Incidence and Molecular Identification of *Escherichia Coli* Harbouring Gentamicin Resistant Gene among Pregnant Women

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Abstract: Pregnant-origin Gram negative enteric bacteria, particularly, *Escherichia coli* isolates were exposed to tests for resistance phenotype using Kirby-Bauer's disk-diffusion assay and for gentamicin resistance genotype using *aacC2*gene, codifying for one specific aminoglycoside-modifying enzyme. The incidence of Enterobacteriaceae was 31 (21.8%), emerged from142 midstream urine (MSU) samples, including symptomatic (17.6%) and asymptomatic (25.7%) bacteriuria. Women with age brackets from 15-44 years were studied. Age group 25-29 years scored the highest incidence rate of infection (29.2%), while age group 35-39 years exhibited the lowest incidence rate. Data described previously were statistically insignificant (*P* value > 0.05). Remarkably, significant differences (*P* value <0.05) observed concerning gestational ages when third (32.81%) and second (14.81%) trimesters were noticed with the highest rate of bacteriuria, respectively. *E. coli* distinguished as the most predominant (41.9%), followed by *Klebssiella pneumoniae* (19.4%) and *Pseudomonas aeruginosa* (12.9%), consecutively. Seven other uropathogens were identified with a lesser frequency. Isolates subjected to numerous antibiotics and results revealed fully resistance to pencillin G and amoxicillin-clavulanic acid, while (96.8%) possessed resistant to amoxicillin, cephlexin, and vancomycin. However, amikacin still acting perfectly (90.3%) against all the isolates, especially towards *E. coli* (92.3%). Concurrently, the antimicrobial potency of streptomycin, ciprofloxacin, and ceftriaxone were (92.3%), (84.6%), and (77%) respectively, when assayed opposite *E. coli* strains solely. Phenotypically, (30.8%) of *E. coli* strains, which stand for about (32.3%) of the whole isolates, were resistance to gentamicin. 75% of phenotypically gentamicin resistance *E. coli* demonstrated genotypically as harbouring the *aacC2* gene.

Keywords: *E. Coli*, UTI, Pregnancy, *Aacc*2 Gene, Antibiogram

1. Introduction

Urinary tract infections (UTI) considered as the second most common clinical problem (Thapa et al., 2015). It is agreed from the past forty decades that UTI is more prevalent in pregnant women than in non-pregnant by about 4 – 10 times (Shulman & Herlinger, 1975). The physio-anatomical and functional perturbation that happen to the urinary system during pregnancy, in particular a decrease in the urine concentration by virtue of the physiological increase in plasma volume, equal to 70% of pregnant women evolve glucosuria, which facilitates bacterial growth in the urine (Murtaza, 2002; Lucas & Cunningham, 1993).

Bacteriuria may exhibit as asymptomatic or symptomatic (Connolly & Thorp, 1999). Asymptomatic infections usually ascribed to pregnant women and the elderly (Al-Dujiaily, 2000). Various factors including low socioeconomic status, multiparty, age, sexual behaviour, urinary tract anomalies, and

previous treatment for UTI, in addition to diabetes, sickle cell anaemia, and immuno-compromised patients had been linked to asymptomatic bacteriuria (Murtaza, 2002; Njoku et al., 1998). Untreated UTI can be associated with serious obstetric complications. These consist of caesarean and preterm deliveries with low birth weight infants, intrauterine growth retardation, fatal death, preeclampsia, maternal sepsis and respiratory insufficiency (Shulman & Herlinger, 1975; Moyo et al., 2010; Mohamed, 2012).

