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# Detection of virulent genes of some food borne pathogens in chicken meat

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**Abstract**

The present study was performed on 120 random samples of fresh chicken meat collected from different supermarkets and retail chicken butchers in both urban and popular areas (60 of each) at Kaliobia Governorate, Egypt, to evaluate their bacteriological profile. The bacteriological examination revealed that, the mean values of Total aerobic bacterial*; Enterobactereceae; Coliform* and *Staph. aureus* counts in the examined samples of fresh chicken meat collected from urban and popular areas were $6.74\times10^5\pm2.8\times10^4$, $8.03\times10^5\pm1.2\times10^4$; $2.85\times10^4\pm6.4\times10^3$, $3.02\times10^4\pm7.6\times10^3$; $8.7\times10^3\pm8\times10^2$, $9.9\times10^3\pm6\times10^2$ and $2.7\times10^3\pm6\times10^2$, $3.9\times10^3\pm7\times10^2$ , respectively. Moreover, a total of 73 (60.8%) isolates of food borne pathogens were recovered from 120 samples (28 from urban and 45 from popular areas) where *S. aureus* were the most isolated (27=22.5%) followed by *E. coli* (10=8.3%), *Ps. aeruginosa* (8=6.7%), *Kl. pneumoniae* (7=5.8%), *B.cereus* and *Enterobacter aerogenes* (6=5.0% for each), Micrococcus spp.(5=4.2%) and *Proteus vulgaris* (4=3.3%) .Meanwhile, *Clostridium perfringens* and *Salmonella* spp. failed to be isolated. In addition, most isolated *Staph. aureus* and *B. cereus* strains were enterotoxigenic ones, as they had haemolytic; amylase; proteolytic; lipolytic; Lecithinase activities beside biofilm formation. PCR results cleared that ***spa*A** and ***bla*Z** virulence genes were detected in all studied *Staph. aureus* strains; ***ica*A**; ***erm*C**; ***sea*** and ***sed*** virulence genes were detected in 5; 3; 2 and 8 out of 10 studied ones, respectively, but, ***pvl*** gene was not amplified in all studied strains. In addition, ***hbl*** virulence gene was detected in *B. cereus* strains, while the ***nhe*** virulence gene was detected in all studied strains. In addition to serotyping of *E. coli* are :2 $O_{1;}$ 4 $O_{55}$ ; 2 $O_{125}$ and 2 $O_{146}$

***Keywords:*** *Chicken meat, bacteriological evaluation, virulent genes, Staph. aureus, B. cereus*

## Introduction

Chicken meat is a common vehicle of pathogenic microorganisms such as *E. coli*; *Salmonella; Staph. aureus; Bacillus cereus*, *Enterobacter; Campylobacter*; *Listeria monocytogenes* and *Pseudomonas* species that considered as the most important causes of foodborne out breaks in people **(Noori and Alwan, 2016)**.

Avian strains of *E. coli* showed many similarities with human extra intestinal pathogenic *E. coli* (ExPEC) strains, in that most of the virulence genes they possess are similar to those identified in uropathogenic *E. coli* and newborn meningitis causing *E. coli* (NMEC) and some studies have also demonstrated that ExPEC strains could belong to the same clones as human EPEC strains and it can be transferred to humans through consumption of contaminated food or food products causing a variety of infections, including bacteremia, pneumonia and septicemia **(Gi *et al.,* 2009*).*** *Salmonella* inhabit the intestinal tract of animals and may be recovered from a wide variety of hosts, especially poultry. Most *Salmonella* strains found in poultry meat are non-host-specific and are considered capable of causing human food poisoning. Salmonellosis (gastroenteritis) is the most common disease in human **(Behravesh, 2008)**. *Staphylococcus aureus* is one of the most common agents in bacterial

food poisoning outbreaks. They produce many extracellular substances some of which are heat stable enterotoxins render the food of high risk even though it appears normal and extensive traditional cooking can kills the bacteria but the toxins can not be destroyed because most of them are heat stable. Virulent genes can be carried on the plasmid. (**Prescott *et al.*, 2005).** *Bacillus cereus* has emerged as a major foodborne pathogen during the last few decades through consuming contaminated foods**.** Its characteristics the same as mesophilic or psychotrophic bacteria; the mesophilic strains tend to be more heat resistant as compared with psychrotrophic ones **(Granum and Baird Parker, 2000).** The pathogenicity of *Staph. aureus* and *B. cereus* food borne pathogens could be attributed to the virulence factors which bacteria produce. For *Staph. aureus* it could be attributed to intracellular adhesion (***ica*A**); protein A (***spa*A**); toxins (enterotoxins, toxic shock syndrome toxin- Panton-Valentine Leukocidin); haemolysin; coagulase, thus clot blood; protease; hyaluronidase, and staphylokinase **(Abdalrahman *et al.*, 2015).** Meanwhile, *B. cereus* produces a large number of secreted cytotoxins and enzymes that may contribute to diarrhoeal disease, that is elicited by heat-labile enterotoxins; the two enterotoxincomplexes ***nhe*** (non-hemolytic enterotoxin) and ***hbl*** (haemolysin BL) and the single protein ***cyt*K** "cytotoxin K" **(Pfrunder *et al*., 2016).** As Egypt has a large chicken production industry and the level of contamination of chicken meat with different food-borne pathogens may constitutes serious problems for consumers, so, the present study was conducted to evaluate the safety of chicken meat in both urban and popular areas at Kaliobia Governorate. The phenotypic characterization of the isolated bacterial strains, beside genotypic characterization and detection of some virulence factors of some isolated *Staph. aureus* and *B. cereus* strains were acheived by PCR technique.

