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PHENOTYPIC SCREENING FOR AMP-C AND EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING *PSEUDOMONAS AERUGINOSA* IN CLINICAL SAMPLES COLLECTED FROM FEDERAL TEACHING HOSPITAL ABAKALIKI (FETHA I AND II)

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Abstract: The aim of this research work was to phenotypically screen for the production of Amp-C β-lactamases and Extended Spectrum Beta Lactamases (ESBLs) in *Pseudomonas aeruginosa* isolates obtained from clinical samples in Federal Teaching Hospital Abakaliki (FETHA I and II) and to determine their susceptibility patterns to different classes of antibiotics. Twelve *Pseudomonas aeruginosa* isolates were obtained from a total of 521 clinical samples that were collected from patients in FETHA I and FETHA II. These isolates were identified using standard microbiology techniques. Antibiotic susceptibility test of these isolates was done on Mueller Hinton agar (Oxoid, England) by disk diffusion technique. Isolates showing blunting of ceftazidime zone of inhibition adjacent to cefoxitin disc were considered Amp-C- positive and were selected for confirmation of Amp-C- β-lactamases production by Amp-C- disc test while ESBL production was determined by DDST method. Nitrocefin tests were performed to detect the production of β-lactamase enzyme in the 12 *Pseudomonas aeruginosa* isolates. The highest prevalence rate of *Pseudomonas aeruginosa* isolation was observed in wound samples. Out of the 12 *Pseudomonas aeruginosa* isolates obtained, 8 (66.7 %) were Amp-C positive while 4 (33.3 %) were positive for ESBL production phenotypically. Result shows that all the 12 isolates obtained were positive for beta lactamase production. Results also show that all the *Pseudomonas aeruginosa* isolates (100 %) were completely resistant to tetracycline, sulphamethoxazole, ciprofloxacin and nitrofurantoin; followed by amoxycillin/clavulanic acid (91.7 %), cefotaxime (91.7 %), cefuroxime (91.7 %), ofloxacin (83.3 %), ceftriaxone (75 %), meropenem (58.3 %), gentamycin (50 %) and imipenem (25 %) being the least observed resistance. This study shows that *Pseudomonas aeruginosa* is one of the most common pathogen causing nosocomial infections; showing increasing resistance to β-lactam antibiotics especially by producing Amp-C β-lactamase, ESBLs and with some significant exhibition of resistance to carbapenems and aminoglycosides in FETHA I and FETHA II.

Keywords: *Pseudomonas aeruginosa*, AmpC, ESBL, antibiotics

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative motile bacillus and belongs to the family Pseudomonaceae. It is found in moist environment, disinfectant solutions, and water due to its ability to utilize many different organic compounds and survive in nutrient deficient conditions (Nadeem *et al*., 2009). It is a leading cause of nosocomial infection especially critical ill and immune-compromised patients (Hugbo *et al*., 1992). It has been implicated in diverse nosocomial pneumonia, urinary tract infection, surgical site infection, severe burns and infection of patients undergoing chemotherapy for neoplastic diseases or those on antibiotics therapy (Erdem, 1999). It has intrinsic resistance to many antimicrobial agents and show resistance to many antibacterial agents. The mechanism of resistance is due to cell wall permeability, production of extracellular chromosomal and plasmid mediated β-lactamases (Livermore, 1989), aminoglycoside modifying enzymes, cephalosporinases (Prince, 1986), and active multidrug efflux mechanism (Li *et al*., 1994). This multidrug resistant *Pseudomonas aeruginosa* causes nosocomial infections which are a global health care problem as it prolongs the duration of hospitalization and increases the cost of the patient care. The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of increasingly resistant strains of microbes. Selective pressure favouring resistant strains arises from misuse and overuse of antimicrobials (File, 1999; Livermore, 2005). The worldwide use of antimicrobials in different fields has created enormous pressure for the selection of resistance among opportunistic bacterial pathogens (Balostescu *et al*., 2003; Sharma *et al*., 2005).

