Phenotypic and molecular detection of metallo-β-lactamase genes of Salmonella enterica strains isolated from poultry meat

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ABSTRACT

The present study was carried out to detect Salmonella enterica in meat samples of commercial boilers (CB) and the spent hens (SH) in Ardabil, Iran. Metallo-β-lactamase (MBL) enzyme produced by Salmonella enterica strains isolated from poultry meat samples were detected by both biochemical and molecular methods. The study included 20 positive samples for Salmonella enterica from boilers (CB) and spent hens (SH). The prevalence of Salmonella enterica for CB was 22% (11/50) and for SH was 18% (9/50). The antibiotic susceptibility testing for both CB and SH showed maximum number of Salmonella enterica isolates were resistant against Augmentin (30 μg), Cotrimoxazole (25 μg) and Tetracycline (25 μg) and susceptible against Ofloxacin (5 μg) and Gentamicin (10 μg). Screening phenotypic confirmatory test for Metallo-β-lactamase (MBL) for CB, (n= 11, 100%) were positive for MBL while for SH (n= 8, 88.88%) samples were positive for MBL. The results showed that MBL positive Salmonella enterica isolates from CB and SH meat samples contained gene blaVIM (n=11, 57.89%), blaIMP (n=6, 31.57%) and blaSPM (n=2, 10.52%). Since Salmonella infections in poultry are high due to large demand and antibiotic resistance to strains, so the purpose of the current study is to focus on the detection of MBL enzyme produced by Salmonella enterica isolates.

Introduction

The advancement in the occurrence and spread of drug resistance in microbial community has impacted negatively to discoveries of medicine. Although antibiotics have saved innumerable lives from mortality and morbidity, however microbial resistance is posing a threat to these discoveries. The rise of Gram-negative carbapenem-resistant bacteria in hospital and communities is an alarming progress in the field of disease control (Bush and Jacoby, 2010; Chika et al, 2014; Walsh, et al, 2005). This threat foretells the jeopardy to public health with potent antibiotics that are used against serious infections.

Metallo-β-lactamases (MBLs) are broad spectrum zinc enzymes that are able to inactivate most clinically useful beta-lactam antibiotics specifically Carbapenems which are considered as the most effective antibiotics against Salmonella enterica (Crowder et al, 2006; Bahar et al, 2010). Carbapenem resistance occurs because of decrease in antibiotics absorption due to lack of an outer membrane porin, as oprD, exclusion from the cell by efflux pump, decrease in outer membrane permeability and production of MBL (Boroumand et al, 2012).

MBLs also divided into several families as follows: IMP, VIM, SPM, GIM, SIM, DIM, AIM, KHM, NDM and KPC. Most of them, if not all, genes encoding IMP, VIM and SPM types as well
as GIM are found as gene cassettes in class 1 integrons, although IMP MBL genes are also found on class 3 integrons (Liakopoulos et al., 2013; Rizek et al., 2014). The VIM, IMP and SPM types are the most clinically significant carbapenemases which encoded by \textit{bla}_{\text{IMP}}, \textit{bla}_{\text{VIM}} and \textit{bla}_{\text{SPM}} genes (Liakopoulos et al., 2013; Moosavian and Rahimzadeh, 2015).

Microbes become resistant to antimicrobial drugs after selective pressure of antibiotics and mutation. Intensive efforts for the control of infection and epidemiological issues are required for multidrug resistance in microbes and their potential spread to the environment. Antibiotics application to the production of livestock and poultry has led to the emergence of drug resistant bacteria in veterinary community (Ejikeugwe et al., 2017).

Multidrug-resistant (MDR) Salmonella strains are characterized by high levels of antibiotic resistance that are often attributed to the acquisition of specific mechanisms (Brunell et al., 2017). Salmonella is one the leading pathogens related to food poisoning and causes major bacterial diseases in poultry resulting in significant losses (Rostango et al., 2006). The integron gene is one of the main multidrug resistance genes in Salmonella (Hu and Li, 2009). Salmonella has been reported to become increasingly resistant to antimicrobials involving \(\beta\)-lactamases and other associated mechanisms (Gonzalez-Sanz et al., 2009; Revathi et al., 1998).

The presence of metallo-\(\beta\)-lactamases was studied in other strains of bacteria i.e. \textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumonia}, which cause resistance to antibiotics (Yong et al., 2009; Ghamgosha et al., 2015). This study is aimed at screening the presence of metallo-\(\beta\)-lactamase genes in Salmonella enterica in poultry meat samples, as well as assessing them on molecular and phenotypic basis.