## Materials and Methods

### 1. Sample collection

A total of 120 random samples of fresh chicken meat collected from different supermarkets and retail chicken butchers in both urban and popular areas (60 of each) at Kaliobia Governorate, Egypt, for bacteriological examination beside the evaluation of their safety and fitness for human consumption.

### 2. Bacteriological Examination

**2.1. Preparation of sample according to ISO 6887-2:2003 and preparation of used media according to ISO/TS 11133:2003**

**2.2. Determination of Aerobic Plate Count (APC)/g** using the standard plate count following **APHA** (**2001**).

**2.3. Determination of Total *Enterobacteriaceae* count** by the surface plating method of **(ISO 21528-2, 2004)** using violet red bile Glucose agar medium (VRBG).

**2.4. Determination of Total *Coliform* count** by the surface plating method of **(ISO 4832, 2006)** using Violet Red Bile agar medium.

**2.5. Determination of Total *Staph. aureus* count (ISO 6888- 1: 1999).**

**2.6. Enumeration of coagulase positive *Staphylococci***: according to **(ISO 6888-1:1999)**

Suspected *Staph. aureus* colonies appeared as circular, smooth, convex, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone on Baird-Parker agar were identified morphologically by Gram stain, biochemically, and coagulase activities

**2.7. Isolation and identification of *E. coli* according to (ISO 16649-2, 2001)**

Typical *E. coli* colonies (bluish green colonies) were picked up for identification morphologically by Gram stain; biochemically, serologically by slide agglutination test (using *E. coli* antisera "SEIKEN" Set 1, consists of polyvalent and (O) antisera of **MAST ASSURE™ *E coli* ANTISERA** mast group ltd., mast Hause DerbyRoad, Bootle North of England according to **(Markey *et al*., 2013)**.

**2.8. Isolation and identification of *B. cereus* strains** according to **(Markey *et al*., 2013)**: Typical *B. cereus* colonies (blue, turquoise to

peacock blue, about 5 mm in diameter and surrounded by a zone of egg yolk precipitation) on Polymyxin–pyruvate-Egg yolk-Mannitol-Bromothymol blue agar "PEMBA" were picked up for identification morphologically by Gram stain and biochemically.

**2.9. Isolation and identification of *Salmonella*** following **(ISO 6579-1:2017)** Suspected Salmonella colonies that appeared as red with black centers on XLD agar and pink colonies on Brilliant Green agar were identified morphologically by Gram- stain and biochemically according to **(Markey *et al*., 2013).**

**2.10. Isolation and Identification of *Clostridium perfringens* strains**: Suspected colonies (showed double zones of haemolysis on neomycin sheep blood agar) were identified morphologically by Gram-stain and biochemically according to **(Paul *et al*., 2009).**

**3. Detection of virulence factors for isolated *Staph. aureus* and *B. cereus* strains:**

**A-Phenotypic virulence of *Staph. aureus* and *B. cereus* strains***:* The haemolytic; amylase; proteolytic (caseinase); lipolytic and lecithinase activities for isolated *S. aureus* and *B. cereus* strains tests were tested as described by **(Yang and Fang, 2003).** In addition to biofilm formation of them using tube method of **(Christensen *et al*., 1982).**

**B-Genotypic virulence of *Staph. aureus* and *B. cereus* strains**:

Genotyping detection of some virulence genes for *Staph. aureus* as intra-cellular adhesion (***ica*A**) gene; protein A (***spa*A**) gene and resistance genes as β lactamase resistance ***(bla*Z)** gene and erythromycin resistance (***erm*C)** gene; also enterotoxine genes as enterotoxins (***sea***, ***sed*** and ***pvl***) gene in 10 random *Staph. aureus* strains. And two toxine genes for Bacillus cerceus, hemolysin BL **(*hbl*)** gene and nonhemolytic enterotoxin (***nhe*)** gene in 4 random *B. cereus* strains using uniplex and duplex polymerase chain reaction, following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR mastermix (Takara, Japan) and 1.5**%** agarose gel electrophoreses **(Sambrook *et al.,* 1989)** using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (0).

**4. Statistical analysis**

Data were subject to statistical analysis with means, standard division, and t-test at $p< 0.05$ using SPSS for Windows 7 version 15 Prevalence. Estimation of commonly isolated pathogens was determined using standard formulae (that is the number of positive animals/samples divided by the total number of animals/ samples examined); descriptive statistics such as percent.

**Results and Discussion**

The obtained results of mean bacterial counts / gm. Table (1) revealed that, mean value of APC; *Enterobactereceae; Coliform* and *Staph. aureus* counts in the examined samples of fresh chicken meat collected from urban and popular areas were $6.74\times10^5\pm2.8\times10^4$, $8.03\times10^5\pm1.2\times10^4$; $2.85\times10^4\pm6.4\times10^3$, $3.02\times10^4\pm7.6\times10^3$; $8.7\times10^3\pm8\times10^2$, $9.9\times10^3\pm6\times10^2$ and$2.7\times10^3\pm6\times10^2$and $3.9\times10^3\pm7\times10^2$, respectively. Moreover, the statistical results revealed that, fresh chicken meat samples from popular area showed a significant ($P\leq 0.05$) increase of APC and *Enterobacteriaceae* counts when compared with that of urban area. Meanwhile, there were no significant difference ($P>0.05$) between samples of both urban and popular area for *Coliform* and *Staph. aureus* counts. For APC and total *Enterobacteriaceae* counts were nearly similar to those obtained by **Mohamed, (2016)**. In addition, the results of *Coliform* plate counts came in parallel with **Javadi and Safarmashaei, (2011).** Moreover, the results of total *Staph. aureus* counts were nearly similar to those obtained by **Mohamed, (2016)**. The difference of mean values in both urban and popular areas may be attributed to sources of chicken, improper handling and bad storage conditions that can lead to toxins production and pathogens proliferation. The results of bacteriological examination of studied samples revealed that foodborne pathogens were isolated from **73** positive samples **(60.8%)**; represented as **28 (46.7%)** from chicken meat samples of urban area,; meanwhile, **45 (75%)** from chicken meat samples of

popular area. The most isolated strains were *Staph. aureus* **(27=22.5%)** followed by *E. coli* **(10=8.3%),** *Ps. aeruginosa* **(8=6.7%),** *Kl. pneumoniae* **(7=5.8%),** *Bacillus cereus* and *Enterobacter aerogenes* **(6 =5.0% for each),** *Micrococcus* spp**. (5 =4.2%)** and *Proteus vulgaris* **(4=3.3%)**. Meanwhile, *Clostridium perfringens* and *Salmonella* spp. failed to be isolated as shown in Table (2).