According to Harberth *et al*. (2000), inadequate and prolonged antimicrobial prophylaxis increases resistance to antimicrobial drugs. Indeed this is more evident in developing countries like Nigeria, where drugs including antibiotics can be purchased over the counter without a doctor's prescription. In addition, antimicrobials are hawked and dispensed at street and market corners by anybody that has the means. In Nigeria, indiscriminate use of antibiotics, poor hygienic practices in hospitals have created suitable conditions for the emergence and uncontrollable spread of the Extended Spectrum Beta Lactamase (ESBL) and AMP-C enzymes (Arzai *et al*., 2008), thus making their detection complicated due to the variable affinity of these enzymes for different substrates and inoculums effects.

One of the mechanisms responsible for the increased antibiotic resistance in clinical isolates of *Pseudomonas aeruginosa* is due to AMP-C enzyme production. In recent years, antibiotic resistance has become a significant human health issue. Multiple antimicrobial resistant bacteria are presently considered as a great global threat to public health (Dundar *et al*., 2010). Resistance to multiple classes of antimicrobials (multidrug resistance) in particular is increasingly common in *P. aeruginosa* with a number of reports on pan-resistant isolates treatable with a single agent, colistin. Acquired resistance in this organism is multifactorial and attributable to chromosomal

mutations and the acquisition of resistance genes via horizontal gene transfer. Mutational changes impacting resistance include up-regulation of multidrug efflux systems to promote antimicrobial expulsion, derepression of Amp-C, Amp-C alterations that expand the enzyme's substrate specificity (i.e., extended-spectrum Amp-C), alterations to outer membrane permeability to limit antimicrobial entry and alterations to antimicrobial targets. Acquired mechanisms contributing to resistance in *P. aeruginosa* include β-lactamases, notably the extended-spectrum β-lactamases and the carbapenemases that hydrolyze most β-lactams, aminoglycoside-modifying enzymes, and 16S rRNA methylases that provide high-level pan-aminoglycoside resistance. (Dundar *et al*., 2010)

The worldwide emergence of multi-drug resistant bacterial strains is a growing concern, especially infections caused by *P. aeruginosa*. *P. aeruginosa* is an opportunistic pathogen with innate resistance to many antibiotics and disinfectants including anti-pseudomonal penicillins, ceftazidime, carbapenems, aminoglycosides and ciprofloxacin (Dundar *et al*., 2010). Pseudomonads are more versatile than Enterobacteriaceae in acquiring drug resistance by various mechanisms. The production of extended-spectrum beta-lactamases (ESBLs) confers resistance at various levels to expanded spectrum cephalosporins (Mirsalehian *et al*., 2010). These enzymes are encoded by different genes located on either chromosomes or plasmids (Aggarwal *et al*., 2008).

Pseudomonas aeruginosa, one of the most common pathogen causing nosocomial infections, has been reported to be increasingly resistant to β-lactam antibiotics, second and third generation cephalosporins especially by producing Amp-C β-lactamases and Extended Spectrum Beta Lactamases (ESBLs). Hence, this study was undertaken to phenotypically screen for the production of Amp-C beta lactamases and ESBLs in *Pseudomonas aeruginosa* isolates obtained from clinical samples in Federal Teaching Hospital Abakaliki (FETHA) and to determine their susceptibility patterns to newer antipseudomonal antibiotics.

Materials and Methods

Collection of Clinical Specimens: A total of 521 clinical samples were collected from patients between the months of January and July, 2015 in Federal Teaching Hospital, Abakaliki (FETHA I and FETHA II). The samples include pus, wound swab, sputum, urine, aspirates, catheter tips, high vaginal swab, endocervical swabs, stool, cerebrospinal fluid (csf), urethral swab, semen, pleural fluid, blood, ear swabs, throat swabs and eye swabs were collected from patients that visited the hospital for health care purposes.

Isolation, identification and characterization of the isolates: The samples were inoculated aseptically into culture media namely blood agar, MacConkey agar, chocolate agar and was incubated at 37^{o}C for 18-24 hours. Isolation and identification of isolates from the clinical samples

were aseptically carried out using standard microbiology techniques as described by Cheesbrough, 2009.

