL) were investigated using the nitrocefin stick and double disk synergy test (DDST) respectively. Plasmid analysis was done on each bacteria isolate showing multidrug resistance. **Result**: Of the sixty one gram negative isolates, 55((90.2%) produced beta- lactamases; 20(32.8%) were found to be ESBL producers while 14(23%) showed AmpC enzyme production. Twenty five out of twenty seven strains harbored plasmids of sizes ranging between 3.0-4.9kb. Statistical analyses showed occurrence of ESBL and AmpC production to be significant. **Conclusion**: The result of this study has shown a high occurrence of beta-lactamase mediated resistance among clinical isolates from cancer patients. Many of these

harbored plasmids which may encode genes for antibiotic resistance or virulence factors which are becoming persistent problems in the healthcare sector. [Academia Arena, 2009;1(5):27-34]. ISSN 1553-992X.

Keywords: ESBL, AmpC, Beta-lactamase, Cancer, multidrug resistance.

INTRODUCTION

Of the various mechanisms of acquired resistance to β-lactam antibiotics, resistance due to β-lactamases is the most prevalent. Gram negative bacteria resistant to agents such as extended spectrum cephalosporin, monobactams, carbapenems and β-lactam-β-lactamase inhibitor combinations have emerged through the production of a variety of β-lactamases(Pitout *et.al*, 1997; Wood ,AJ. 1996) Emergence of resistance to these agents has resulted in a major clinical crisis(D'agata,FEMC.2000).
Bacteria infections associated with multi-drug resistance in cancer patients have been reported as high (Figuera *et.al*, 2006) and this is caused mostly by the effect of the cytotoxic chemotherapy and radiotherapy which lowers the immunity(Rice *et.al*,1990). With the increased use of β-lactams among these patients an increase in bacteria resistance has developed. Extended spectrum beta-lactamase resistances are now a problem among patients with chronic cases and carcinomas.(Naumovski *et al*. 1992)

In this study, we examined the occurrence of different β-lactamases among gram negative isolates from patients with cancer of the breast and cervix. We also determined the plasmid profiles of isolates producing extended spectrum beta-lactamase.

MATERIALS AND METHODS

Isolation and Identification

A total of 61 gram negative bacteria isolates from 119 breast and cervical cancer patients samples who attended the Radiology and Oncology Clinic of the Lagos University Teaching Hospital, Nigeria between April, 2006 and November, 2006 have been included in this study. These isolates were from the urine, breast wound swabs and cervical swabs of non-hospitalized cancer patients attending the Radiology and Oncology Clinic of the Lagos University Teaching Hospital.
The strains were identified by conventional methods and confirmation to the species level was done biochemically with the use of API 20E and 20NE system. (API bio merieux, Nurtingen, Germany).

Beta-lactamase was determined using a chromogenic cephalosporin method (Nitrocephin-stick oxoid, UK) and positive control of *Staphylococcus. aureus* ATCC 29213.

Susceptibility testing was performed using the standard agar disc diffusion method as described by the NCCLS standard. (2000). ESBL production was detected using the double disk synergy test as described by Jarlier *et.al*. (1988) with modification by Thomson and Sanders. (1992). All strains showing resistance to third generation cephalosporins was screened fro ESBL production. An amoxicillin-clavulanate disk was placed at the centre of inoculated plate and disks containing ceftazidime (30μg), cefotaxime (30μg) ceftriaxone (30μg) and aztreonam (30μg) were placed 20mm apart (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-β-lactam caused by the synergy with the clavulanate in the amoxycilin clavulanate disk was considered as an evidence of ESBL production. (Jarlier *et.al*.1988., Thompson and Sanders., 1992). *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Presumptive Phenotypic Determination of CTX-M ESBL: Bacteria isolates resistant to cefotaxime, ceftriaxone and aztreonam were presumptively identified as producers of CTX-M ESBL (Livermore and Brown., 2001)

The inducibility of AmpC β-lactamase production was performed by disk antagonism tests in which disks containing an inducing agent, cefoxitin (30μg) and ceftazidime (30μg), were placed on Mueller Hinton agar plates. Blunting of the ceftazidime zone at adjusted distance between the disks and resistance to cefoxitin was observed. (Livermoreand Brown., 2001)

Plasmid DNA of isolates producing ESBL was extracted using the alkaline phosphate method of Birnboin and Doly(1979). Agarose gel electorphoresis was used to determine the profiles of the plasmid. The profiles were compared to a DNA of known molecular weight plasmids.