Similar results were recorded by (**Olukemi *et al.,* 2015*;* Noori and Alwan, 2016** and **Suleiman *et al.,* 2016).** The results of *E. coli* isolation (Table, 2) showed that, **10** strains were isolated, **3 (5.0%)** from chicken meat samples of urban area and **7 (11.7%)** from chicken meat samples of popular area. Nearly similar results were obtained by **(Olukemi *et al.*, 2015; Noori and Alwan, 2016; Suleiman *et al.*, 2016 and Abd El-Alim, 2017).** In addition, the results of serological examination declared that, out of *E. coli* strains, 4 serotyped as ($O_{55}$,$O_{125}$, $O_1$ and $O_{146}$) as in table (4). These results came in harmony with those of **(Abd El -Tawab *et al.*, 2015 and Abd El-Ali, 2017).** The results of *Staph. aureus* isolation (Table 2) revealed that, 27 strains were isolated, **12 (20.0%)** from chicken meat samples of urban area and **15 (25.0%)** from chicken meat samples of popular area. These results were nearly similar to (**Zargar *et al.,* 2014*;* Suleiman *et al.,* 2016** and **Abd El-Alim, 2017)**. The presence of *Staph. aureus* in foods commonly indicates direct contamination from worker's hands with abrasion and wounds or inadequately cleaned equipment and they grow without pronounced change in odor or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhoea and gastroenteritis among consumers **Argudin *et al.,* (2010).** The results of *B. cereus* isolation (Table, 2) revealed that, 6 strains were isolated, **2 (3.3%)** from chicken meat samples of urban area and **4 (6.7%)** from chicken meat samples of popular area. while the prevalence of *Bacillus cereus in other studies was* in percentages. 15**%** and 9.4**%.** Nearly similar results were obtained by (**Hanna *et al.*, 2014** and **Ben Amor Maroua *et al.*, 2018).**
Most isolated *Staph. aureus* and *B. cereus* strains have phenotypic Virulence factors, as they had haemolytic; amylase; proteolytic; lipolytic; Lecithinase activities and had the ability for biofilm production. Which have role in the pathogenesis of bacterial infections **(Markey *et al.*, 2013)** and biofilm formation of isolates consider sources for the transmission of *bacteria* as biofilms is a relevant risk factors due to the potential contamination of food products with pathogenic and spoilage microorganisms. The majority of bacteria are able to adhere and to form biofilms, where they can persist and survive for days to weeks or even longer **(Winkelströter *et al.,* 2014).**

In case of isolated *Staph. aureus* as in (Table 3) showed that, all **27** *Staph. aureus* (100%) had haemolytic activity and lipolytic activity, where 25 isolate (92.6%) have amylase activity, proteolytic (caseinase) activity detected in 24 *Staph. aureus* (88.9%), 22 *Staph. aureus* (81.5%) had Lecithinase activity these virulence factors detected by **Barretti *et al.,* (2009)** who detected β Hemolysin, 29 (82.3%), Lipase 34 (97.1), Lecithinase 34 (97.1%) (**Eftekhar and Dadaei; 2011 and Pinto *et al.*, 2015; Abd El-Tawab *et al.*, 2015; Abd El-Alim, 2017 and Gündoğan and Devren 2010).**

Moreover**, 20** *Staph. aureus* **(77.8%)** had the ability for biofilm production. In case of isolates of *B. cerceus*, all sex *B. cereus* (100**%**) isolated strains had haemolytic activity, starch hydrolysis, protease enzyme, lipolytic activity and Lecithinase activity These also detected by **(Celenk *et al.*, 2009; Sharaf Eman *et al.*, 2014** and **Sarita and Prabir, 2014).** Moreover**, 4** *B. cereus* **(66.7%)** isolated strains had the ability for biofilm production These results came in harmony with those of **Özdemir Fatma and Seza Arslan, (2018)** who detected **8** out of **24 (33.3%)** *B. cereus* isolates were considered as biofilm producers**.**

The genotypic identification and detection of some virulence genes in isolated *Staph. aureus* and *B. cereus* strains using PCR results showed that for *Staph. aureus* PCR using 7 sets of primers was used for detection of 7 virulence genes that may play a role in virulence of *S. aureus*. and formation of biofilm (icaA

and spa), food toxication (leukocidin (*pvl*), sea and sed) and resistance genes (erythromycin resistance gene (*remC*) and β lactemase resistance gene (*blaZ*). It was applied on 10 random isolated in Table **(5)** and (Fig. 1-6). In addition, PCR using 2 sets of primers was used for detection of 2 toxins of *B. cereus* genes that may play a role in food toxication the non-hemolytic enterotoxin Nhe and the hemolysin BL (Hbl) toxin which responsible for inducing rapid cell lysis the result of PCR were hbl gene (2\4) and nhe 4\4 as in (Fig. 7&8).