Among diverse pathogens that cause UTIs during pregnancy, *E. coli* with its multidrug resistant strains considered as predominant (Dalzell & Lefevre, 2000; Kariuki et al., 2007). *Klebsiella*, *Enterobacter*, and *Proteus* species, in addition to gram positive isolates, such as *Staphylococcus saprophyticus* and *Enterococcus* account for the remaining cases (Gilstrap & Ramin, 2001; Masinde et al., 2009). It is strongly recommended that screening and treatment of asymptomatic bacteriuria in pregnant women should be performed on time to minimize the unfavourable consequences (Nicolle, 2006). Unfortunately, urinary tract pathogens evolved resistance to antibiotics, and it has been known to increase over the time (Kahlmeter, 2003). Moreover, the manner by which these pathogenic strains gain resistance to the antibiotics varied depending on the position of isolation and the geographical area (Jamie et al., 2002). In 2009, resistance of infectious agents to highly potent aminoglycosides was of special concern, since they are used widely for the management of various infections. Diverse mechanisms formulated to explain the resistance that can cohabit in the same cell; they are mediated by the enzymatic modification via acetyltransferases, nucleotidyltransferases, and phosphotransferases (Dias-Goncalves et al., 2015; Davis et al., 2010). Genetically, in most of the instances, the *aacC2* (gentamicin-resistance) gene identified most frequently in strains of gram-negative Enterobacteriaceae separated from clinical specimens (Lindemann et al., 2011). Additionally, it has been detected that these genes are often located on transposons, which plays an important role in the dissemination of drug resistance across inter and intra species boundaries (Ramirez & Tolmasky, 2010). Interestingly, intrinsic resistance of *Streptomyces* and *Micromonospora* species, the aminoglycoside-producing bacteria to aminoglycosides explained by the methylation of nucleotides situated within the A site of the 16S rRNA, thus preventing the disruption of translation process by aminoglycosides (Davis et al., 2010).

Due to impractical and incredible prescription of gentamicin by Gynecologist and Obstetrics to women suffered from UTI before or even during pregnancy in some area of Asia, in particular Iraq, we found it of great issue to undertake this study. We aimed to determine the incidence of Gram negative bacteriuria in a population of pregnant women with investigating various attributed pathogenic microbes, in addition to checking for the antibiotic susceptibility while focusing on gentamicin resistant *E. coli* to allocate it with the molecular pattern that confer gentamicin resistance.

2. Materials and Methods

2.1 Study Population

A study was conducted from November 2015 to February 2016 at both Sheray Naqeeb Maternity Hospital and Sherwana Health Centre, Kalar, Kurdistan region, Iraq. Pregnant women regardless of symptoms of UTI between the ages of 15 to 43 years with no gestational age limits were included, while those who received antibiotics therapy within at least 72 hours and non-pregnant women were excluded in this study. Information about age, duration of gestation and symptoms of UTI was obtained from each pregnant woman using standard questionnaires and kept confidential during the

research.

2.2 Sample Collection and Bacterial Identification

Total of 142 mid-stream urine (MSU) samples were collected aseptically in sterile disposable containers. Specimens labelled and transported to the microbiology laboratory. A calibrated sterile platinum wire loop was used to transfer 1µl of uncentrifuged urine specimen and streaked on MacConkey agar. All inoculated plates were incubated at 37°C for 18-24 hours. Significant bacteriuria in both symptomatic and asymptomatic pregnant women was defined by $\geq 10^5$ colony forming units (CFU) per mL of urine (Stamm et al., 1982). Whole isolates were identified using classical biochemical methods (Cheesebrough, 2004; Prescott et al., 2008), while investigation of *E. coli* strains carried out biochemically and by molecular techniques (PCR) using primers designed for 16S rRNA (Table, 1).

2.3 Antibiotic Susceptibility Testing

Antimicrobial susceptibility test (AST) was performed for all the isolates by Kirby-Bauer's disk-diffusion method (Bauer et al., 1966) as recommended by Clinical and Laboratory Standard Institute (CLSI, 2012). Disks of 16 different antibiotics (Bioanalyse, Turkey) including vancomycin (10µg), amikacin (30µg), amoxicillin (25µg), amoxicillin/clavulanic acid (30µg) gentamicin (10µg), rifampin (5µg), azithromycin (15µg), cephalexin (30µg), trimethoprim (10µg), nalidixic acid (30µg), nitrofurantoin (100µg), tetracycline (10µg), and penicillin G (10µg) were spread on fresh plates of Mueller-Hinton agar and incubated overnight at 37°C. *E. coli* ATCC25922 was used as a reference strain for the identification and AST.