Disc diffusion susceptibility test: An 18-24 hour old broth culture of the *Pseudomonas aeruginosa* isolate was standardized by diluting to 0.5 Mcfarland's standard. Twenty milliliter each of molten Mueller Hinton agar was poured aseptically into sterile Petri dishes and then allowed to gel and labeled. A sterilized wire loop was used to inoculate the standardized culture of the organism on plates of Mueller Hinton agar medium. The surface of the medium was streaked in four directions while the plate was being rotated approximately 60º to ensure even distribution. With the Petri dish lid in place, the surface of the Mueller Hinton agar medium was allowed to dry for 25 minutes. A sterilized forceps was used to place the antibiotic discs evenly on the inoculated Mueller Hinton agar so that the disc should be about 15 mm from the edge of the plate and not close than 25 mm from disc to disc. After 30 minutes, the plates were inverted and incubated for at least 18-24 hours. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The interpretation as 'Sensitive' or 'Resistant' was done on the basis of diameters of zones of inhibition of bacterial growth as recommended by Clinical and Laboratory Standards Institute (CLSI, 2005). The following standard antibiotic discs were used against the isolates: Cefoxitin disc (30 µg), Imipenem (IPM 10 µg), Gentamycin (CN 30 µg), Meropenem (MEM 10µg), Sulphamethoxazole/Trimethoprim (SXT 25 µg), Amoxycillin/Clavulanic acid (TIM 30 µg), Cefotaxime (CTX 30 µg), Ofloxacin (OFX 5 µg), Ceftriaxone (CRO 30 µg), Cefuroxime (CXM 30 µg), Nitrofuratoin (F 200 µg), Ciprofloxacin (CIP 5 µg) and Tetracycline (TE 10 µg).

Presumption test for determining AMP-C beta lactamases: The test organism from overnight cultures was inoculated into nutrient broth and incubated at 35^{o}C for 3 hours. A standardized wire loop of about 4 mm diameter was used to pick a loopful of the test organism suspension and dropped at the centre of the Mueller Hinton Agar plate. A sterile dry swab stick was used to spread the inoculums evenly across the centre of the plate and was allowed for 15 minutes to pre-diffuse into the agar. Cefoxitin (30µg) disc was placed on Mueller Hinton Agar plate and incubated overnight. This procedure was used to test for Amp-C production using disc diffusion method and the result was interpreted as per the Clinical Laboratory Standard Institute Criteria (CLSI, 2005). Isolates that yielded a zone diameter less than 18mm (screened positive) was further subjected to confirmatory test using the Amp-C disc test technique.

AMP-C disc test: All isolates from the presumptive test was subjected to the AMP-C test for confirmation as described by Black *et al*., 2004.

ESBL (Extended Spectrum Beta Lactamase) test: Extended spectrum beta lactamase production was determined among isolated *P. aeruginoas* using the double disc synergy test method.

SCREENING FOR BETA LACTAMASE PRODUCTION USING NITROCEFIN STICK

The nitrocefin stick was allowed to cool to room temperature and a colony of *Pseudomonas aeruginosa* from the isolation medium was selected. A nitrocefin stick (the colour coded end) was used to touch the colony and the stick was rotated to pick mass of the cells. Two drops of distilled water was used to moisten the tip of the stick and was allowed for 5 minutes and further observed for pink-red colour development.

RESULTS

Table 1: Clinical samples collected from Federal Teaching Hospital, Abakaliki (FETHA I)

S/N	Sample source	No of samples collected	No of *Pseudomonas aeruginosa* isolated
1.	Urine	140	1
2.	Wound swab	13	6
3.	Endocervical swab	6	-
4.	High vaginal swab	12	-
5.	Sputum	30	-
6.	Eye swab	3	-
7.	Throat swab	4	-
8.	Aspirates	4	-
9.	Ear swab	2	-
10.	Pus	1	-
11.	Blood	6	-
12.	Pleural fluid	1	-
13.	Semen	10	1
14.	Urethral swab	1	-
15.	Stool	6	-
	Total	239	8