Statistical analysis using analysis of variance (ANOVA) as test of significance was done. All statistical analysis was performed at 95% confidence interval and significant p values less than 0.05 were considered significant.

RESULT

Sixty one gram negative bacteria made up of seventeen (17) different species were recovered from one hundred and nineteen (119) clinical samples. Amongst them were *Klebsiella pneumoniae, Citrobacter amalonaticus, E.coli, P. mirabilis, A.baumanii, Serratia liquefaciens* and *Stenotrophomonas maltophilia*. Out of the sixty one (61) isolates 55, (90.2%) produced beta-lactamase (TEM/SHV like type), 20 (32.8%) and 14 (23%) were found to be extended spectrum beta-lactamase and AmpC producers respectively (Table 1). Most of the ESBL-producers were multi-resistant to the fluoroquinoles and aminoglycosides, 93.4% of isolates were found to be sensitive to imipenem and all isolates showed resistance to tetracycline (100%) (Table 2) . Plasmid analysis was performed on each bacteria showing multi-drug resistance (Table 3). Twenty five (25) of twenty seven (27) strains harboured plasmids of sizes ranging between 3.0 – 4.9kb. (Table 4)

Statistical analysis showed occurrence of ESBL and AmpC production among isolated strains from cancer infections to be significant.

TABLE 1:BETA-LACTAMASES DETECTED IN ORGANISMS ISOLATED

Organisms	Beta-lactamase enzyme detection using nitrocephin.n=55	ESBL enzyme detection using double disk synergy test (DDST).n=20	AmpC (enzyme detection using cefoxitin resistance.n=14
E.coli (16)	16 (29%)	11 (55%)	6 (42.9%)
Enterobacter aerogenes (2)	2 (3.6%)	0%	0%
Enterobacter agglomerans (1)	1 (1.8%)	0%	0%
Enterobacter cloacae (2)	2 (3.6%)	0%	2 (14.3%)
Citrobacter amalonaticus / koserii (3)	3 (5.5%)	1 (5%)	1 (7.2%)
Citrobacter freundii (1)	1 (1.8%)	1 (5%)	0%
Klebsiella pneumoniae (6)	6 (10.9%)	4 (20%)	2 (14.3%)
Klebsiella planticola (6)	3 (5.5%)	2 (10%)	2 (14.3%)
Klebsiella oxytoca (4)	4 (7.3%)	0%	0%
Klebsiella rhinoscleromatis (1)	1 (1.8%)	0%	0%
Klebsiella ozaene (3)	1 (1.8%)	1 (5%)	0%
Pseudomonas aeruginosa	9 (16.4%)	0 (0%)	0%
Acinetobacter iwofii (1)	1 (1.8%)	0%	0%
Acinetobacter Baumanil (1)	1 (1.8%)	0%	0%
Proteus mirabilis (3)	2 (3.6%)	0%	0%
Serratia liquefaciens (1)	1 (1.8%)	0%	1 (7.2%)
Stenotrophomonas maltophilia (1)	1 (1.8%)	0%	0%