2.4 DNA Extraction

Genomic DNA was extracted from clinical isolates of *E. coli* using boil preparation method (a single colony from each isolates was carefully suspended in 50µl of deionized water, then boiled for 10 min at 95°C and spun down at 10,000 × g for 5 min. supernatant was used directly as a DNA template for PCR).

2.5 PCR Primers

Primers in (Table, 1) were provided by CinnaGen and designed based on the complete annotation sequence of *Escherichia coli* 16SrRNA and Gentamicin resistant (*aacC2*) gene exhibited by the National Centre for Biotechnology Information (Accession No. J01859.1, Accession No.X51534.1).

Bacteria	Primers	Primer sequence(5′-3′)	Product size(bp)	Reference
Escherichia coli	16SrRNA-F 16SrRNA-R	GAAGACTGACGCTCAGGTGCGAA CCGTGGCATTCTGATCCACGATTA	627	This study
Escherichia coli	16SrRNA-F 16SrRNA-R	GAA GAA GCT TGC TTC TTT GCT G GAG CCC GGG GAT TTC ACA T	544	(Sabat et al., 2000)
*aacC*2 gene	F-*aacC*2 R-*aacC*2	CGG AAG GCA ATA ACG GAG GCA AT CGT TTC TTC CAA GCA TCG GCA TC	573	This study
*aacC*2 gene	F-*aacC*2 R-*aacC*2	TAG AGG AGA TAT CGC GAT GC ATT ATC ATT GTC GAC GGC CT	896	(Ho et al., 2010)

Table 1: Sequence, symbol, and product size of designed and reference primers

2.6 PCR Amplification (16S rRNA and *aacC2* gene)

PCR assay performed in 25µl final reaction volume, consisted of 2µl of DNA template, 0.5µl for each primer, 12.5µl of One PCR^{TM} master mix (GeneDirex), consisted of Taq DNA polymerase, PCR buffer, dNTPs, gel loading dye, enhancer, and fluorescence dye. The volume of the reaction was completed with the addition of nuclease free water. The PCR mixtures were spun down shortly for 5-10 seconds, then placed in thermal cycler (TCY, Crealcon, NL) and subjected to the following cycling conditions: initial denaturation at 94°C for 4minutes, followed by 35 cycle of denaturation at 94°C for 30 seconds, annealing at 55°C for 1minute and extension at 72°C for 2minutes and a final extension step at 72$^{°C}$ for 5minutes.

Amplified DNA fragments were visualized in 1.5% agarose gel electrophoresis containing ethidium bromide at 90 volts for 60 minutes at room temperature. Amplicon size determined by comparison with 100 bp DNA ladder (GeneDirex).

2.7 Statistical analysis

Data were analysed using Statistical Package for Social Sciences (SPSS, 2009). Probability values of $P< 0.05$ were considered as statistically significant.

3. Results and Discussion

3.1 Incidence of Bacteriuria

During the embarked study, 142 MSU samples gathered from recruited pregnant women with symptomatic and asymptomatic bacteriuria. Our data (Table, 2) declared 31 (21.8%) samples positive for Enterobacteriaceae, 19(25.7%) of them accounted as ASB, while 13 (17.6%) were SB. Previous finding overwhelmingly was not statistically significant (*P* value > 0.05). Results of (Rizvi et al., 2011) are underpinning our findings via showing higher incidence of ASB (74.8%) than SB (25.2%). This might be due to silent infection that has been left without being investigated at early stages, the fact that supports the concurrent recommendation of routine screening for UTI in pregnant women during the first visit to the hospital to avoid unwanted complications (McIsaac et al., 2005). However, it contradicts an earlier report by (Masinde et al., 2009) who displayed the incidence of SB and ASB as 17.9% and 13.0%, respectively.

