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Detection Of β-lactamases Enzymes Responsible For β-lactam Resistance In Some Gram Negative Bacteria By PCR

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Detection of β- lactamases enzymes responsible for β-lactam resistance in some gram negative bacteria by PCR

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ABSTRACT

β- lactamases enzymes are well studied in literature since they lead to β-lactam resistance among many pathogens, hence global concern of human and animal health issues is rising. The aim of study is to detect these kinds of enzymes among some gram negative bacteria isolated locally. Thirty isolate of each gram negatives *Citrobacter spp., Serratia spp.,* and *Enterobacter spp.* were obtained from different clinical samples in Baghdad and Al-Najaf cities through March 2020 until January 2021. Isolates were identified biochemically and by Vitek2 system. β- Lactamases encoding genes like (bla_{SHV} (231bp), bla_{IMP-1} (500), bla_{VIM} (382), bla_{Amp} (1150), bla_{CMY} (1014), bla_{oxa23} (501), and bla_{oxa51} (353bp) were detected by PCR. It was found that (80%, 75%, 30%, 50%, 38%, 55%, and 69%) of *Citrobacter spp* isolates had β- Lactamases encoding genes (bla_{SHV}, bla_{IMP-1}, bla_{VIM}, bla_{Amp}, bla_{CMY}, bla_{oxa23}, and bla_{oxa51}), respectively. *Serratia spp.* showed having (bla_{SHV}, bla_{IMP-1}, bla_{VIM}, bla_{Amp}, bla_{CMY}, bla_{oxa23}, and *bla oxa51*) among (20%, 85%, 60%, 28%, 40%, 67%, and 58%), respectively. There were also (60%, 50%, 25%, 33%, 60%, 81%, and 73%) of *Enterobacter spp.* isolates harbored (bla_{SHV}, bla_{IMP-1}, bla_{VIM}, bla_{Amp}, bla_{CMY}, bla_{oxa23}, and bla_{oxa51}), respectively. Our findings revealed that most of gram negative bacteria isolated locally had different β- Lactamases encoding genes, leading to increase health problems among patients infected with those pathogens.

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1. Introduction

It is fact that β-lactam antibiotics are always prescribed in many countries since they have wide spectrum and few side effects [33], and also lack of producing new drugs since past fifteen years [6]. Bacterial cell wall; specifically D-alanyl-D-alanine carboxypeptidase transpeptidase is the target of these antibiotics; therefore, any changes are made by bacteria in this site would turn them inactive even with β-lactam inhibitors combinations [33 and 20]. B-lactamase enzymes have been classified according to two criteria;

Bush/Jacoby/Medeiros and Ambler system (A, B, C, and D) [20and 7]. The Ambler class A is penicillinases, B is metalloenzymes MBL which can catalyze and hydrolyze carbapenems, C is AmpC enzymes or called cephalosporinases, and D is oxacillinases [23 and 19]. Also, it could be classified as described in literature according to the active-site serine β-lactamases like classes A, C, and D and zinc-dependent metallo-β-lactamases class B [32 and 30]. Most of these enzymes are found in gram-negative bacteria like *Escherichia coli, Salmonella spp, Citrobacter freundii, Enterobacter spp., Morganella, Proteus, Serratia* spp, *Pseudomonas aeruginosa*, and others of *Enterobacteraceae* family [7, 23, and 19]. Many threats have been arising from gram-negative bacterial pathogens having b-lactamase mediated by plasmids [21], especially MBL enzymes [26]. Furthermore, extended spectrum b-lactamases (ESBL) producing gram negative pathogens possess a challenge of infection treatments [25], especially high mortality rate causing by nosocomial infections [25, 14 , and 15]. The ESBL enzymes can also inhibit aminoglycosides and sulphonamides [8 and13] besides degrading penicillins, cephalosporins,

carbapenems, and monobactams [27, 10, and 9]; therefore, the aim of study is to detect these kinds of enzymes among some Iraqi gram negative bacteria clinical isolates.

2.Material and Methods: 30 isolate of each Gram negatives *Citrobacter spp., Serratia spp., and Enterobacter spp.* were obtained from different clinical samples in Baghdad and Al-Najaf cities through March 2020 until January 2021. Isolates were identified biochemically and by Vitek2 system. β- Lactamases encoding genes (table 1) like (bla_{SHV} (231bp), bla_{IMP-1} (500), bla_{VIM} (382), bla_{Amp} (1150), bla_{CMY} (1014), bla_{oxa23} (501), bla_{oxa51} (353bp) were detected genetically by only PCR in this study.

Table 1: β- Lactamases encoding genes [1 and 2]

Primer	Sequences (5'…….. 3') F.	Sequences (5'…….. 3') R.	size (bp)	Tm
bla_{SHV}	AAGATCCACTATCGCCAGCAG	ATTCAGTTCCGTTTCCCAGCGG	231	59
bla_{IMP-1}	CTACGCCAGCAGAGTCTTTG	AACCAGTTTTGCCTTACCAT	500	55
bla_{VIM}	GTTTGGTCGCATATCGCAAC	AATGCGCAGCACCAGGATAG	382	57
bla_{Amp}	ATGCAACAACGACAATCCATC	GTTGGGGTAGTTGCGATTGG	1150	58
bla_{CMY}	GACAGCCTCTTTCTCCACA	TGGAACGAAGGCTACGTA	1014	50
bla OXA23	GATCGGATTGGAGAACCAGA	ATTTCTGACCGCATTTCCAT	501	53
bla OXA 51	TAATGCTTTGATCGGCCTTG	TGGATTGCACTTCATCTTGG	353	53

3.Results

3.1. β- Lactamases encoding genes among *Citrobacter spp*

It was found that 80% of isolates had bla_{SHV} gene (figure 1), 75% of isolates had bla_{IMP-1} gene (figure 2), 30% of them had bla_{VIM} (figure 3), 50% had bla_{Amp} gene (figure 4), and other b-lactamse genes tested like (bla_{CMY}, *bla* $_{oxa23}$, *bla* $_{oxa51}$) were found among (38%, 55%, and 69%) of *Citrobacter spp* isolates (figure 5, 6, and 7), respectively

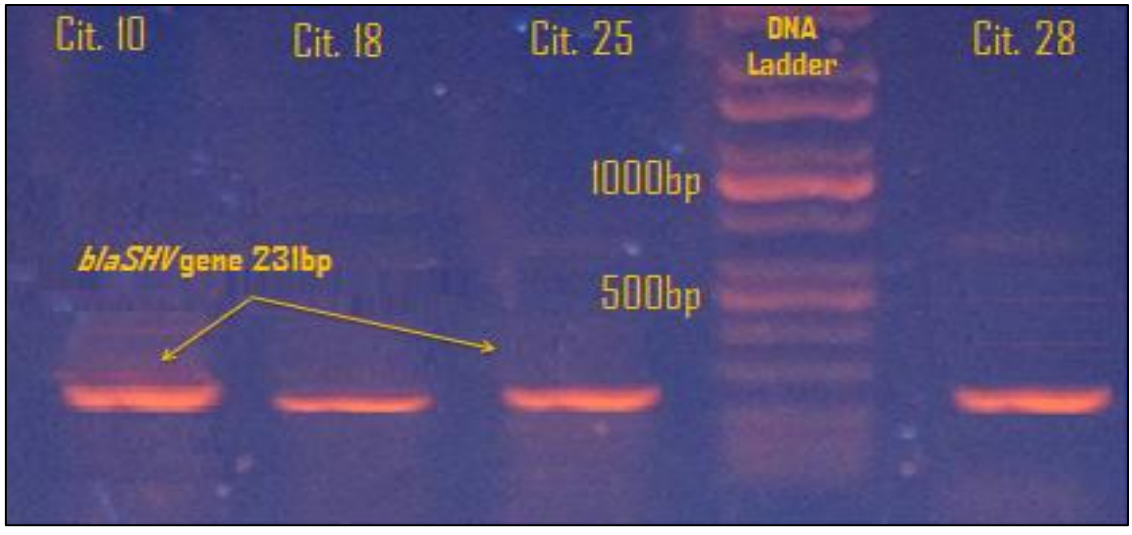

Figure 1: bla_{SHV} gene amplified at 231bp among 80% of *Citrobacter spp* isolates