Table 2: Clinical samples collected from Federal Teaching Hospital, Abakaliki (FETHA II)

S/N	Sample source	No. of samples collected	No. of *Pseudomonas aeruginosa* isolated
1.	Urine	144	1
2.	Wound swab	6	3
3.	Endocervical swab	44	-
4.	High vaginal swab	18	-
5.	Sputum	23	-
6.	Eye swab	3	-
7.	Throat swab	8	-
8.	Aspirates	1	-
9.	Ear swab	4	-
10.	Blood	3	-
11.	Semen	12	-
12.	Urethral swab	3	-
13.	Stool	5	-
	Total	282	4

Table 3: Amp-C beta lactamase producing *Pseudomonas aeruginosa* isolated from FETHA I and FETHA II

FETHA I			
S/No	Isolate code	Identity of organism	ESBL production
1	Fet 1-a	*Pseudomonas aeruginosa*	Positive
2	Fet 1-b	*Pseudomonas aeruginosa*	Positive
3	Fet 1-c	*Pseudomonas aeruginosa*	Positive
4	Fet 1-d	*Pseudomonas aeruginosa*	Negative
5	Fet 1-e	*Pseudomonas aeruginosa*	Negative
6	Fet 1-f	*Pseudomonas aeruginosa*	Negative
7	Fet 1-g	*Pseudomonas aeruginosa*	Negative
8	Fet 1-h	*Pseudomonas aeruginosa*	Negative

FETHA II

S/No	Isolate code	Identity of organism	ESBL production
9	Fet 2-a	*Pseudomonas aeruginosa*	Positive
10	Fet 2-b	*Pseudomonas aeruginosa*	Negative
11	Fet 2-c	*Pseudomonas aeruginosa*	Negative
12	Fet 2-d	*Pseudomonas aeruginosa*	Negative

Table 4: Phenotypic screening for Extended Spectrum Beta-Lactamase (ESBL) producing *Pseudomonas aeruginosa* isolated from FETHA I AND II

FETHA I			
S/No	Isolate code	Identity of organism	ESBL production
1	Fet 1-a	*Pseudomonas aeruginosa*	Positive
2	Fet 1-b	*Pseudomonas aeruginosa*	Positive
3	Fet 1-c	*Pseudomonas aeruginosa*	Positive
4	Fet 1-d	*Pseudomonas aeruginosa*	Negative
5	Fet 1-e	*Pseudomonas aeruginosa*	Negative
6	Fet 1-f	*Pseudomonas aeruginosa*	Negative
7	Fet 1-g	*Pseudomonas aeruginosa*	Negative
8	Fet 1-h	*Pseudomonas aeruginosa*	Negative

FETHA II

S/No	Isolate code	Identity of organism	ESBL production
9	Fet 2-a	*Pseudomonas aeruginosa*	Positive
10	Fet 2-b	*Pseudomonas aeruginosa*	Negative
11	Fet 2-c	*Pseudomonas aeruginosa*	Negative
12	Fet 2-d	*Pseudomonas aeruginosa*	Negative

Table 5: Beta lactamase producing *Pseudomonas aeruginosa* isolated from FETHA I AND II

S/No	Isolate code	Identity of organism	β-lactamase production
1	Fet 1-a	*Pseudomonas aeruginosa*	Positive
2	Fet 1-b	*Pseudomonas aeruginosa*	Positive
3	Fet 1-c	*Pseudomonas aeruginosa*	Positive

4	Fet 1-d	*Pseudomonas aeruginosa*	Positive
5	Fet 1-e	*Pseudomonas aeruginosa*	Positive
6	Fet 1-f	*Pseudomonas aeruginosa*	Positive
7	Fet 1-g	*Pseudomonas aeruginosa*	Positive
8	Fet 1-h	*Pseudomonas aeruginosa*	Positive
9	Fet 2-a	*Pseudomonas aeruginosa*	Positive
10	Fet 2-b	*Pseudomonas aeruginosa*	Positive
11	Fet 2-c	*Pseudomonas aeruginosa*	Positive
12	Fet 2-d	*Pseudomonas aeruginosa*	Positive