Regarding to *Staph. aureus* strains, the PCR results for amplification of ***spa*A** gene (Fig., 1) showed that, it was amplified in all 10 studied *Staph. aureus* strains giving product of 226 bp. and similar findings were recorded by **(Podkowik *et al*., 2012** and **Krupa *et al*., 2014)**. Meanwhile, the results of PCR for amplification of ***ica*A** gene (Fig. 2) showed that, it was amplified in 5 out of 10 *Staph. aureus* studied strains giving product of 103 bp., these results came in harmony with those of **(Febler *et al.*, 2011** and **Pinto *et al*., 2015).** For ***bla*Z** gene, it was amplified in all 10 studied *Staph. aureus* strains giving product of 173 bp. (Fig. 3). These results agreed with those of **(Podkowik *et al*., 2012**; **Momtaz *et al*., 2013** and **Krupa *et al.*, 2014)**. Also**,** the ***erm*C** gene was amplified in 3 out of 10 *Staph. aureus* studied strains giving product of 295 bp. as shown in Fig. (4). Similar results were recorded by (**Podkowik *et al*., 2012** and **Krupa *et al*., 2014).** In addition, the results of PCR for amplification of ***sea*** and **sed** genes in *Staph. aureus* strains (Fig. 5) showed that, it was amplified in 2 out of 10 *(sea)* and *8\10 (sed) Staph. aureus* studied strains giving product of 102 bp (sea"). And sed at 278 bp and these results came in harmony with those of **(Abdalrahman *et al.*, 2015; Afifi Dina, 2016 and Moustafa *et al*., 2016).** Meanwhile, the results were disagreed with **(Febler *et al.*, 2011)** who failed to detect ***sea*** virulent gene in these strains that isolated from poultry and poultry products. Also, Fig. (5) showed that, the ***sed*** gene was amplified in 8 out of 10 *S. aureus* studied strains giving product of 278 bp. Similar results were decided by **(Abdalrahman *et al.*, 2015** and **Moustafa *et al.*, 2016)**. Moreover, the PCR results for amplification of ***pvl*** gene (Fig. 6) showed that, it was not amplified in all studied *Staph. aureus* strains at 433 bp. and these results were agreed with those recorded by **(Febler *et al*., 2011)** but disagreed with **(Abdalrahman *et al*., 2015 and Afifi Dina, 2016)** who detected ***pvl*** gene in *Staph. aureus* strains isolated from poultry meat. Meanwhile, for *B. cereus* strains, the obtained PCR result for amplification of ***hbl*** gene in *B. cereus* strains revealed that, it was amplified in 2 out of 4 studied strains giving product of 1091 bp.as shown in Fig. (7). In addition, the results of PCR amplification of nonhemolytic enterotoxin (***nhe***) gene (Fig. 8) cleared that, it was amplified in all 4 studied *B. cereus* strains giving product of 766 bp. These results were agreed with those obtained by **(Aragon-Alegero *et al*., 2008; Forghani *et al.*, 2014** and **Tewari *et al.*, 2015).**

**Conclusion and Recommendation**

Finally, the present study proved that, chicken meat is considered of public health hazard and the presence of aerobic bacteria; coliforms mainly pathogenic *E. coli* enterotoxigenic coagulase positive *Staph. aureus* and enterotoxigenic *B. cereus* may be due to the mishandling and the negligence of hygienic aspects. Therefore, it was concluded that these pathogens are meat borne pathogens of public health importance.

| Target M.O. | Target gene | | Primer sequence (5'-3') | Amplified segment (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Secondary denaturation | Annealing | Extension | | |
| *Staph. aureus* | *spa*A | F | TCA ACA AAG AAC AAC AAA ATG C | 226bp | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | Wada *et al.*, 2010 |
| | | R | GCT TTC GGT GCT TGA GAT TC | | | | | | | |
| | *ica*A | F | ACA GTC GCT ACG AAA AGA A | 103bp | 95˚C 1 min. | 945˚C 1 min | 60˚C 1 min | 72˚C 1 min | 72˚C 8 min. (40cycl) | Park *et al.*, 2008 |
| | | R | GGA AAT GCC ATA ATG ACA AC-3') | | | | | | | |
| | *pvl* | F | ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A | 433bp | 94˚C 5 min. | 94˚C 45 sec. | 55˚C 45 sec | 72˚C 45 sec | 72˚C 7 min. | Park et al., 2008 |
| | | R | GCA TCA AST GTA TTG GAT AGC AAA AGC | | | | | | | |
| | *blaZ* | F | ACTTCAACACCTGCTGCTTTC | 173bp | 94˚C 5 min. | 94˚C 30 sec. | 54˚C 30 sec | 72˚C 30 sec. | 94˚C 5 min. | Duran *et al.*, 2012 |
| | | R | TGACCACTTTTATCAGCAACC | | | | | | | |
| | *ermC* | F | ATCTTTGAAATCGGCTCAGG | 295bp | 94˚C 5 min. | 94˚C 30 sec. | 51˚C 30 sec | 72˚C 30 sec. | 72˚C 10 min. | *et al.*, 2008 |
| | | R | CAAACCCGTATTCCACGATT | | | | | | | |
| | *sea* | F | GGTTATCAATGTGCGGGTGG | 102bp | 94˚C 5 min. | 94˚C 45 sec | 55˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | Mehrotra et al., 2000 |
| | | R | CGGCACTTTTTTCTCTTCGG | | | | | | | |
| | *sed* | F | CCAATAATAGGAGAAAATAAAAG | 278bp | 94˚C 5 min. | 94˚C 45 sec | 50˚C 45 sec | 72˚C 45 sec | 72˚C 7 min. | Mehrotra et al., 2000 |
| | | R | ATTGGTATTTTTTTTCGTTC | | | | | | | |
| *B. cereus* | *hbl* | F | GTA AAT TAI GAT GAI CAA TTTC | 1091bp | 94˚C 5 min. | 94˚C 30 sec | 49˚C 40 sec | 72˚C 1 min. | 72˚C 10 min. | Ehling-Schulz *et al.*, 2006 |
| | | R | AGA ATA GGC ATT CAT AGA TT | | | | | | | |
| | *nhe* | F | AAG CIG CTC TTC GIA TTC | 766 bp | 94˚C 5 min. | 94˚C 30 sec | 49˚C 40 sec. | 72˚C 45 sec | 72˚C 10 min | Ehling-Schulz *et al.*, 2006 |
| | | R | ITI GTT GAA ATA AGC TGT GG | | | | | | | |