Concerning age groups, individuals of the age group 25-29 years had the highest incidence of infection (29.2%). The second highest age group was 40-44 years (28.6%), and lesser rates were with the remaining age groups (Table, 2), which is statistically insignificant. Our observation tends to agree with that of Akobi et al., (2014). On the other hand, it is in contrast with the findings of Kawser et al., (2011) in which the age group 21-25 years occupied the first foremost of the age bracket 16 to 40 years. This discrepancy could be due to multiparity as well as more sexual activity as two possible factors influencing the fluctuation of UTI among pregnant women. Interestingly, significant outcomes were observed when samples distributed over gestational periods. Pregnant women in third trimester had the highest incidence (32.81%), accompanied by second trimester (14.81%). This is consistent with previous report by Okonko et al., (2009). Surprisingly, Turay et al., (2014) revealed the first and second trimesters as having the greatest rate of infection, which counteract current observation. Races, geographical distribution, poverty, and different locations

where studies had been executed might explain the differences.

It is predicted that the endangerment of UTI possibly commence within week six, and reach maximum peak within 22-24th week of pregnancy (Rahimkhani et al., 2006). Furthermore, as a consequence of increased in uterus weight and development, passage of urine from the bladder can be obstructed leading to infection. In addition, dilatation of the uterus caused by muscular flexibility as a result of a raise in progesterone level, in turn minimize the flow of urine (Fasalu et al., 2015).

Table 2: Incidence of bacteriuria among pregnant women distributed depending on various parameters

Characteristics	Significant Bacteriuria		Total No. (%)	Chi-square	P-value
	Positives No. (%)	Negatives No. (%)			
Symptoms of UTI					
SB	12(17.6)	56(82.3)	68(47.9)	**1.339**	**.247**
ASB	19(25.7)	55(74.3)	74(52.1)		
Total	**31(21.8)**	**111(78.2)**	**142(100)**		
Age (Years)					
15-19 Years	2(25)	6(75)	8(5.6)	**4.222**	**.518**
20-24 Years	6(15.4)	33(84.6)	39(27.5)		
25-29 Years	14(29.2)	34(70.8)	48(33.8)		
30-34 Years	6(22.2)	21(77.8)	27(19)		
35-39 Years	1(7.7)	12(92.3)	13(9.2)		
40-44 Years	2(28.6)	5(71.4)	7(4.9)		
Total	**31(21.8)**	**111(78.2)**	**142(100)**		
Trimester					
First Trimester	2(8.3)	22(91.7)	24(16.9)	**8.643**	**.013**
Second Trimester	8(14.81)	46(85.19)	54(38)		
Third Trimester	21(32.81)	43(67.19)	64(45.1)		
Total	**31(21.8)**	**111(78.2)**	**142(100)**		

3.2 Gram Negative Bacterial Profile

Within 31 isolates of Enterobacteriaceae identified by biochemical tests, suspicious one confirmed using BIOMÉRIEUX VITEK 2 system. 13(41.9%) *E. coli* strains ranked as the dominant organism. *K. pneumoniae* 6(19.4%) ranked next. 4(12.9%) of the isolates were *P. aeruginosa* and 2(6.5%) were *P. vulgaris*, respectively. The rest (Table, 3) were found equally. Of a special concern, strains of *E. coli* were molecularly identified by PCR (Figure, 1). This observation seems to agree to high extent with the findings of numerous other researchers. For example, Okonko et al., (2009) and Manjula et al., (2013) independently reported *E. coli*, *Klebsiella* sp. and *P. aeruginosa* as the 3 most common organisms implicated in UTIs, while results exhibited by Battikhi and Battikhi, (2015) revealed *E. coli*, and *K. pneumonia* with the highest incidence rate. In contrary, Ezeigbo et al., (2016) announced that *P. mirabilis* occupied next to *E. coli* as the most predominant organisms. Stamey and Sexton, (1975) stated that uropathogens are most likely originated from elementary tract and rise through