**Material and Methods**

**Samples collection and processing**

A total of 100 meat samples positive for \textit{Salmonella enterica} (50 samples of CB and 50 samples of SH) were collected from Ardabil, Iran. Samples were transported to the Microbiology Lab for microbiological analysis. Part of each collected meat sample (0.5 g) was placed into test tube containing 6 ml buffered Peptone and the tubes were covered with cotton wool and incubated at 37°C for 24 hrs.

After incubation, 50 \(\mu\)l of overnight grown culture was used to spread on already prepared and solidified Brilliant green agar (BG) (LabM, UK) and XLD. Plates were incubated at 37°C for 24 hours after labeling properly with sample number. If there are single colonies which caused the color of the medium to change from yellow to red/pink were sub-cultivated onto nutrient agar and incubated overnight for 24 hours at 37°C. Then it was passed in differential culture media to perform biochemical tests.

**Antibiotics sensitivity for Salmonella isolates**

Disk diffusion antibiotic sensitivity test was used in determining sensitivity of the \textit{Salmonella enterica} isolates obtained from different meat samples to various antibiotics at different concentrations. Augmentin (30 \(\mu\)g), Ofloxacin (5 \(\mu\)g), Gentamicin (10 \(\mu\)g), Nalidixic acid (30 \(\mu\)g), Nitrofurantoin (200 \(\mu\)g), Cotrimaxazole (25 \(\mu\)g) and Tetracycline (25 \(\mu\)g) were used to determine sensitivity pattern.

**Serotyping for potential Salmonella strains**

Serotyping was performed on Muller-Hinton agar by obtaining pure cultures of \textit{Salmonella} (RS, 2003). Agglutination was tested on a clean glass slide using Polyvalent O and H antisera. According to the Kauffmann-White scheme, slide cohesion tests were used to examine and study the serotypes in \textit{Salmonella} isolates from the poultry meat (Brenner et al., 2000; Schrader et al., 2008).

**Screening for the presence of Metallo-\(\beta\)-lactamases (MBL)**

The susceptibility of the test isolates to the carbapenems was evaluated by the Kirby-Bauer disk diffusion method as per the CLSI criteria (Gautam et al., 2013; Clinical Laboratory Standard Institute, 2011). MBL-producing isolates was suspected when the test organism(s) was resistant to any of the carbapenems (imipenem and meropenem). As per the CLSI criteria, isolates showing inhibition zone diameter (IZD) of \(\leq 23\)mm were suspected to produce MBL enzymes and these isolates were subjected to phenotypic confirmation test (Chika et al., 2014; Clinical
Laboratory Standard Institute, 2011). For *Salmonella enterica* isolates obtained from commercial boilers (CB) and the spent hens (SH), 20 samples each were suspected to produce MBL enzymes and subsequently subjected to phenotypic detection of MBL as described below.

**Phenotypic detection of MBL**

MBL production was phenotypically evaluated by the inhibition-based assay using EDTA (Chika et al, 2014). The test bacteria isolates (adjusted to 0.5 MacFarland turbidity standards) were aseptically swabbed on MH agar plates, and imipenem (10μg) and meropenem (10μg) disks impregnated with EDTA (0.5μg) was aseptically placed on the MH agar plates. Supplementary imipenem (10μg) and meropenem (10μg) disks without EDTA were also placed alongside antibiotic disks impregnated with EDTA. Antibiotic disks were placed 20mm apart, and all the plates were incubated at 30 °C for 24hors. A difference of ≥7mm between the zones of inhibition of any of the carbapenem disks tested alone compared to disks with EDTA infers MBL production phenotypically (Chika et al, 2014; Chika et al, 2016).

**DNA extraction**

The DNA extraction was carried out using commercial DNA kit, Fermentas genomic DNA purification kit (Fermentas, USA). The manufacturer’s instructions were carefully followed at each step. First of all, lysis buffer 400 μl was added to bacterial broth cultures (200 μl). Then 600 μl of chloroform was added and the suspension was inverted for about 3 to 5 times. Centrifuge was performed afterwards for 2 minutes at 12,800 g. The supernatant obtained as a result of centrifugation was removed. NaCl solution (100 μl) was added and afterwards 70% cold ethanol was added in the quantity 300 μl. The mixture was placed for 10 minutes at -20 °C. Centrifugation was performed again at 12,800 g for three to four minutes. The DNA pellet was obtain that was air-dried and then dissolved in 100 μl TE buffer and saved for futher use in the refrigerator at 4 °C.