Table (0): Primers sequences, target genes, amplicons sizes and cycling conditions

**Table (1).** Mean bacterial counts /gm. in the examined samples of fresh chicken meat collected from urban and rural (popular) areas (n=60 for each sample)

| Samples | Fresh chicken meat from urban area | Fresh chicken meat from popular area |
|---|---|---|
| **Aerobic plate counts** | $6.74\times10^5 \pm 2.8\times10^4$ [b] | $8.03 \times 10^5 \pm 1.2\times 104$ [a] |
| ***Enterobactereceae* counts** | $2.85\times10^4 \pm 6.4\times10^3$ [b] | $3.02 \times 10^4 \pm 7.6\times10^3$ [a] |
| ***Coliform* counts** | $0.87\times10^4 \pm 8\times10^2$ [a] | $9.9 \times 10^3 \pm 6\times10^2$ [a] |
| ***Staph. aureus* counts** | $2.7\times10^3 \pm 6\times10^2$ [a] | $3.9 \times 10^3 \pm 7\times10^2$ [a] |

Value in the same raw carring different super script letter are significantly different with each other at $P \leq 0.05$

**Table (2).** Prevalence of foodborne pathogens in examined chicken meat samples

| Samples | Samples from urban area | | Samples from popular area | | Total | |
|---|---|---|---|---|---|---|
| **Isolates** | **No.** | **%*** | **No.** | **%*** | **No.** | **%**** |
| ***Bacillus cereus*** | 2 | 3.3 | 4 | 6.7 | 6 | 5.0 |
| ***Clostridium Perfringens*** | 0 | 0 | 0 | 0 | 0 | 0 |
| ***E. coli*** | 3 | 5.0 | 7 | 11.7 | 10 | 8.3 |
| ***Enterobacter Aerogenes*** | 2 | 3.3 | 4 | 6.7 | 6 | 5.0 |
| ***Kl. Pneumonia*** | 2 | 3.3 | 5 | 8.3 | 7 | 5.8 |
| ***Micrococcus spp.*** | 2 | 3.3 | 3 | 5.0 | 5 | 4.2 |
| ***Proteus vulgaris*** | 2 | 3.3 | 2 | 3.3 | 4 | 3.3 |
| ***Ps. aeruginosa*** | 3 | 5.0 | 5 | 8.3 | 8 | 6.7 |
| ***Salmonella spp.*** | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Staph. Aureus*** | 12 | 20.0 | 15 | 25.0 | 27 | 22.5 |
| **Total** | 28.0 | 46.7 | 45.0 | 75.0 | 73.0 | 60.8 |

%* Percentage in relation to total number of each sample (60 )
%**Percentage in relation to total number of samples ( 120)

**Table (3).** Phenotypic virulence factors of *Staph. aureus* and *B. cereus* isolates

| Phenotypic virulence activities | ***Staph. aureus*** **( 27 )** | | ***B. cereus*** **( 6)** | |
|---|---|---|---|---|
| | **No.** | **%*** | **No.** | **%*** |
| **Haemolytic activity(β- haemolysis)** | 27 | 100.0 | 6 | 100.0 |
| **Starch hydrolysis** | 25 | 92.6 | 6 | 100.0 |
| **Lipolytic activity** | 27 | 100.0 | 6 | 100.0 |
| **Proteolytic (caseinase) activity** | 24 | 88.9 | 6 | 100.0 |
| **Lecithinase activity** | 22 | 81.5 | 6 | 100.0 |
| **Biofilm production** | 21 | 77.8 | 4 | 66.7 |

*Percentage in relation to number of each isolated strain

**Table (4).** The results of specific polyvalent and mono valent antisera used for serotyping of *E.coli* isolated :-

| Polyvalent antisera | Mono valent antisera | No. of isolate |
|---|---|---|
| **Polyvalent 1** | $O_1$ | 2 |
| **Polyvalent 2** | O 125 | 2 |
| **Polyvalent 2** | O 146 | 2 |
| **Polyvalent 2** | O55 | 4 |

O (somatic antigens)

**Table (5).** Genotypic factors of 10 strains *Staph. aureus* isolates

| ***Sed*** | ***Sea*** | ***ica* A** | ***Spa*** | ***Pvl*** | ***remC*** | ***blaZ*** | **Serial** |
|---|---|---|---|---|---|---|---|
| + | - | - | - | - | - | + | |
| + | + | + | + | - | + | + | |
| + | - | + | + | - | - | + | |
| + | - | - | + | - | + | + | |
| + | - | + | + | - | - | + | |
| - | - | - | + | - | - | + | |
| + | - | - | + | - | - | + | |
| + | - | + | + | - | + | + | |
| - | - | - | + | - | - | + | |
| + | + | + | + | - | - | + | |
| 8 | 2 | 5 | 10 | 0 | 3 | 10 | Total No. |
| 80.0 | 20.0 | 50.0 | 100.0 | 0 | 30.0 | 100.0 | % |

*remC:* erythromycin resistance gene *blaZ*: β lactemase resistance gene *spa*: protein A *pvl***:** leukocidine
*sea and sed: S.aureus* enterotoxins A and D *icaA :* intra-cellar adhesion