Table 6: Antibiotic sensitivity profile of *Pseudomonas aeruginosa* isolates

	Frequency (%)	
Antibiotics	Resistance	Susceptible
Imipenem (10 µg)	25	75
Gentamycin (30 µg)	50	50
Meropenem (10 µg)	58.3	41.7
Ceftriaxone (30 µg)	75	25
Tetracycline (10 µg)	100	0
Sulphamethoxazole/Trimethoprim (30 µg)	100	0
Ofloxacin (5 µg)	83.3	16.7
Amoxycillin/Clavulanic acid (30 µg)	91.7	8.3
Cefotaxime (30 µg)	91.7	8.3
Ciprofloxacin (10 µg)	100	0
Nitrofurantoin (200 µg)	100	0
Cefuroxime (30 µg)	91.7	8.3

DISCUSSION

Pseudomonas aeruginosa infection is a major cause of mortality and morbidity in hospitalized patients of developing countries (Rastegar *et al*., 2000). The emergence of multi-drug resistant bacterial strains is a growing concern, especially infections caused by *P. aeruginosa* and the development of bacterial resistance is a major worldwide problem complicating the use of chemotherapeutic agents and the control of infectious diseases (Russell *et al*., 1999).

Eight *Pseudomonas aeruginosa* isolates were obtained from the 239 clinical samples collected from FETHA I (Table 1). The highest prevalence rate of *Pseudomonas aeruginosa* isolation in FETHA I was observed in wound samples as 6 (75 %) isolates were recovered from the wound samples unlike the urine and semen samples in which 1 (12.5 %) *Pseudomonas aeruginosa* isolate was each observed (Table 1).

Four *Pseudomonas aeruginosa* isolates were obtained from the 282 clinical samples collected from FETHA II (Table 2). The highest prevalence rate of *Pseudomonas aeruginosa* isolation in FETHA II was again observed in wound samples as 3 (75 %) were recovered from the wound samples unlike the urine sample in which 1 (25 %) *Pseudomonas aeruginosa* isolate was observed. No *Pseudomonas aeruginosa* isolate was observed in semen samples and other clinical samples collected in FETHA II. This result, again, explains that *Pseudomonas aeruginosa* are more prevalent in wound samples than urine, semen and other clinical samples collected.

In 2011, the isolation frequency of *Pseudomonas aeruginosa* in clinical samples by Bashir *et al*. was 27.3 % from urine, followed by 24.2 % from wound infections. The report of this research work agrees in part with the report of Bashir *et al*. in 2011 as *Pseudomonas aeruginosa* isolates were obtained from both urine and wound samples. The result of Bashir *et al*. shows that *Pseudomonas aeruginosa* is more prevalent in urine samples than wound samples but the result of this research work does not agree with the report of Bashir *et al*. as more *Pseudomonas aeruginosa* isolates were obtained from wound samples (75 %) when compared to urine samples (12.5 %). In 2008, Javiya *et al*. reported that the highest number of *Pseudomonas* infection was found in urine followed by pus and sputum which indicates that wound infections and Urinary tract infections are the most common hospital acquired infections. This research work is also in concord with the report of Javiya *et al*., in 2008.

Pseudomonas aeruginosa is an opportunistic pathogen with innate resistance to many antibiotics and disinfectants including anti-pseudomonal penicillins, ceftazidime, carbapenems, aminoglycosides and ciprofloxacin (Dundar *et al*., 2010). Resistance to extended spectrum cephalosporins may arise from over-expression of the naturally occurring cephalosporinase or acquired beta-lactamases such as extended-spectrum β-lactamases (ESBL) and Amp-C β-lactamases (Amp-C). In this study, 8 (66.7 %) out of the 12 *Pseudomonas aeruginosa* isolates

obtained from both FETHA I and FETHA II were positive for Amp-C β-lactamase production (Table 3).