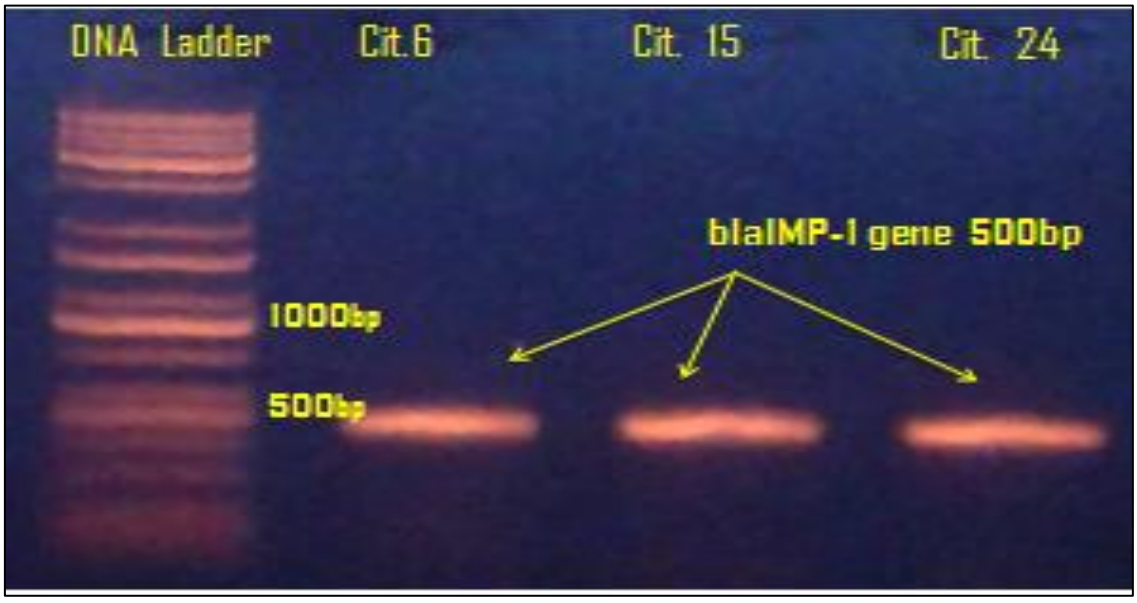

Figure 2: *bla*$_{IMP-1}$ gene amplified at 500bp among 75% of *Citrobacter spp* isolates

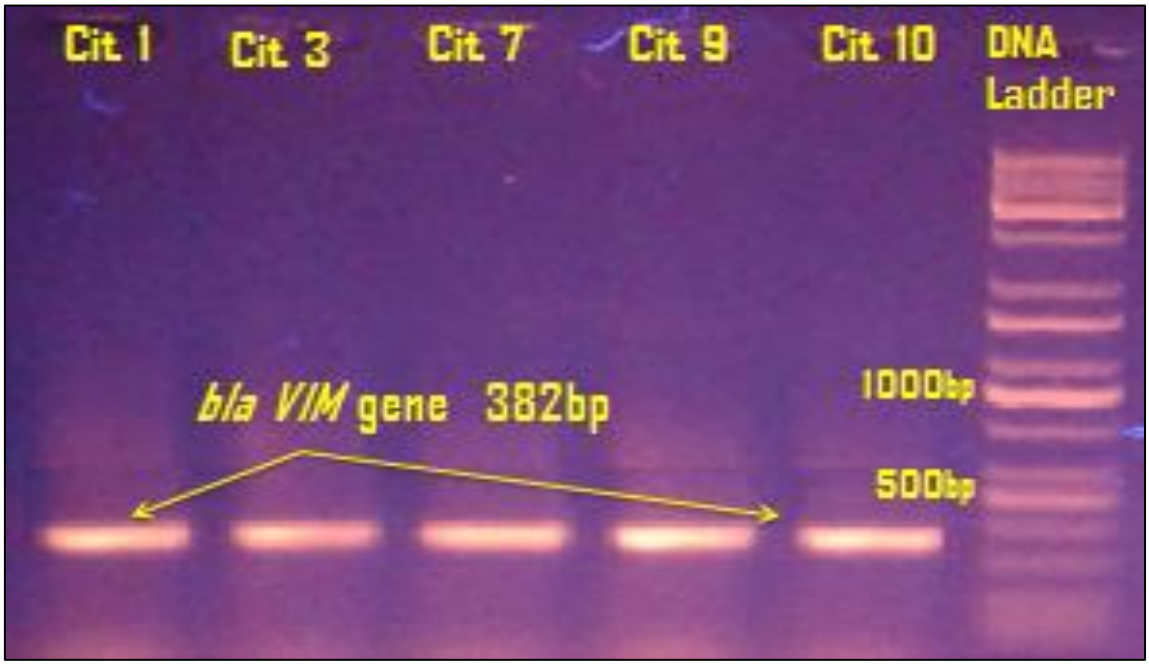

Figure 3: *bla*$_{VIM}$ gene amplified at 382bp among 30% of *Citrobacter spp* isolates

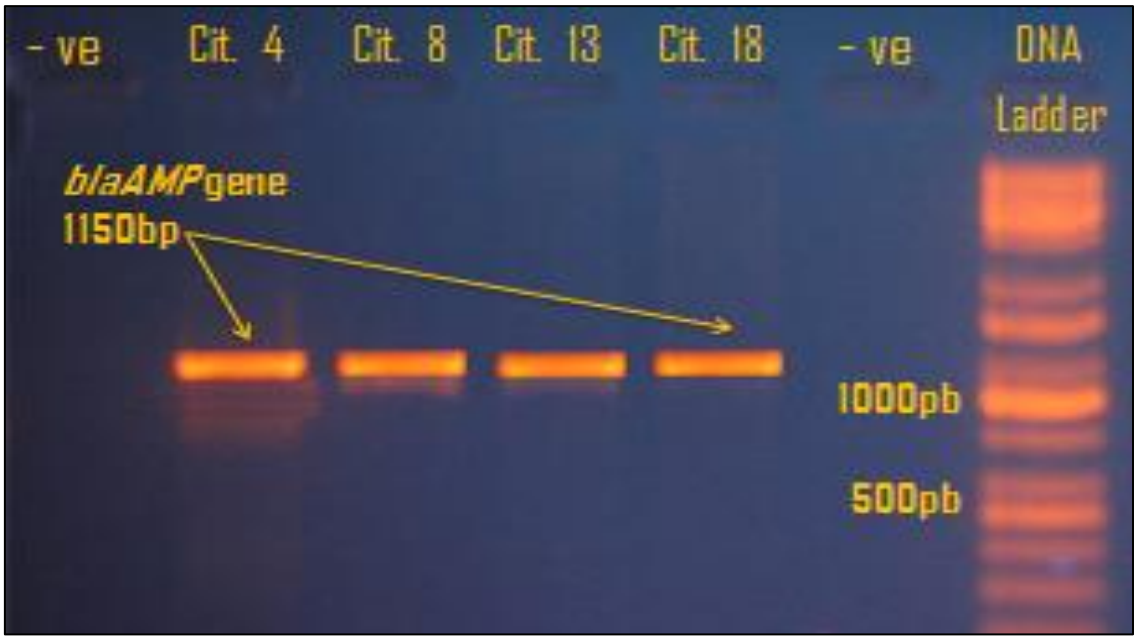

Figure 4: bla_{AMP} gene amplified at 1150bp among 50% of *Citrobacter spp* isolates