**Molecular detection of *bla*<sup>VIM</sup>, *bla*<sup>IMP</sup> and *bla*<sup>SPM-1</sup> genes**

PCR amplification for the detection of MBL genes (*bla*<sup>VIM</sup>, *bla*<sup>IMP</sup> and *bla*<sup>SPM-1</sup>) was performed using specific primers (Table 1) from Sinaclone, Iran. PCR was performed according to the method reported by Senda et al. (1996) (Senda et al, 1996). PCR mixture (25 μL) consisted of 1 μL DNA, 0.24 Mm dNTPs, forward and reverse primers 5pm and Taq polymerase (1 unit) (Qiao et al, 2017).

**Table 1- Nucleotide sequences of primers used for detection of metalo-beta lactamase genes.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers Sequence (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla</em>&lt;sup&gt;VIM&lt;/sup&gt;</td>
<td><strong>Forward</strong> 5′- AGTGGTGAGTATCCGACAG-3′&lt;br&gt;<strong>Reverse</strong> 5′- ATGAAAGTGGTGGAGAC-3′</td>
</tr>
<tr>
<td><em>bla</em>&lt;sup&gt;IMP&lt;/sup&gt;</td>
<td><strong>Forward</strong> 5′- CTACCAGCGAGAGTCTTTG-3′&lt;br&gt;<strong>Reverse</strong> 5′- AACCAGTTTTGCCTTACCAT-3′</td>
</tr>
<tr>
<td><em>bla</em>&lt;sup&gt;SPM-1&lt;/sup&gt;</td>
<td><strong>Forward</strong> 5′- GCCGTTTGTGGTTGTC-3′&lt;br&gt;<strong>Reverse</strong> 5′- TTGGGAGTGTGGACTAC-3′</td>
</tr>
</tbody>
</table>

**Results**

The prevalence of *Salmonella enterica* in CB samples was 22% (11/50) and for SH samples was 18% (9/50). In the current study, 100 meat samples (50 samples from CB and 50 samples from SH) were collected from Ardabil, which were positive for *Salmonella enterica*. Sensitivity of the *Salmonella enterica* isolates obtained from different meat samples to various antibiotics at different concentrations was determined by disk diffusion antibiotic sensitivity test shown in Fig.1.

**Fig. 1- Antibacterial activity assay against Salmonella strains by the disk diffusion method.**

In case of both CB and SH, maximum number of *Salmonella enterica* isolates were found to be resistant against Augmentin (30 μg) while resistance against Cotrimaxazole (25 μg) and Tetracycline (25 μg) was found to be at second and third number respectively (Table2). Maximum number of *Salmonella enterica* isolates of both CB and SH were found to be susceptible against Ofloxacin (5 μg) and Gentamicin (10 μg).
(Table 2) indicating that these two drugs were effective to kill *Salmonella enterica* than any other antibiotic.

### Table 2- Antimicrobial susceptibility of *Salmonella enterica* isolates

<table>
<thead>
<tr>
<th>Antibiotics (µg)</th>
<th>Commercial boilers (CB) (n=50)</th>
<th>Spent hens (SH) (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible n (%)</td>
<td>Intermediate n (%)</td>
</tr>
<tr>
<td>Augmentin (30 µg)</td>
<td>0(0) 5(10) 45(90) 0(0) 3(6) 47(94)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin (5 µg)</td>
<td>34(68) 3(6) 13(26) 28(56) 2(4) 20(40)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td>25(50) 5(10) 20(40) 28(56) 2(4) 20(40)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid (30 µg)</td>
<td>0(0) 20(40) 30(60) 0(0) 15(30) 35(70)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin (200 µg)</td>
<td>10(20) 15(30) 25(50) 8(16) 12(24) 30(60)</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole (25 µg)</td>
<td>2(4) 5(10) 43(86) 1(2) 7(14) 42(84)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (25 µg)</td>
<td>5(10) 10(20) 35(70) 0(0) 12(24) 38(76)</td>
<td></td>
</tr>
</tbody>
</table>

### Screening of Metallo-β-lactamases (MBL) and phenotypic detection

The *Salmonella enterica* isolates were suspected to produce MBL when these were resistant to either imipenem or meropenem. The *Salmonella enterica* isolates showing inhibition zone diameter (IZD) of ≤ 23mm were suspected to produce MBL enzymes and these isolates were subjected to phenotypic confirmation test (Fig. 2, Table 3). For *Salmonella enterica* isolates obtained from commercial boilers (CB) and the spent hens (SH), 20 samples each were suspected to produce MBL enzymes and subsequently subjected to phenotypic detection of MBL (Table 3). MBL production was phenotypically evaluated by the inhibition-based assay using EDTA. A difference of ≥7mm between the zones of inhibition of any of the carbapenem disks tested alone compared to disks with EDTA inferred phenotypically MBL positive.