TABLE 2:RESISTANCE PATTERN OF ISOLATES

Isolates	Antibiotic Resistance Profile
E. coli	TZP, CAZ, CRO, CTX, ATM, PEF, OFX, CIP, AMX, GEN, NIT, COT, CXM, AUG, TET
Enterobacter spp	AMX, CXM, AUG, TET
Serratia liquefaciens	TZP, FOX, AK, GEN, COT, AUG, TET
Klebsiella spp	TZP, CTX, CIP, AMX, GEN, NIT, COT, CXM, AUG, TET
Proteus mirabilis	AK, PEF, OFX, CIP, GEN, NIT, CXM, TET,
Pseudomonas aeruginosa	TZP, FOX, CAZ, CRO, ATM, AK, PEF, OFX, CIP, AMX, GEN, NIT, COT, CXM, AUG, TET
Stenotrophomonas maltophilia	TZP, PEF, OFX, CIP, AMX, GEN, NIT, COT, TET
Citrobacter spp	TZP, FOX, CTX ATM PEF CIP AMX GEN, NIT, COT, CXM, AUG, TET
Acinetobacter spp	TZP, CTX, IMP, AMX, GEN NIT, COT CXM AUG, TET

TABLE 3: ANTIMICROBIAL RESISTANCE PATTERN OF ISOLATES IN RELATION TO PLASMIDS.

Isolates	Antimicrobial Resistance Pattern	Number of Isolates	Number with Plasmids.
Pseudomonas aeruginosa	TZP, FOX, CAZ CRO, ATM, AK, PEF, OFX, CIP, AMX, GEN, NIT, COT, CXM, TET	4	4
Citrobacter spp	TZP, FOX, CTX, COT, ATM, PEF, CIP, AMX, GEN, NIT, CXM, TET	2	2
E.coli	TZP, CAZ, CTX, ATM, PEF, OFX, CIP, AMX, GEN, NIT, COT, CXM, AUG, TET	11	11
		7	7
Klebsiella spp	TZP, CTX, CIP, AMX, GEN, NIT, COT, CXM, AUG, TET		
		1	1
Acinetobacter spp	TZP. CTX, IMP, AMX, GEN, NIT, COT, CXM, AUG, TET		
		2	0
Enterobacter spp	AMX, CXM, AUG, NIT, TET		

TABLE 4: PLASMID SCREENING RESULTS OF ISOLATES

Isolates	Source	Number with Plasmid	Plasmid Sizes (kb)
E .coli (1)	Urine	1	4.4
E. coli (2)	Urine	1	3.0,3.6, 4.4
E. coli (3)	Urine	1	4.3
E. coli (4)	Urine	1	4.6
E. coli (5)	Urine	1	4.5
E. coli (6)	Urine	1	4.8
E. coli (7)	Urine	1	4.6
E. coli (8)	Urine	1	4.6
E. coli (9)	Urine	1	4.9
E. coli (10)	Cervical swab	1	4.7
E. coli (11)	Cervical swab	1	4.6
Citrobacter spp (1)	Urine	1	4.4
Citrobacter spp (2)	Urine	1	4.6
K. pneumoniae (1)	Urine	1	4.5
K. pneumoniae (2)	Urine	1	4.4
K. pneumoniae (3)	Urine	1	4.7
K. pneumoniae (4)	Urine	1	4.7
K. planticola (1)	Urine	1	4.1, 4.7
K. planticola (2)	Swab	1	4.4
K ozaenae (1)	Urine	1	4.6
P. aeruginosa (1)	Urine	1	4.6
P. aeruginosa (2)	Urine	1	4.6
P. aeruginosa (3)	Urine	1	4.6
P. aeruginosa (4)	Swab	1	4.6
Acinetobacter spp (1)	Urine	1	4.6

Discussion

Chemotherapy and radiotherapy methods of treatment adopted for patients with carcinoma of the cervix and breast have been implicated as an agent of immunosupression. Hence, it's been known to contribute to infections in patient with an underlying debilitating effect of cancerous growth. (Rice *et.al*.,1990) With the emergence and increase in bacterial resistance, surveillance of the prevalent pathogen and their resistance pattern is of utmost importance to reduce the mortality rate due to bacterial infections and also improve the quality of life of affected patient. (Figuera *et.al*.,2006)

Reports of ESBL producing strains have been appearing for about a decade among outpatients and in patients in this environment (Aibinu *et.al*., 2003) but no data exists on ESBL bacteria isolates from cancer patients in this environment. Therefore, it is noteworthy that this study isolated 61 gram negative pathogens and out of these 32.8% were found to be ESBL producers while 23% showed AmpC enzyme.