In a similar study from India, Amp-C production was observed in antibiotic resistant *Pseudomonas* spp (Parveen *et al*., 2010). Our study correlated well with reports from Aligarh by Shahid *et al*., in 2004 as 20 % and from Kolkata by Arora *et al*., in 2005 as 17.3 % and from Varanasi as 22 %.

Amp-C β-lactamase producing bacteria can cause major therapeutic failure if they remain undetected because in routine Kirby- Bauer Disc diffusion test for antibiotic sensitivity, they may show false sensitivity zone. But the fact is that Amp-C β-lactamase producing organisms particularly *Pseudomonas aeruginosa* is on the rise and poses a major therapeutic challenge due to treatment failure, and have been responsible for several nosocomial outbreak (Arora *et al*., 2005).

In this study, all the 12 *Pseudomonas aeruginosa* isolates obtained from FETHA I and FETHA II produced beta lactamases and this explains their high resistance to the β-lactam antibiotics (Amoxycillin/Clavulanic acid) used with a resistance frequency of 91.7 % (Table 5 and 6). This research work also reported that 4 (33.3 %) *Pseudomonas aeruginosa* isolates out of the 12 *Pseudomonas aeruginosa* isolates were positive for ESBLs production (Table 4). The detection of extended spectrum β-lactamases (ESBLs) in Amp-C producing *Pseudomonas* species isolates is another major problem.

In a study from Brazil, 84 *P. aeruginosa* isolates were found to produce ESBL (Pellegrino *et al*., 2004). In the present study, 4 (33.3 %) isolates of *Pseudomonas aeruginosa* isolates produced ESBLs (Table 4). Likewise, in two other studies from India and Brazil, similar high rates of ESBL production of about 21 % was observed among *P. aeruginosa* isolates (Aggarwal *et al*., 2008). These observations suggest that the ESBLs which are generally widespread among members of Enterobacteriaceae are also increasingly found in *P. aeruginosa*. Horizontal gene spread is considered to be responsible for the high frequency of ESBLs detected in *P. aeruginosa* (Jiang, 2006). In our study, antibiotic resistant *P. aeruginosa* isolates were noted to produce ESBLs and Amp-C β-lactamases. The report of our study corroborates the report of other researchers.

All the ESBL-producing *P. aeruginosa* isolates were multi-drug-resistant as they were resistant to at least two different classes of antibiotics. Isolates were 100 % resistant to tetracycline, sulphamethoxazole/trimethoprim, ciprofloxacin and nitrofurantoin (Table 6). This was closely followed by amoxicillin/clavulanic acid (91.7 %), cefotaxime (91.7 %), cefuroxime (91.7 %), ofloxacin (83.3 %), ceftriaxone (75 %), meropenem (58.3 %), gentamycin (50 %) and imipenem (25 %) (Table 6). This antibiotic sensitivity result shows that carbapenems and aminoglycosides are the most active antibiotics for the treatment of *Pseudomonas aeruginosa* infections. The most effective antibiotic from the report of this study is imipenem in which 25 % resistance by

Pseudomonas aeruginosa isolates was observed. This is in harmony with the findings of Okesola and Oni (2012) and also in agreement with our study.

The role of carbapenems in the treatment of serious bacterial infections caused by β – lactamase resistant bacteria is a great advancement. The carbapenems used in this research work are imipenem and meropenem. In this study, the resistance of *Pseudomonas aeruginosa* to imipenem and meropenem antibiotics is 25 % and 58.3 % respectively, showing that imipenem was the most effective carbapenems antibiotic used. A Latin America multicenter study, the SENTRY – Antimicrobial Surveillance Program – reported in 2001 that *P. aeruginosa* presented resistance of 37.8 % to imipenem and 35.6 % to meropenem (Sader *et al*., 2005).