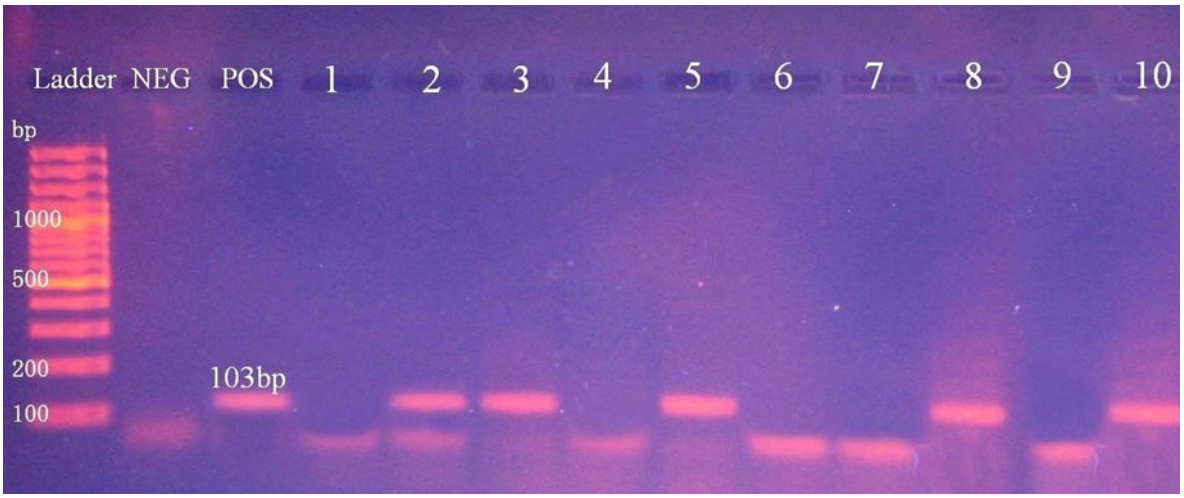


**Fig. (1):** Intra-cellular adhesion (*ica*A) gen
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S. aureus* ATCC25923)
Lane 2, 3, 5, 8 & 10 *S. aureus* (Positive at 103 bp.)
Lane1, 4, 6, 7, 9: s.aureus (Negative)

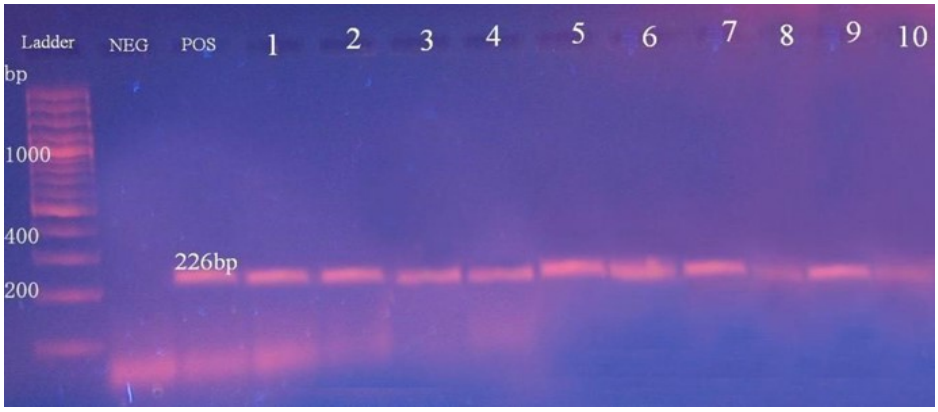


**Fig. (2 ): protein A (*spa*A) gene.**

Lane M: 100 - 1500 bpDNA Ladder.
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S. aureus* ATCC25923) Lane 1 -10 : *S. aureus* (Positive at 226 bp.).

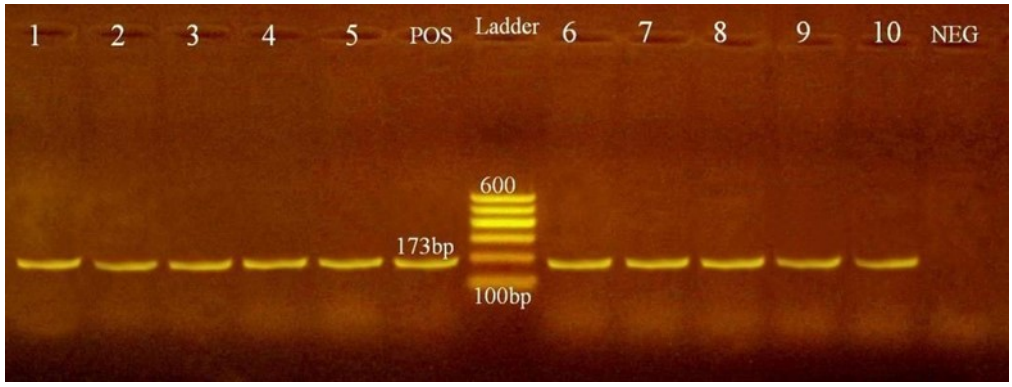


**Fig (3): β lactamase resistance (*blaZ*) gene.**

Lane L: 100 - 600 bp DNA Ladder.
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S. aureus* ATCC25923) Lane 1 - 10 : *S. aureus* (Positive at 173 bp.).