In case of commercial boilers and spent hens *Salmonella* positive samples 11(22%) and 9(18%) respectively were suspected to be MBL positive too. Out of these, CB samples were 100% (11/11) MBL positive while SH samples gave 88.88% (8/9) MBL positive result (Table 3).

### Table 3- MBL detection in *Salmonella enterica* isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Host positive for <em>Salmonella</em></th>
<th>Suspected MBL producers n (%)</th>
<th>MBL Positive n (%)</th>
<th>MBL Negative n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enterica</em> Commercial boilers(CB) (n=20)</td>
<td>11(22%)</td>
<td>11(100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enterica</em> Spent hens (SH) (n=20)</td>
<td>9(18%)</td>
<td>8(88.88%)</td>
<td>1(0.1%)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2- Imipenem disk test for *Salmonella enterica* isolates.
Serotypes detected

Out of the 20 positive samples of Salmonella, two serotypes were detected of which 15 were *Salmonella enteritidis* and 5 serovar *Salmonella typhimurium*.

*b*la*VIM*, *bla*IMP and *bla*SPM-1 genes molecular detection

The *bla*VIM, *bla*IMP and *bla*SPM-1 genes were detected in MBL phenotypically positive samples by PCR based amplification detection method. The results showed that *S. enterica* isolates from commercial boilers meat samples and spent hens contained meat samples contained gene *bla*VIM (11, 57.89%), *bla*IMP (6, 31.57%) and *bla*SPM-1 (2, 10.52%) (Fig. 3, Table 4).

**Table 4** - *bla*VIM, *bla*IMP and *bla*SPM-1 Genes were detected in MBL phenotypically positive samples

<table>
<thead>
<tr>
<th>MBL Positive n (%)</th>
<th>Total samples positive for salmonella</th>
<th><em>bla</em>VIM genes obtained by PCR</th>
<th><em>bla</em>IMP genes obtained by PCR</th>
<th><em>bla</em>SPM-1 genes obtained by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>20</td>
<td>57.89% n=11</td>
<td>31.57% n=6</td>
<td>5.60% n=2</td>
</tr>
</tbody>
</table>

Discussion

Resistance to antibiotics in non-hospital environments is a growing concern that has risked the efficacy of potential antibiotics. *Salmonella* species are notorious gram negative bacteria responsible for a number of infections that are becoming resistant to available antibiotics. *S. enterica* in meat samples is major health concern and in current research meat samples of commercial boilers (CB) and the spent hens (SH) from Ardabil, the city of Ancient Iran were studied for the detection of *S. enterica*. Metallo-β-lactamase (MBL) enzyme produced by *S. enterica* strains isolated from poultry meat samples were detected by both biochemical and molecular methods. The 50 positive samples each of CB and SH for *S. enterica* were included in the current research. The prevalence of *S. enterica* for CB was 22% (11/50) and for SH was 18% (9/50).

The isolated *S. enterica* strains were subjected to antibiotic susceptibility testing and for both CB and SH, maximum number of *S. enterica* isolates were found to be resistant against Augmentin (30 μg) while resistance against Cotrimaxazole (25 μg) and Tetracycline (25 μg) was found to be at second and third number respectively. Maximum number of *S. enterica* isolates from both CB and SH were found to be susceptible against Ofloxacin (5 μg) and Gentamicin (10 μg) indicating that these two drugs were effective to kill *S. enterica* than any other antibiotic. This is in agreement with Ezekiel et al. (2011) who showed that Salmonella found in poultry meat was found to be 100% resistant to augmentin (Ezekiel et al, 2011). In another study, tetracycline was found to be 90%–100% resistant against Salmonella (Sakaridis et al, 2011). Miranda et al. (2008) in a study also reported the development of resistant strains of Salmonella of poultry meat (Miranda et al, 2008). The rising numbers of resistant Salmonella strains in poultry can progress towards an epidemic (Velge et al, 2005; White et al, 2001).

In addition to poultry resistant *Salmonella* strains are also find in cow meat and milk which showed resistance to Gentamicin, Nitrofurantoin, Cotrimaxazole and Tetracycline (Addis et al, 2011).