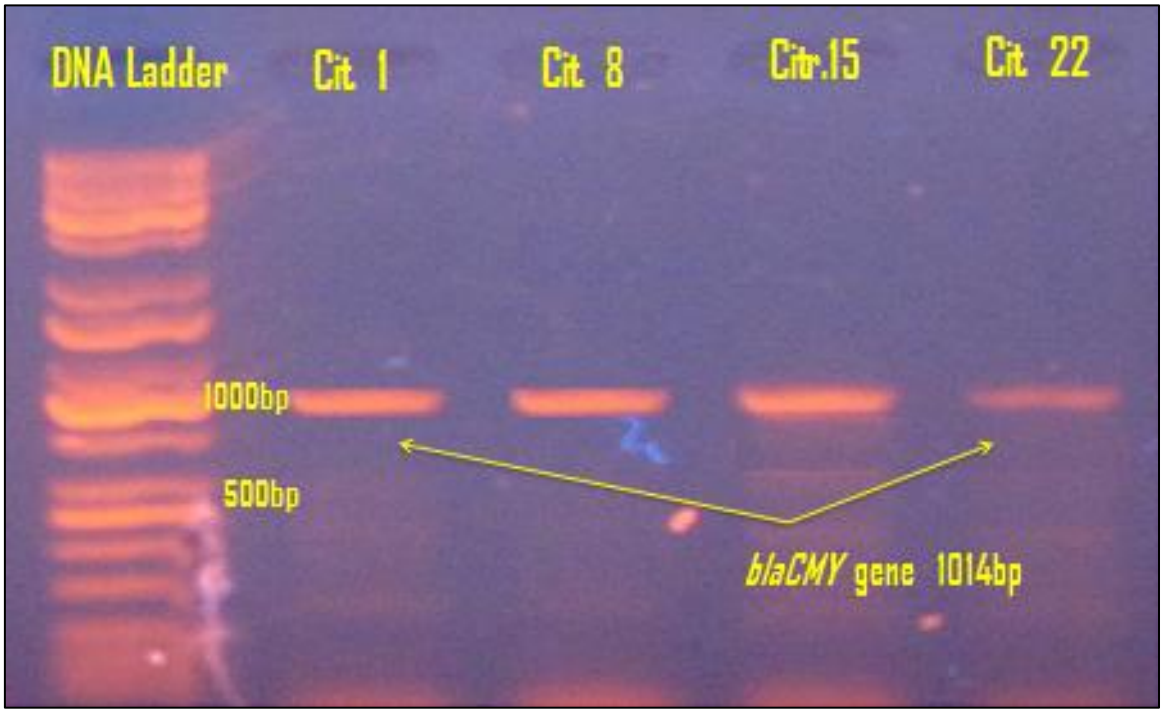

Figure 5: bla_{CMY} gene amplified at 1014bp among 38% of *Citrobacter spp* isolates

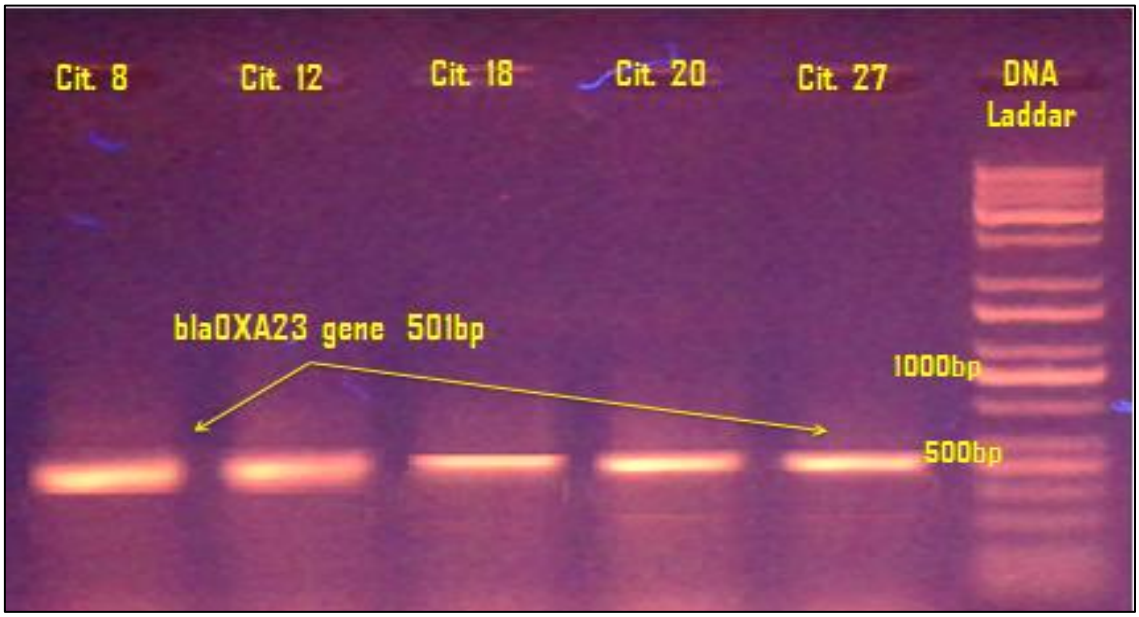

Figure 6: *bla*$_{oxa23}$ gene amplified at 501bp among 55% of *Citrobacter spp* isolates

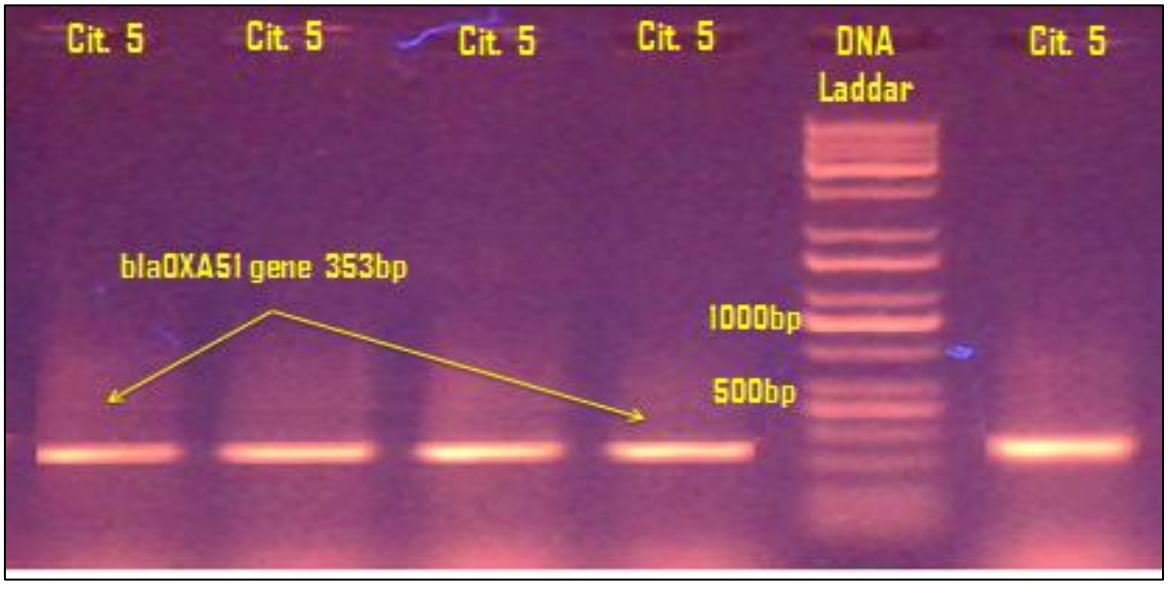

Figure 7: *bla*$_{oxa51}$ gene amplified at 353bp among 69% of *Citrobacter spp* isolates