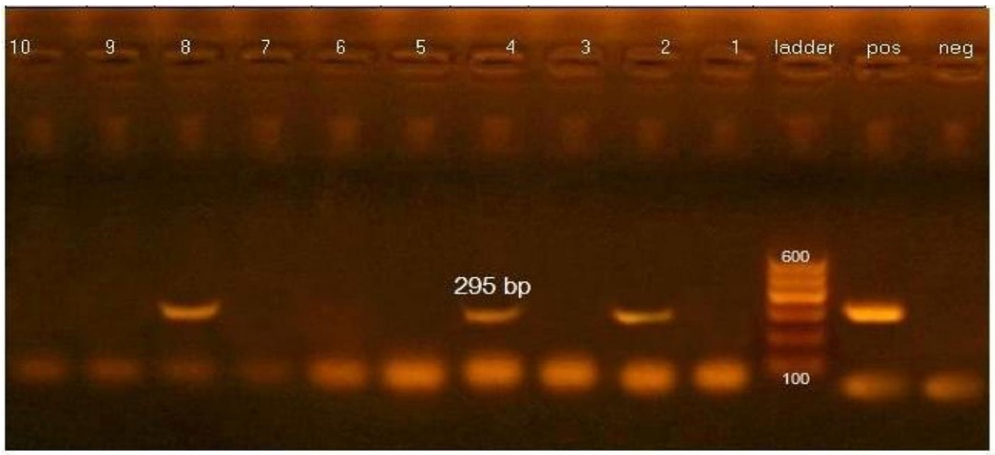


**Fig. (4): Erythromycin resistance (*ermC*) gene**

Lane L: 100 - 600 bp. DNA Ladder
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S. aureus* ATCC25923)
Lane 2, 4 & 8 : *S. aureus* (Positive at 295 bp.)
Lane 1, 3, 5, 6, 7, 9 & 10 : *S. aureus* (Negative)

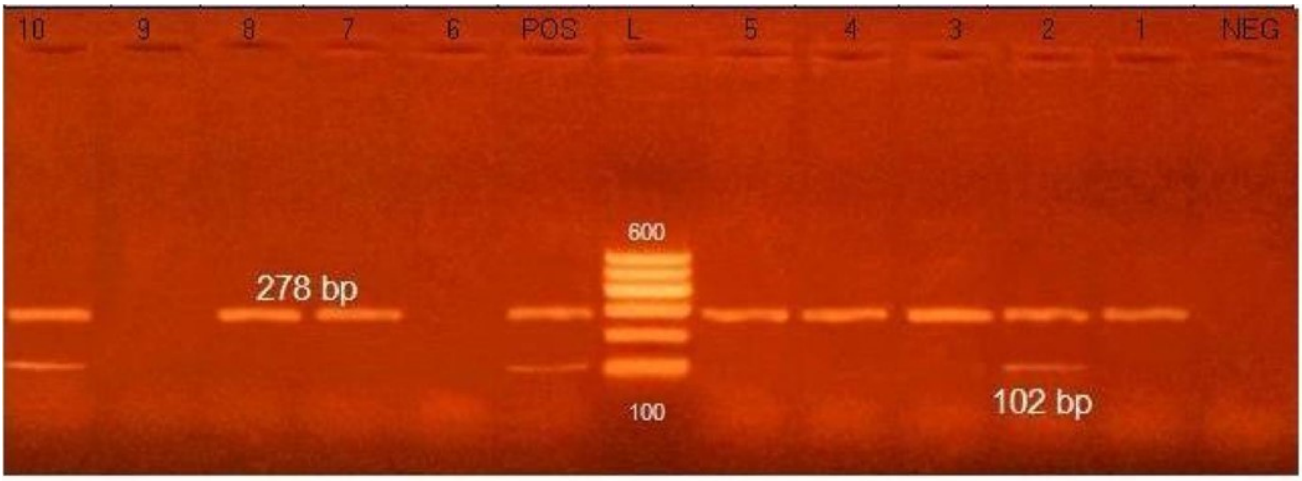


**Fig. (5):** Enterotoxins (*sea, and sed)* genes
Enterotoxin **(*sea*)** gene •
Lane L: 100 - 600 bp. DNA Ladder
Neg.: Negative control (*E.coli* AJ413986 )
Pos.: Positive control (*S. aureus* ATCC25923)
Lane 2 & 10 : *S. aureus* (Positive at 102 bp.) Lane1 ,3 -9 : *S. aureus* (Negative)
**B.** Enterotoxin **(*sed*)** gene
Lane L:100 - 600 bp. DNA Ladder
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S.aureus* ATCC25923) Lane 1 - 5 ,7 ,8 &10 : *S. aureus* (Positive at 278 bp.)
Lane 6 & 9 : *S. aureus* ( Negative )

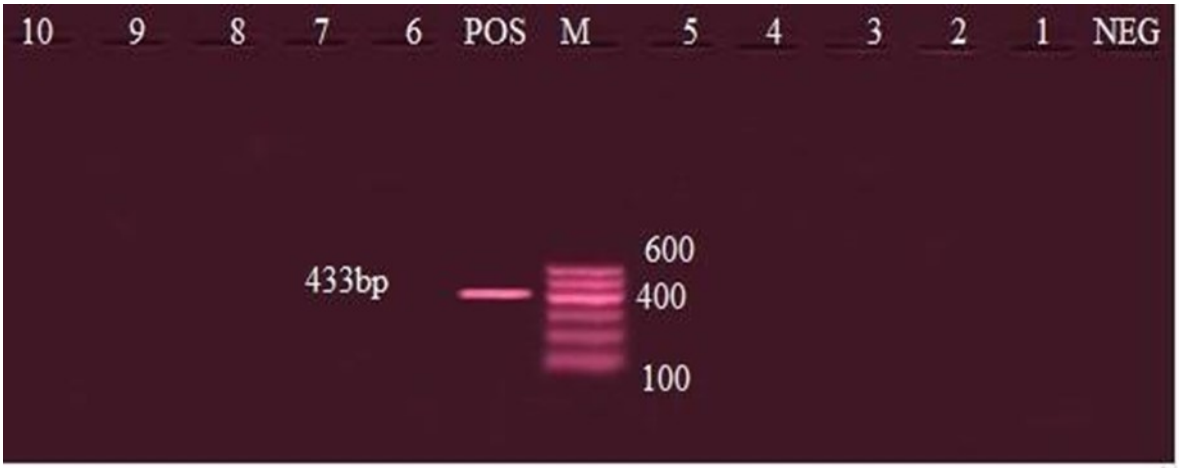


**Fig (6 ): Panton-Valentine Leukocidin (*pvl*) gene**
Lane M: 100 -600 bp. DNA Ladder
Neg.: Negative control (*E.coli* AJ413986)
Pos.: Positive control (*S. aureus* ATCC25923)
Lane1-10:S.aureus(Negative at 433bp)