faecal-urethral path. Additional factor that encourage microbial growth and predisposes females to bladder contamination, in particular during pregnancy, is the female's perineum with moist environment (Ebie et al., 2001). Moreover, pregnant women suffers from difficulties to clean the perineum properly contribute to increase in the incidence of UTIs (Demilie et al., 2012). Since implication of *E. coli* in UTI is becoming a fact, it could be due to various factors that had been proposed by investigators at different times and locations. pH and osmotic pressure of the females urine was found as two factors promotes the growth of *E. coli* (Asscher, 1981; Obiogbolu et al., 2009). It is predicted that a significant increase in the level of lactose and amino acids during the gestational period enhance the growth of *E. coli* in urine (Weatheral et al., 1988).

Table 3: Uropathogenic Gram negative bacteria isolated from pregnant women

No.		ASB	SB	No(%)
1	*Escherichia coli*	**4**	**9**	13(41.9)
2	*Klebssiella pneumonia*	**4**	**2**	6(19.4)
3	*Pseudomonas aeruginosa*	**3**	**1**	4(12.9)
4	*Proteus vulgaris*	**2**	**0**	2(6.5)
5	*Citrobacter koseri*	**1**	**0**	1(3.2)
6	*Serratia marcescens*	**1**	**0**	1(3.2)
7	*Enterobacter spp.*	**1**	**0**	1(3.2)
8	*Morganella morganii*	**1**	**0**	1(3.2)
9	*Proteus mirabilis*	**1**	**0**	1(3.2)
10	*Salmonella typhimurium*	**1**	**0**	1(3.2)
		19	**12**	

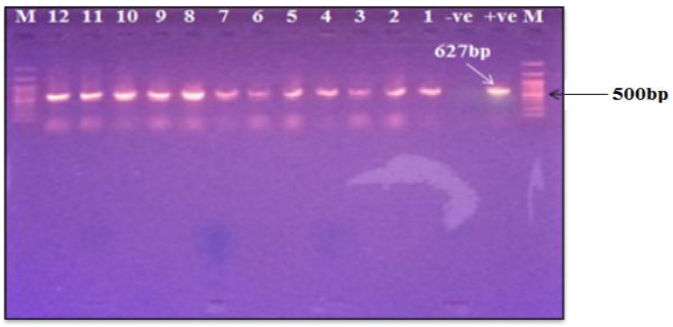

Figure 1: Molecular identification of UPEC isolates. M: 100bp DNA marker. +ve; positive control (*E. coli* ATCC 25922), -ve; negative control. Lanes 1 to 12; PCR amplification of 16SrRNA of *E. coli* isolates.

3.3 Antimicrobial Susceptibility Pattern

Results (Table, 4) illustrate the response of 31 isolates to 13 different antimicrobial agents. Microorganisms of the current study were highly resistance to AMC and PG with a rate of (100%), while expressed less resistant (96.8%) to AX, CL, and VA. Moreover, progressive resistance to RA (74.2%), F (71%), and NA (61.3%) has been achieved. These results are worrisome since it might means excessive and unnecessary use of antibiotics, which most of them tend to be safe for treating patients with UTIs, especially pregnant women. Therefore, in order to minimize resistant dissemination, non-

Table 4, Antimicrobial resistance pattern among uropathogenic Gram negative isolates from pregnant women