This result records so far the highest number of gram negative species to be recovered from cancer patients compared to previous studies on isolates from this environment that studied anaerobes and gram positive organisms. (Rotimi *et.al*., 1984.,Oduyebo *et.al*.,2001)

Of the 61 isolates recovered from urine, and swabs of these patients, 49 (80.3%) were from urine. *Klebsiella* species had the highest occurrence in urine among these cancer patients which is in agreement with Podschunn and Ulmann(1998)that reported *Klebsiella* species as an opportunistic pathogen, which primarily attack immunocompromised individuals . On the other hand, *Proteus mirabilis* was the most frequently isolated organism from wound swabs of these cervical and breast cancer patients. This was followed by *Enterobacter* spp (E. *aerogenes* and *E. cloacae*); *Pseudomonas aeruginosa* and *Escherichia coli*. All these organisms have been implicated in wound infections (Goosens, H. 2005). *Klebsiella planticola* was the only species of *Klebsiella* isolated from wound swabs in this study. This is in agreement with the report of Podschunn and Ullmann(1998)which reported *K. planticola* to have a high frequency of recovery from clinical samples of wound. This findings highlights the organisms most commonly implicated in wound swabs of cervical and breast cancer and the organisms most commonly implicated in infections among cancer patients in this environment.

Multi-drug resistance was found among the 9 groups of organisms isolated. The best coverage against these organisms was obtained with imipenem(100%), except for *Pseudomonas aeruginosa* and *Acinetobacter* species which showed resistance to the carbapenem with 33% and 50% resistance respectively.

High resistance to amoxicillin clavulanic acid, a beta-lactam beta- lactamase inhibitor was observed among the isolates and all other beta-lactam agents. The third generation cephalosporin were effective against *S. maltophilia, Serratia* spp with over 75% sensitive, multi-drug resistance occurred in E. *coli, Pseudomonas aeruginosa, Acinetobacter* spp, *Serratia liquefaciens* with complete resistance to over 7 antibiotics.

Most of the ESBL isolates were multi-resistant but susceptibility to imipenem and amikacin (>60%) was high. However, imipenem is not easily available in Nigeria because of its high cost. Resistance to the quinolones (ofloxacin, ciprofloxacin, pefloxacin) was detected in most of the ESBL and AMPC producers (>50%). This further limits the choice of effective antibiotics among these patients.

Emergence of these resistance and production of ESBLS and AmpC is of concern among these patients. Plasmid profiles showed that all the strains were diverse in nature with respect to transmission of antibiotic resistance except for *P.aeruginosa* isolates which had all strains harbouring only one plasmid of same molecular weight. It was also observed that *Enterobacter cloacae* showing AmpC did not harbour plasmid suggesting that the resistance may be chromosomally borne.

This emergence of ESBLs and stable derepressed mutants that hyperproduced chromosomal beta-lactamases have the potential to diminish the activity of all extended spectrum cephalosporins (Goosens.,2005) and these important pathogens are currently on the rise in critically ill group of patient (Patterson and Bonomo2005).

It is of concern in this study that proliferation of beta-lactamase resistance among strains may have been due to misuse of antibiotics, proliferation of multiply resistant clones, transfer of resistance-carrying plasmids and inability to detect emerging phenotypes in developing countries as stated by Croft *et.al.*, 2007.

Conclusion

This study has been able to show an emergence of different strains of multi-resistant bacteria producing beta-lactamases among clinical isolates of cancer patients. Many of these multiresistant species harboured plasmids which may encode genes for antibiotic resistance or virulence factors and may predispose to high morbidity and mortality of the disease. The resistance has paralleled the introduction, administration misuse and overuse of broad spectrum of antibiotics. All physicians should be obligated to prescribe antimicrobial agents more deliberately. Also, there is need for antibiotic surveillance in this population of patients to ensure judicious use of antibiotics.

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