Extensive research has been done on serotypes in *Salmonella* and a variety of serotypes including Enteritidis, Typhimurium, Derby, Anatum and

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**Fig. 3- PCR for detection of blaVIM gene in Salmonella enterica isolates.**

M: DNA size marker; Lane 1: Psotive control blaVIM; Lane 2: Negative control; Lane 3-4: PCR product of blaVIM gene(261 bp).
Albany have been implicated in Salmonellosis of the poultry. Antibiotic-resistant rate in Salmonella isolates identified in humans is steadily increasing in recent years. They also in antibiotic susceptibility test have shown multiple resistances in most isolated samples. Regarding the importance of Salmonella contamination in poultry breeding and its impact on public health and increasing antibiotic resistance of Salmonella (Graziani et al, 2008), serotyping and investigation of antibiotic resistance pattern of isolates was performed in this study that S. enteritidis was the dominant serotype by a high ratio 75%.

Screening phenotypic confirmatory test used for detection of Metallo-β-lactamase (MBL) enzyme for CB, (n= 11, 22%) were positive for MBL while for SH (n= 9, 18%) samples were positive for MBL. The results showed that MBL positive S. enterica isolates from CB and SH meat samples contained gene gene blaVIM (11, 57.89%), blaIMP (6, 31.57%) and blaSPM-1 (2, 10.52%) of MBL. This evidence has been supported in a study by Murphy et al., (2003) where MBL gene SPM-1 from Pseudomonas aeruginosa (48-1997A) was cloned (Murphy et al, 2003). The results showed that it had ORF (open reading frame) homologous with S. enterica serovar Typhimurium suggesting that its origin was Salmonella sp. Another similar study done by C. Rizek et al., (2014) have also identified carbapenemase genes blaSPM and blaVIM in cardapenem-resistant Pseudomonas aeruginosa isolates which further supports the results of this study (Rizel et al, 2014).

Similarly, another member of the Enterobacteriaceae family, Klebsiella isolated from non-hospital environments was reported to produce MBL suggesting the evidence for multidrug resistance being spread to other sources (Eijkewugwu et al, 2017).Thus Salmonella has a significant potential to become increasingly resistant towards a range of antimicrobials. Research is needed to further evaluate the different strains of Salmonella that prevail in the meat samples as it can lead to the increased transfer and spread of MBL-producing bacteria in the food chain. This will help the scientific community to plan strategies accordingly.

I would like to thank the central laboratory pathobiology for supporting this research.

References


Burns, 36(6), 826-30.


Antimicrobial resistance in Salmonella enterica serovar Typhimurium from human and animal sources in Italy. Veterinary microbiology, 128(3-4), 414-418.


تشخیص فنوتیپ و مولکولی زنهای متالوپتالاقتناز در گونه‌های سالمونلا انتاریکا

چکیده

مطالعه حاضر برای شناسایی سالمونلا انتاریکا در نمونه‌های گوشته جوجه گوشتی (SH) و مرغ گوشتی (CB) در شهرستان اردبیل انجام شد. آزمیخت متابولیت‌های انتاریکا (MBL) تولید شده توسط سویه‌های سالمونلا انتاریکا جدای شده از نمونه‌های گوشت مرغ توسط روش های بیوشیمیایی و مولکولی شناسایی شد. این مطالعه شامل 20 نمونه مثبت برای سالمونلا انتاریکا از نمونه‌های گوشت جوجه کبابی (CB) و مرغ گوشتی (SH) بود. شیوع سالمونلا انتاریکا برابر 22/47% (n=50) و برابر 18/10% (n=9/5) بود. میزان حساسیت تکنیک گرفتن نتایج از گوشت SH و CB (50/11) بود. (CB) مقاوم بوده و نسبت به افولکسانین (5 میکروگرم) کوتربیوم اکزرول (25 میلی گرم) و نتراسیلین (25 میکروگرم) مقاوم بوده و نسبت به افولکسانین (5 میکروگرم) و جنیمیسین (10 میکروگرم) حساسیت دارند. در مطالعه سایر همکاران (MBL) توسط آزمون PCR دارای 87/SH (n=88.88 مثبت بود، در حالت که برابر 100/100 % مثبت بود. نتایج نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در گوشت طیور به دلیل نتایج بالا و مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%) در ۱ (n=2.1-12.52 %) و %blalMP در bamPG در bamPG در مثبت نشان داد که جدایی های مثبت CB برای 100/11 بود. در برابر آزمون VIM 6/2 (n=6.57.89%