Isolated bacteria`	No.	AK(%)	AX	TMP	F	CN	AMC	NA	CL	RA	AZM	TE	VA	PG
Escherichia coli	13	1(7.7)	13(100)	8(61.5)	7(53.8)	4(30.8)	13(100)	9(69.2)	13(100)	10(76.9)	2(15.4)	3(23)	13(100)	13(100)
Klebsiella pneumoniae	6	1(16.7)	6(100)	0(0)	3(50)	3(50)	6(100)	2(33.3)	6(100)	4(66.7)	3(50)	3(50)	6(100)	6(100)
Pseudomonas aeruginosa	4	0(0)	4(100)	4(100)	4(100)	1(25)	4(100)	3(75)	4(100)	4(100)	1(25)	3(75)	4(100)	4(100)
Proteus vulgaris	2	0(0)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)
Citrobacter koseri	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)
Serratia marcescens	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	(0)	1(100)	1(100)
Enterobacter spp.	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	1(1000	1(100)
Morganella morganii	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)
Proteus mirabilis	1	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)
Salmonella typhimurium	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
Total	31	3(9.7)	30(96.8)	16(51.6)	22(71)	10(32.3)	31(100)	19(61.3)	30(96.8)	23(74.2)	9(29)	12(38.7)	30(96.8)	31(100)

AK: Amikacin, Ax: Amoxicillin, TMP: Trimethoprim, F: Nitrofurantoin, CN: Gentamicin, AMC: Amoxicillin-clavulanic acid, NA: Nalidixic acid, CL: Cephalexin, RA: Rifampin, AZM: Azithromycin ,TE: Tetracycline , VA: Vancomycin , P: Penicillin G

essential use and prescription of antibiotics should be avoided. Since this study carried out in a developing country, the case looks quite similar to that in Vietnam and Uganda, as the antibiotics can be obtained via private providers without been prescribed by professionals from health sector. However, the case is clearly different in developed countries such as in Sweden. Thus, low level of resistance among bacterial isolates may be a consequence of both less use of antibiotics and carefully monitored by clinical health practitioners (Ramos et al., 2012). Fortunately, Amikacin scored the highest rate of antibacterial activity (90.3%) spreading over all the isolates, in particular against *E. coli* (92.3%). This achievement is appropriate with the data presented by Rizvi et al., (2011) when they showed that AK had a strongest antimicrobial activity. More specifically, Soleimani et al., (2016) revealed that the rate of resistance to aminoglycosides is approximately high, in spite of that; amikacin appeared to be the most effective one towards the UPEC.

It is of essential to point out that only strains of UPEC subjected to three extra antibiotics from various groups (Figure, 2). Those were Streptomycin, Ciprofloxacin, and Ceftriaxone. Rate of resistance of UPEC to them were 7.3%, 15.4%, and 23%, respectively. Our data is in agreement with earlier reports, for example, May, (2011) revealed that 9.1% of the isolates were resistance to streptomycin, and Masinde et al., (2009) showed that 11.8%, and 29.4% of their isolates were resistance to ciprofloxacin, and ceftriaxone. Although, these findings demonstrate that we have multi-drug resistance (MDR) *E. coli*, it also explains the effectiveness of the mentioned antibiotics towards the target bacteria. However, we should not neglect their side effects, especially to pregnant women, such as the foetal arthopathy of ciprofloxacin (Masinde et al., 2009). Resistance rate of UPEC isolates of Akobi et al., (2014) contradict ours, who showed that 70.9%, and 64.9% of them were resistance to streptomycin, and ceftriaxone, respectively. *E. coli* isolates from surgical wound infections exhibited different antimicrobial susceptibilities. Their resistance against streptomycin, Ciprofloxacin, and ceftriaxone were (72.4%), (75.9%), and (82.8%), respectively (Muhammad et al., 2009). On the contrary, all the 57 *E. coli* strains from slaughtered commercial chickens were susceptible to streptomycin (Momtaz et al., 2012). Therefore, these discrepancies in the behaviour of

antimicrobial susceptibility of *E. coli* strains could only be interpreted on the basis of the proper origin of the strains.