3.2. β- Lactamases encoding genes among *Serratia spp.*

Interestingly, (20%, 85%, 60%, 28%, 40%, 67%, and 58%) of ***Serratia spp.*** isolates had *bla*$_{SHV}$ (figure 8), *bla*$_{IMP-1}$ (figure 9), *bla*$_{VIM}$ (figure 10), *bla*$_{Amp}$ (figure 11), *bla*$_{CMY}$ (figure 12), *bla* $_{oxa23}$ (figure 13), *bla* $_{oxa51}$ (figure 14), respectively.

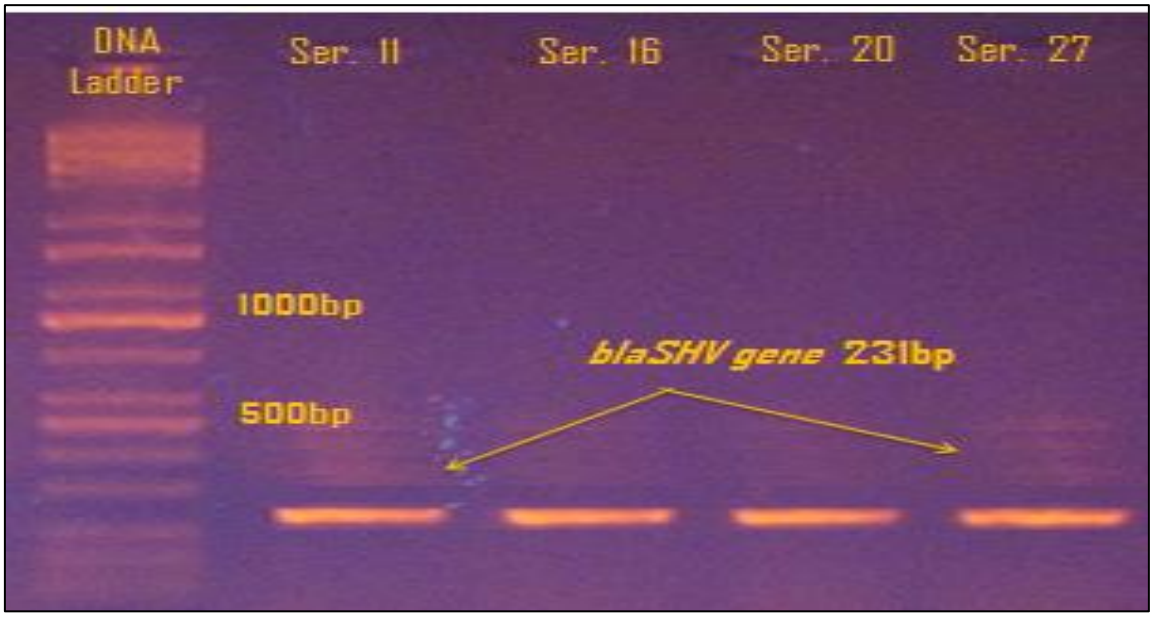

Figure 8: *bla*$_{SHV}$ gene amplified at 231bp among 20% of *Serratia spp.* Isolates

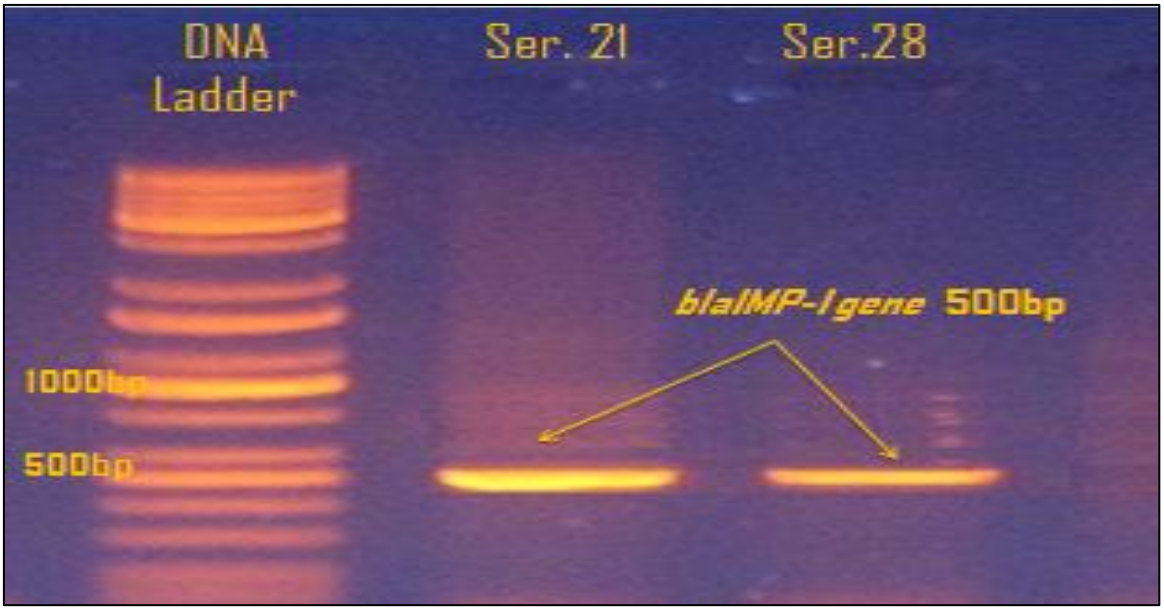

Figure 9: *bla*$_{IMP-I}$ gene amplified at 500bp among 85% of *Serratia spp* isolates