In the present study, maximum no of strains (50 % - 75 %) were susceptible to imipenem, gentamycin and meropenem compared to 86 % susceptibility to imipenem in the study conducted in BHU (Bhattacharjee *et al*., 2008). At Saint Joseph Martin Hospital in Buenos Aires from 1998 to 2001, Rodriguez *et al.*, reported that *P. aeruginosa* showed an increasing resistance to imipenem from 15.4 % to 68 %. Imipenem was found to be the most efficacious drug against *Pseudomonas* spp. in our study and this is in accordance with the findings of Ullah *et al*. in 2009. However, notably in our study, imipenem underperformed compared to the 100 % susceptibility found in ESBL-producing Gram negative isolates, including *P. aeruginosa*, in different studies (Winokur *et al*., 2001). Decreased susceptibility to imipenem is a matter of great concern for treating infections caused by *Pseudomonas aeruginosa* and indicates the urgent need for improved infection control strategies.

Until the end of the 1980s, fluoroquinolones had excellent activities against *P. aeruginosa* but extensive use of the antimicrobial, in particular ciprofloxacin, had led to an increasing incidence of ciprofloxacin–resistant isolates (Dalhoff, 1994). The current study showed that the *Pseudomonas aeruginosa* isolates were 100 % resistant to ciprofloxacin. This finding is higher than that in a study in India which found that the resistance of ciprofloxacin against *P. aeruginosa* was (73.2 %) (Arora, 2011). The resistance of *Pseudomonas aeruginosa* isolates to ciprofloxacin in our study exhibited similar resistance trends as those isolated from hospital patients as reported by Bouza *et al*. (1999), Cavallo *et al*. (2000) and Strateva *et al*. (2007). Although, *P. aeruginosa* strains exhibit an intrinsic susceptibility to β-lactams, imipenem, aminoglycosides and flouroquinolones, *P. aeruginosa* resistance to these antibiotics has emerged and is widespread (Pagani *et al*., 2005).

In this study, the *Pseudomonas aeruginosa* isolates showed moderate resistance to the aminoglycosides. This is in contrast to Anjun *et al*. (2010) and Strateva *et al*. (2007), where the resistance rates of their hospital isolates to gentamicin were much higher at 66 % and 79.7 % respectively. The present study is in agreement with a study which found that *P. aeruginosa*

isolated from patients demonstrated resistance to gentamycin (Drenkard, 2003). According to Boffi *et al*. (2000), substantial regional variation in resistance patterns has been observed and is probably related to antibiotic treatment regimen.

The report of this research work also showed that the *Pseudomonas aeruginosa* isolates tested exhibited resistance frequency of 75 % to 91.7 % to the second generation (cefuroxime) and third generation (cefotaxime and ceftriaxone) cephalosporin antibiotics used in this study.

In 2010, Parveen *et al*. reported that 55 % of *P. aeruginosa* isolates were also resistant to third generation cephalosporins. These reports corroborate the report of this study.

Antibiotic resistance is now generally accepted as a major public health issue and *P. aeruginosa* infection is considered a major problem because of its resistance to relatively high levels of most antibiotics in use, particularly due to the combination of the following mechanisms: beta-lactamase production, a strong barrier to diffusion at the outer bacterial membrane and bacterial efflux (Owens *et al*., 2008).

CONCLUSION

Pseudomonas aeruginosa continues to be leading cause of serious infections particularly nosocomial infections. The present study has demonstrated that *Pseudomonas aeruginosa* are ESBL and AMP-C β-lactamase producers and are surprisingly developing resistance to life saving antimicrobial agents such as imipenem, meropenem and gentamycin. From this study, we conclude that there is the presence of Amp-C and ESBL- β-lactamase producing *P. aeruginosa* in FETHA I and FETHA II. This has important implications, as carbapenems remain the only choice of treatment for infections caused by *Pseudomonas aeruginosa*. Also, the emergence of multidrug resistant *Pseudomonas aeruginosa* due to the indiscriminate use of antibiotics is a challenging clinical problem which leads to the development of resistance to the routinely used antibiotics. Control measures to control the spread of these multiple antibiotic resistant *Pseudomonas aeruginosa* strains in the hospital should include judicious use of antibiotics and implementation of surveillance strategies to know the susceptibility pattern and to detect the ESBL and AMP-C β-lactamase producers.

AUTHORS DISCLOSURE STATEMENT

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