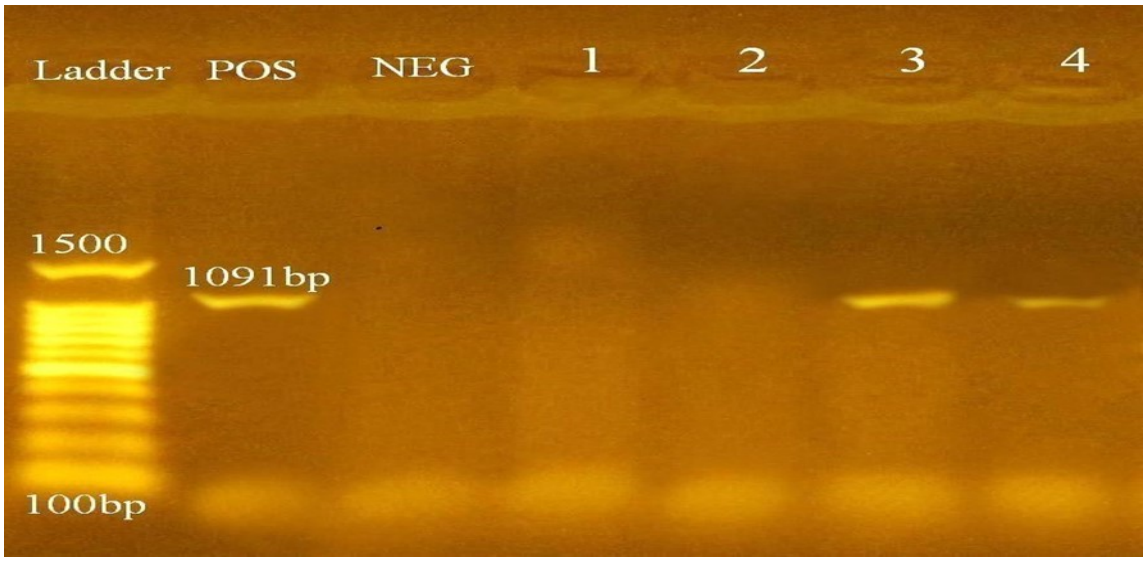


**Fig. (7 ): Hemolysin BL (*hbl*) gene**

Lane L:100 -600 bp. DNA Ladder
Neg.: Negative control *E.coli* AJ413986 )
Pos.: Positive control (*B. cereus* form Ahri.)
Lane 3 &4 : *B. cereus* (Positive at 1091 bp.)
Lane 1 & 2 : *B. cereus* (Negative)

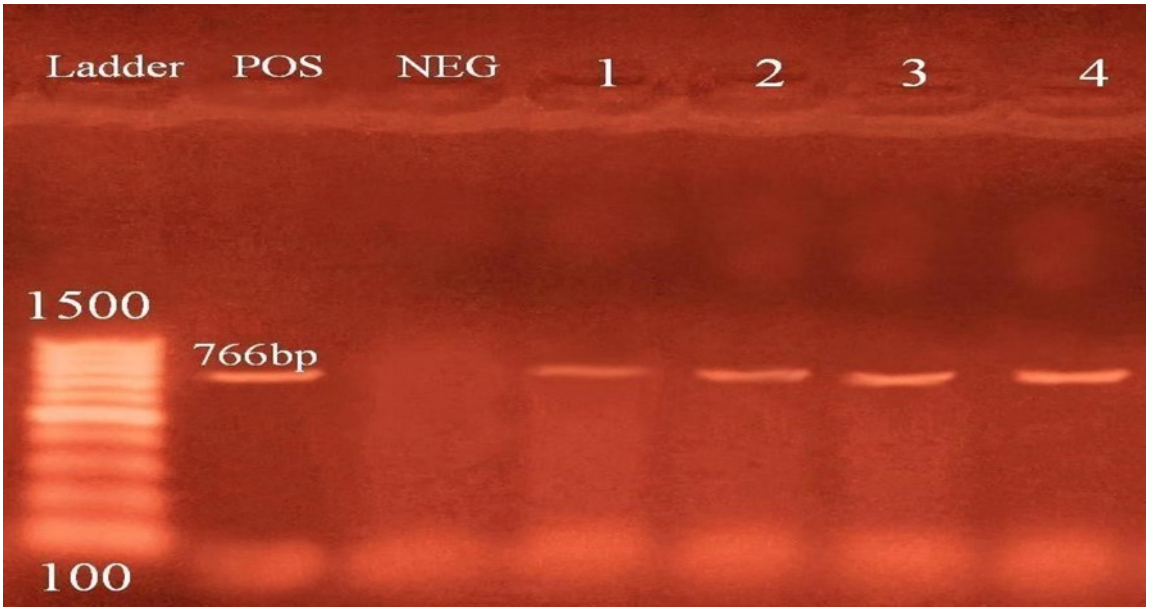


**Fig (8 ): Non-hemolytic enterotoxin (*nhe*) gene**

Lane L:100 - 1000 bp. DNA Ladder
Neg.: Negative control (*E. coli* AJ413986 )
Pos.: Positive control (*B. cereus* form Ahri.)
Lane 1 -4 : *B. cereus* (Positive at 766 bp.)

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