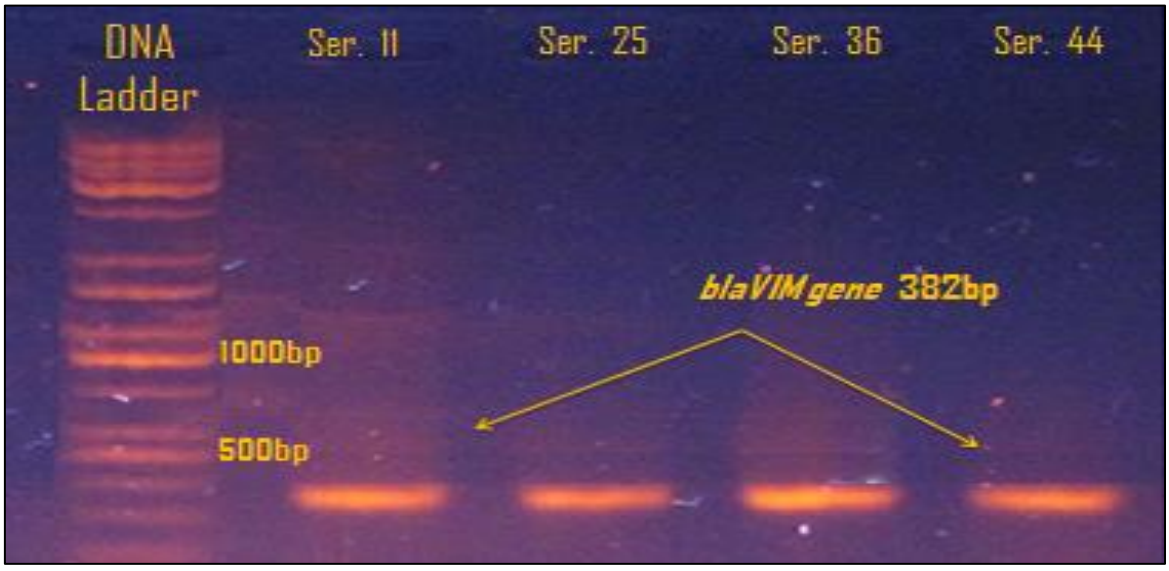

Figure 10: *bla*$_{VIM}$ gene amplified at 382bp among 60% of *Serratia spp* isolates

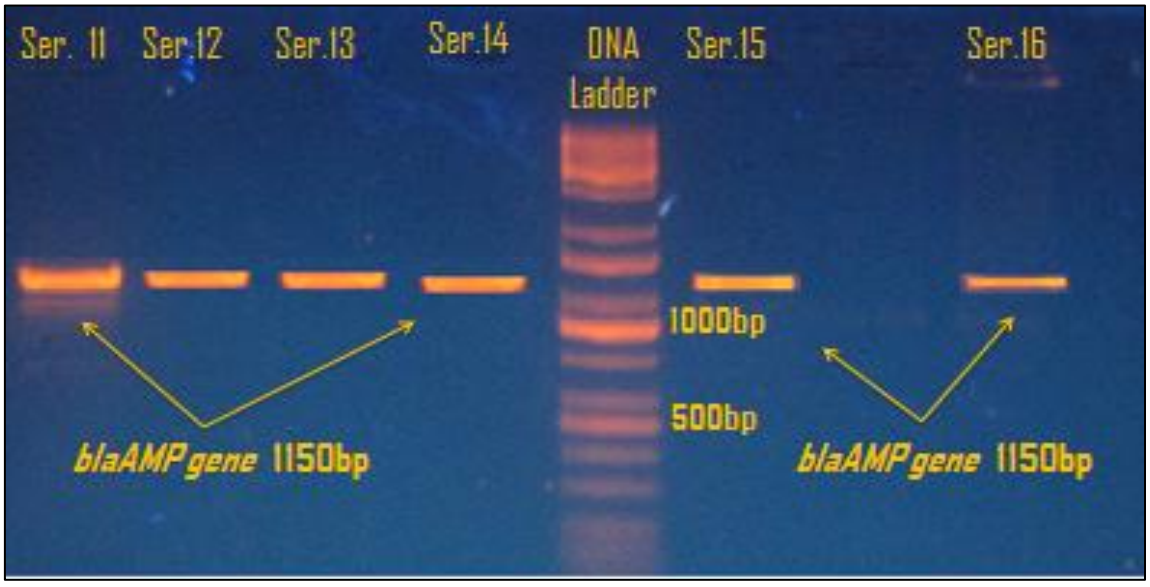

Figure 11: *bla*$_{AMP}$ gene amplified at 1150bp among 28% of *Serratia spp* isolates

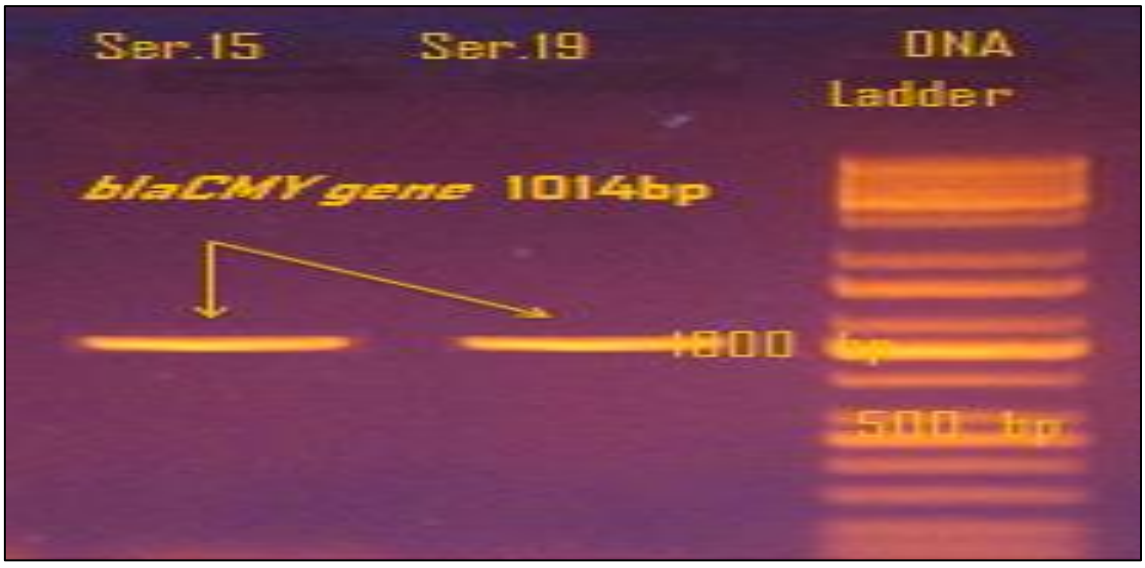

Figure 12: bla_{CMY} gene amplified at 1014bp among 40% of *Serratia spp* isolates

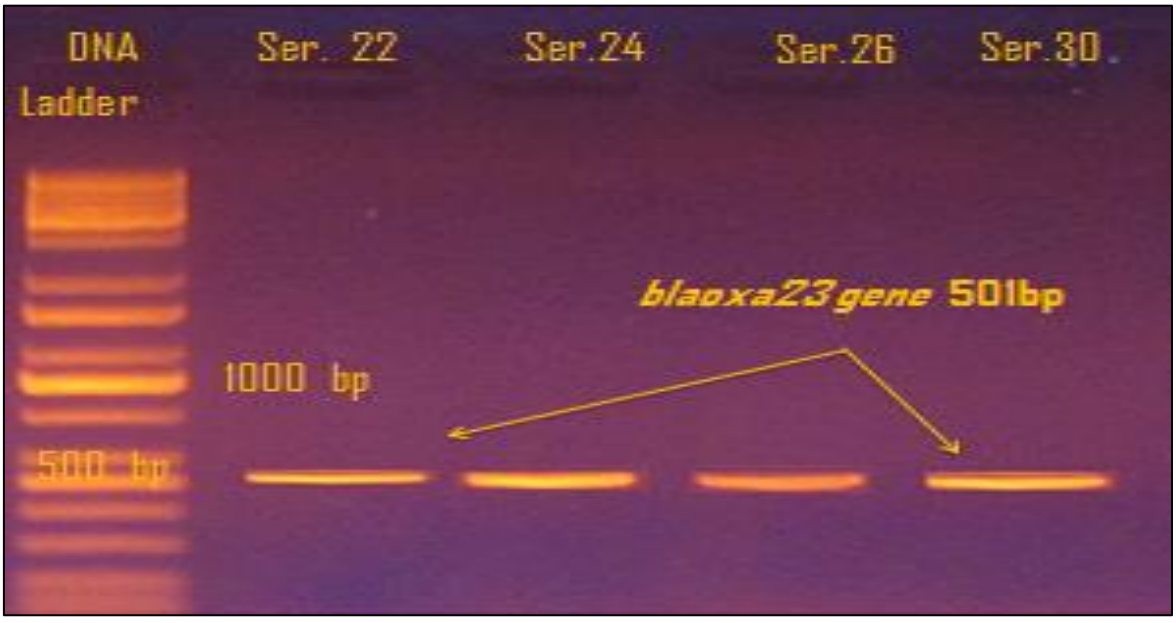

Figure 13: bla_{oxa23} gene amplified at 501bp among 67% of *Serratia spp* isolates