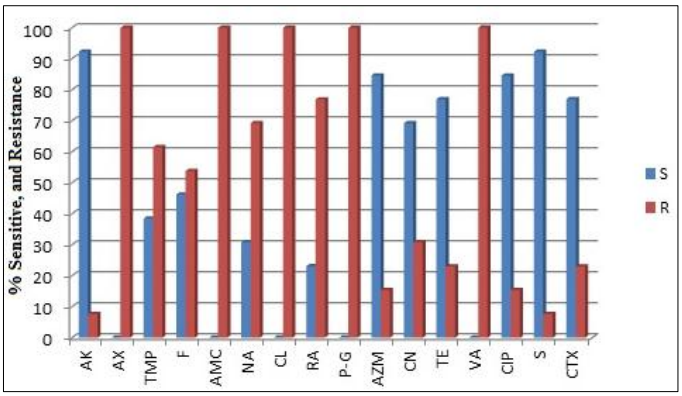

Figure 2: Susceptibility of *E. coli* isolates to antibiotics from various groups. S; sensitive, R; resistance

3.4 Molecular Detection of *aacC(2)* by PCR

One of the most important purposes of this study was to analyse antibacterial resistance of UPEC isolates phenotypically, and at molecular level, especially to investigate the occurrence of genes conferring resistance to aminoglycosides, specifically to gentamicin. Plate bioassay showed 4(30.8%) of UPEC isolates resistance to CN. This achievement tend to be in close conformity with AST profiles since 3(23.1%) of the described isolates were positive for the *aacC(2)* gene (Figure, 3). It also means that 75% of them harbouring the gene of concern. Earlier reports by Dias-Goncalves et al., (2015) exhibited that 80% of the gentamicin-resistant *E. coli* possessed the *aacC(2)*gene. Further report by Ho et al., (2010) monitor the presence of *aacC(2)*gene in isolates from human (84.1%) and animal (75.5%), respectively. The high dissemination of the studied gene in genetically diverse strains signifies to high extent the substantial role of horizontal gene transfer in the increase of the level of gentamicin-resistance. Worthwhile, the sole isolate, which account for (25%) of CN resistant strains that we were unable to demonstrate the *aacC(2)* gene in, might be due to one of the possibilities other than aminoglycoside enzymatic modifications; including reduced uptake and increased efflux pump, and conversion of the target RNA (Houghton et al., 2010). The incidence of a new aminoglycoside acetyltransferase gene suggested by Cabrera et al., (2009) is another prospect.

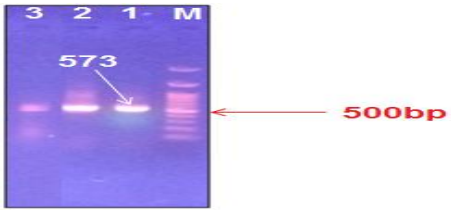

Figure 3: PCR identification of *aacC(2)* gene in UPEC strains resistant to gentamicin by disc diffusion assay.M: 100bp DNA marker. Lanes 1 to 3; PCR amplification of *aacC(2)* gene

4. Conclusion & Recommendations

It can be concluded from the current study that, incidence of ASB was 25.7%. *E. coli* were revealed as the most common organism among all the isolates of Enterobacteriaceae regardless of the cases

studied, whether SB or ASB. Majority of *E. coli* strains were sensitive to amikacin, streptomycin, ciprofloxacin, azithromycin, ceftriaxone and tetracycline. It is worth mentioning that a huge number of the strains were resistant to penicillin G, amoxicillin, amoxicillin-clavulanic acid, cephalexin, vancomycin. This indicates that multidrug resistance *E. coli* evolved at risky level. Molecular approach conducted to ascertain the presence of *aacC(2)* gene. The current situation is mostly contributed to both the non-controlled and non-licensed use of antibiotics. So it imposed the duty on the government to put optimum censoring on antibiotics administration, especially in developing countries and more specifically for pregnant women.

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