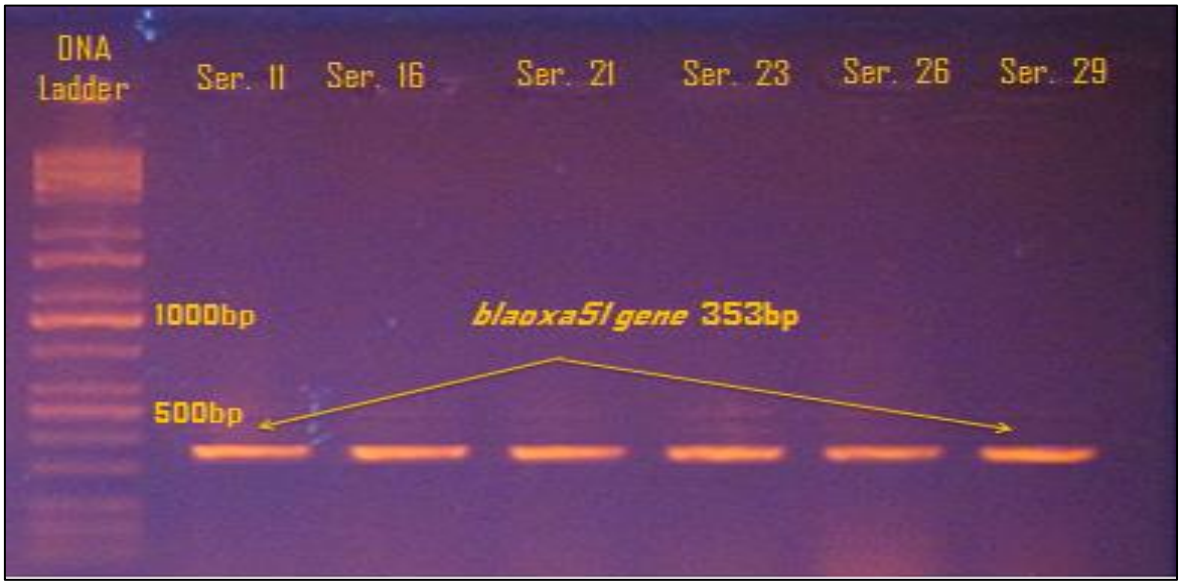

Figure 14: *bla*$_{oxa51}$ gene amplified at 353bp among 58% of *Serratia spp* isolates

1.3. β- Lactamases encoding genes among *Enterobacter spp.*

There were also (60%, 50%, 25%, 33%, 60%, 81%, and 73%) of *Enterobacter spp.* harbor *bla*$_{SHV}$ (figure 15), *bla*$_{IMP-1}$ (figure 16), *bla*$_{VIM}$ (figure 17), *bla*$_{Amp}$ (figure 18), *bla*$_{CMY}$ (figure 19), *bla* $_{oxa23}$ (figure 20), *bla* $_{oxa51}$ (figure 21), respectively.

Figure 15: *bla*$_{SHV}$ gene amplified at 231bp among 66% of *Enterobacter spp* isolates

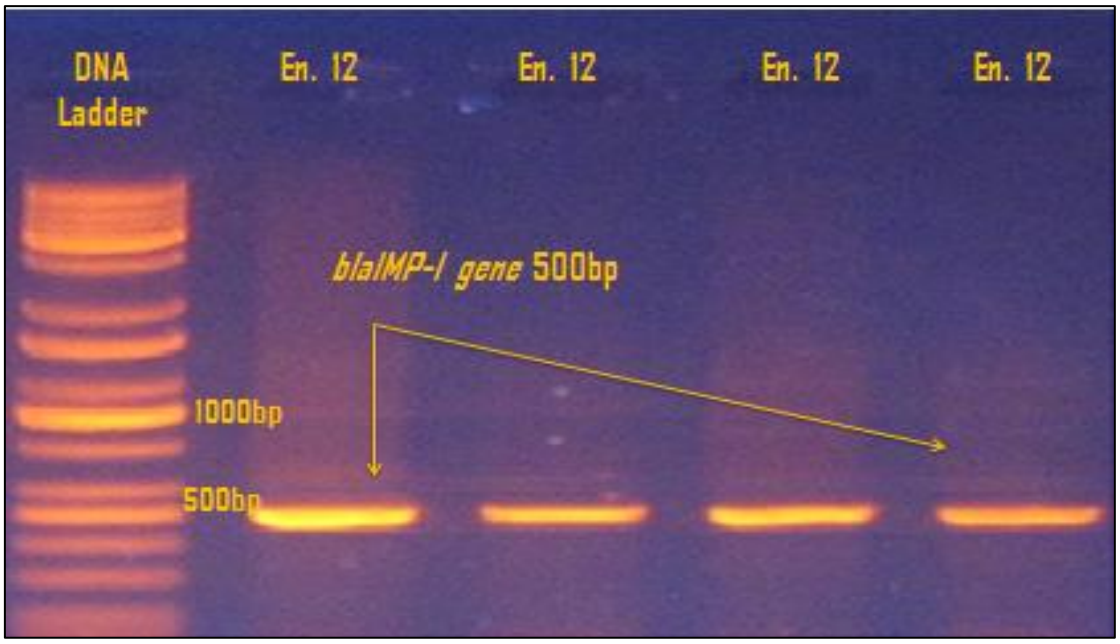

Figure 16: *bla*$_{IMP-I}$ gene amplified at 500bp among 50% of *Enterobacter spp* isolates

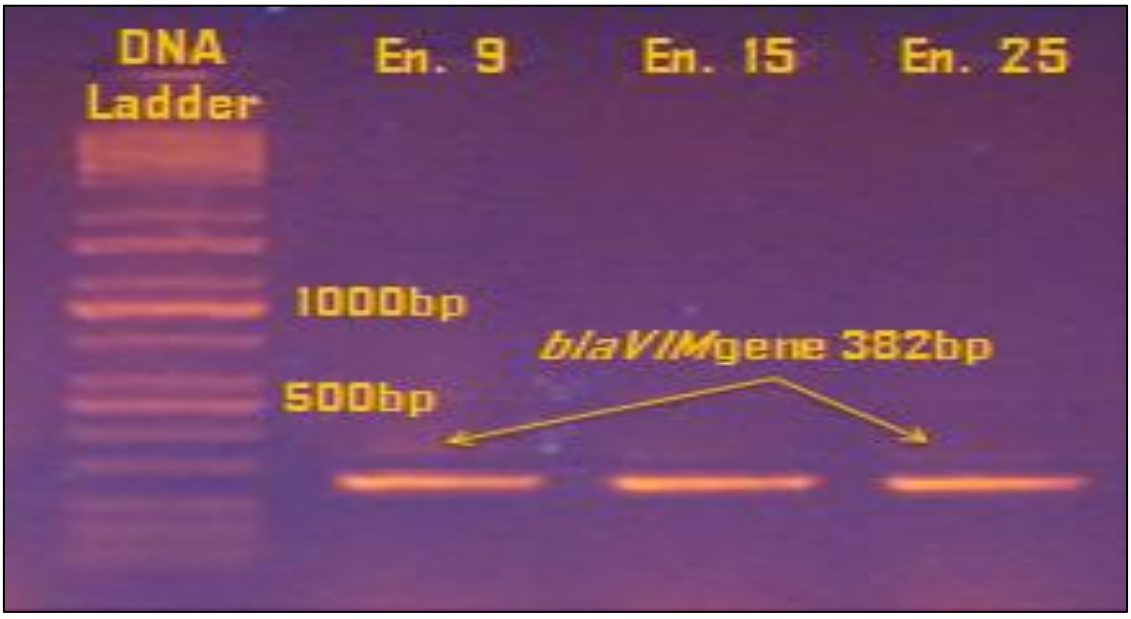

Figure 17: *bla*$_{VIM}$ gene amplified at 382bp among 25% of *Enterobacter spp* isolate

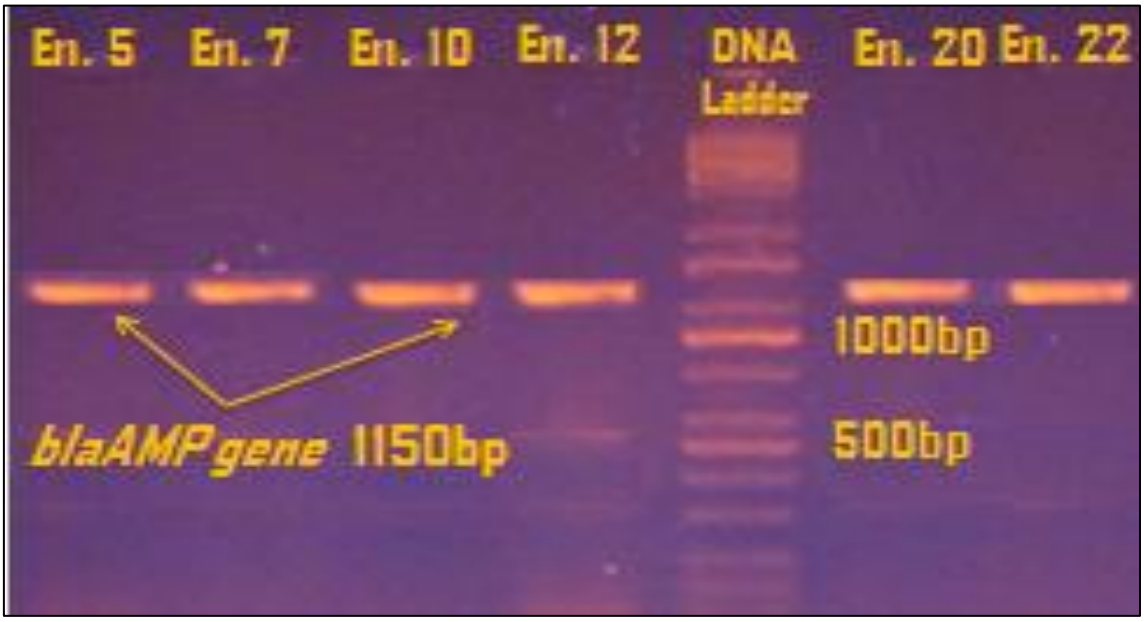

Figure 18: *bla*$_{AMP}$ gene amplified at 1150bp among 33% of *Enterobacter spp* isolates

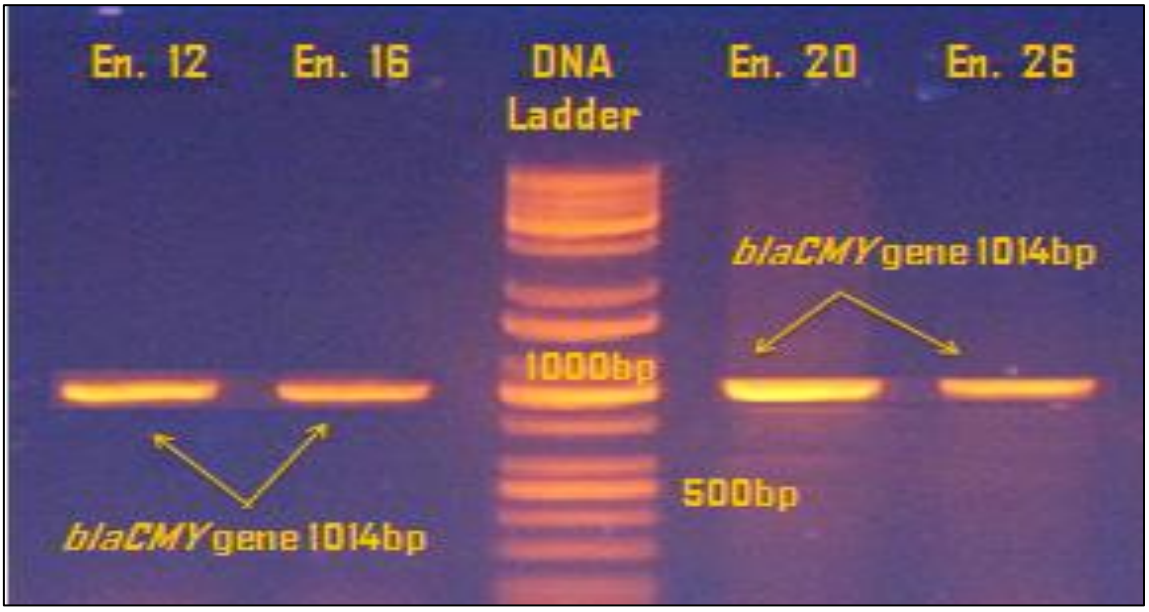

Figure 19: *bla*$_{CMY}$ gene amplified at 1014bp among 60% of *Enterobacter spp* isolates

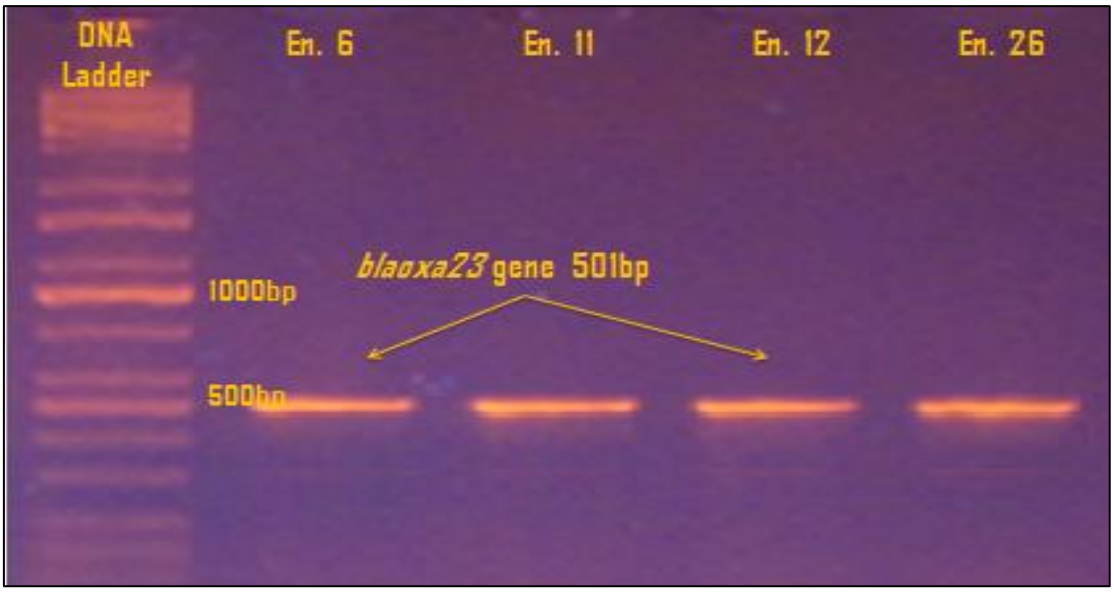

Figure 20: *bla*$_{oxa23}$ gene amplified at 501bp among 80% of *Enterobacter spp* isolates

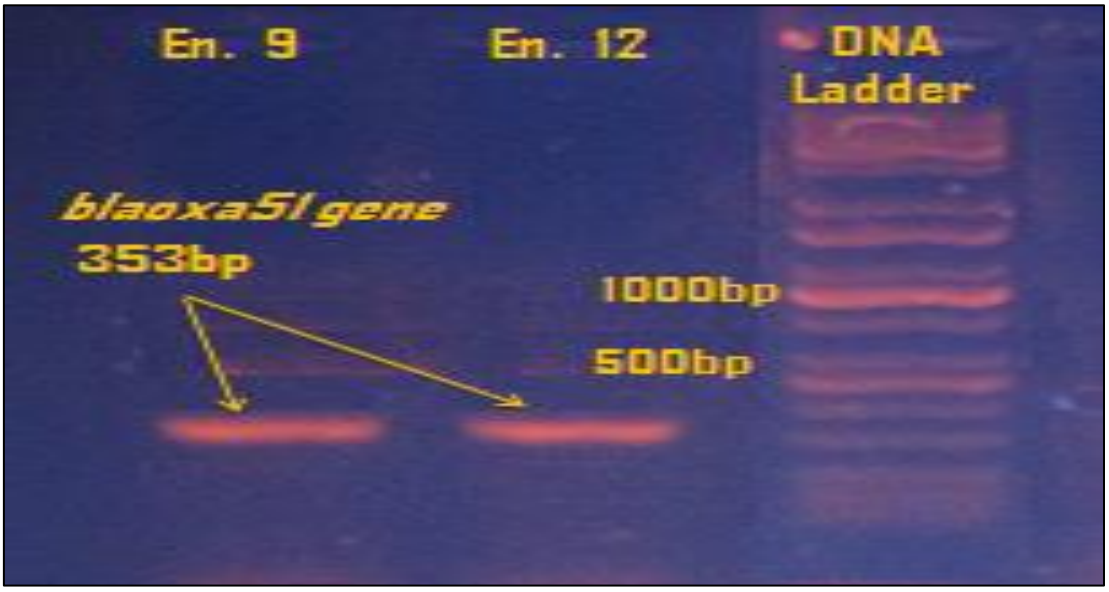

Figure 21: *bla*$_{oxa51}$ gene amplified at 353bp among 73% of *Enterobacter spp* isolates

4. Discussion

It is reported that Ambler class could be disseminated among gram negatives in Asia, Europe, North America, and South America, especially IMP-, VIM-, SPM- and GIM-types confirm high resistancy levels among bacteria towards most b-lactams but azetronam [26]. Our findings revealed that different classes of beta-lactamase genes such as (*bla*$_{SHV}$, *bla*$_{IMP-1}$, *bla*$_{VIM}$, *bla*$_{Amp}$, *bla*$_{CMY}$, *bla* $_{oxa23}$, and *bla* $_{oxa51}$) tested in this study were found among some local gram negatives pathogens like *Citrobacter spp.*, *Serratia spp.*, and *Enterobacter spp.* similarity with other studies conducted by [23, 19, 26, and 29]. It is found that

Citrobacter youngae isolated from different hospitals in China had IMP-type MBL enzyme like blaIMP-4 [26]. It was also demonstrated that *Serratia marcescens* isolated from clinical samples had bla_{IMP} and bla_{VIM} genes [26]. Further, it was revealed that gram negatives bacteria isolated clinically could resist many b-lactams agents due to having AmpC enzymes [19]. Singhal *et al.* [29] demonstrated that the presence of ESBL and AmpC b-Lactamase among 173 (64%) gram negatives pathogens could give the rise of high level of antibiotic resistance among useful marketing drugs [29]. Some studies showed that *Serratia marcescens* isolated from different locations throughout USA could resist different b-lactams like cephalosporins, imipenem and aztreonam due to having bla_{SME} gene encoding resistance enzymes [19 and 4]. Moreover, 93 gram negative pathogens like *Escherichia coli*, *Enterobacter spp*, *Pseudomonas spp*, *Proteus spp*, and *Citrobacter freundii* isolated from hospitals had ESBLs enzymes such as (SHV-12, SHV-28 and CTX-M15) [25]. Haidar *et al.* [13] also, reported that there were 19% of gram negative bacteria tested had bla_{SHV}, 38% had bla_{OXA}, and 80% had bla_{OXA} genes. He and his colleagues further conclude that the presence of these kinds of b-lactamase genes encoded by plasmids or chromosome conferred multi-drug resistance level [13]. It is found that the presence of bla_{OXA} enzymes, especially bla_{OXA23} among pathogens contributes with serious threat to human health [11 and 22]. It is found that VIM and IMP related to MBL enzymes are wide spread among gram negatives like *P. aeruginosa*, and *A. bumannii* pathogens [28]. It is fact grounded on studies that $bla_{OXA\ 23}$ gene presence guarantee carbapenem resistance in pathogens [3]; therefore, gram negatives pathogens having such a gene were difficult to be eradicated [16, 17, and 33]. It has also found that bla_{SHV} gene presence did not affect oxyimino cephalosporine and monobactams but inactivate penicillins and cephalosporines [12 and 31]. Studies confirmed that presence of MBLs genes on integrons give the ability to disseminate among bacteria, leading to give rise to antibiotic resistance levels [31]. Besides, it was reported that gram negative bacteria isolated from septicemic pediatric patients could resist imipenem, ciprofloxacin, and cefoxin since having acquired MBL genes [5]. Besides, ceftriaxone, cefotaxime, ceftazidime, aztreonam, and some oxymino-β- lactam antibiotics could be inactivate by altering the active site of them by MBL enzymes producing bacteria such as *Enterobacteraceae* family [24].

2. Conclusion

Wide range of antibiotic resistance levels has been appeared among local pathogens from which gram negative bacteria [1 and 2]. Our findings confirm the widespread of b-lactamases enzymes among some local gram negative pathogens. Therefore, more phenotypic and genotypic studies should be done to screen different classes of b-lactamase and trying to find an appropriate antibiotic regime for treating infected patients with such producing pathogens.

Conflict of Interest: The authors declare that they have no conflict of interest.

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