Prevalence of *bla*TEM and *bla*SHV genes in genomic and plasmid DNA of ESBL producing *Escherichia coli* clinical isolates from chicken

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ABSTRACT

A total of 102 chicken samples were collected from different farms in Qalubia, Behera, Cairo, Assuit and Menofia Governorates. The samples were represented by liver, spleen, lungs, heart blood, intestine and kidneys and subjected for isolation and identification of *Escherichia coli*. The bacteriological examination of the samples indicated the isolation of 55 *E. coli* represented as 31, 6, 8, 5, and 5 from Qalubia, Behera, Cairo, Assuit and Menofia, respectively. Different serotypes of *E. coli* (O_{158}, O_{125}, O_{111}, O_{27}, O_{20}, O_6, O_{25}, O_{26}, O_{145}, and O_{159}) and12 untyped strains were demonstrated. Thirty-Two *E. coli* isolates were subjected to initial screening test for extended-spectrum beta-lactamases (ESBLs) production by disc diffusion method with various cephalosporins. The results showed that 46.9% of samples were sensitive to ceftriaxone and cefotaxime and 53.1% to ceftazidime. By double disc synergy test, 19 *E. coli* strains were identified as ESBL producers. PCR results of 32 *E. coli* isolates showed that *bla*TEM gene was detected in the genomic DNA of all isolates and in plasmid DNA of 18 isolates. While, *bla*SHV was detected in the genomic DNA of 12 isolates and in plasmid DNA of 9 isolates. We can conclude from the current results that amplification of both genomic and plasmid DNA increase the positivity of detection in comparison with amplification of each of DNAs alone.

Key words: *E. coli*, ESBL, Plamid, *bla*TEM and *bla*SHV.

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1. INTRODUCTION

Escherichia coli is a Gram-negative rod-shaped bacterium that belongs to the *Enterobacteriaceae* family. It is one of the most prevalent pathogenic agents in avians (Janssen et al., 2003, Barnes, et al., 2008 and Arab, et al., 2013). *E. coli* infections are responsible for significant losses in the poultry industry in many parts of the world (Janßen et al., 2001). Avian pathogenic *E. coli* (APEC) is mostly associated with infection of extraintestinal tissues in chickens and other avian species with the exception of a possible relationship with the development of enteritis. Consequences of *E. coli* infection in poultry includes yolk sac infection, embryonic mortality or death of the young birds for up to 3 weeks following hatching, omphalitis, airsacculitis, pericarditis and perihepatitis, reduced growth and feed efficiency and an increased condemnation rate at the abattoirs. In laying birds, APEC may cause salpingitis and loss of egg laying ability. APEC also associated with swollen head syndrome and cellulitis (Cheville., et al., 1978, Morley et al., 1984, Randall et al., 1984, Leitner et al., 1992 and Peighambari et al., 1995). Susceptibility to *E. coli* infections increased by several factors as mycoplasmosis, viral infections like infectious bronchitis virus and New castle disease virus as well as environmental factors (Gross, 1990 and Dho-Moulin et al., 1999). Antimicrobial therapy is used to reduce both the incidence and mortality associated with *E. coli* infection in poultry. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Blanco et al., 1997). Widespread use of broad-spectrum cephalosporins and other beta-lactams is the major cause of the induction of ESBLs production in *E. coli* and other members of Family *Enteobacteraceae* (Thornsberry, 1995 and Chaudhary and Aggarwal, 2004). This results in increased resistance to broad-spectrum β lactams. Extended-spectrum β-lactamases (ESBLs) are Ambler class A penicillinases, which are capable of hydrolyzing the expanded-spectrum cephalosporins like ceftazidime, cefotoxime, monobactam-azteronam and related oxyimino β-lactams as well as olderpenicillins and

cephalosporins. They arise from mutations in the genes for common plasmid-mediated β-lactamases, especially Temoniera (TEM) and sulfhydryl variable (SHV) enzymes, which alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the β-lactamase for oxyimino compounds while simultaneously weakening the overall enzyme efficiency (Jacoby and Han, 1996, Emery and Weymouth, 1997 and Giriyapur et al., 2011).

As the level of resistance to beta lactam antibiotics is increasing, the present study was conducted to shed light over the prevalence of *E. coli* infection in chickens and detection of ESBL production by isolated strains using DDST and PCR of *bla*TEM and *bla*SHV genes in both genomic and plasmid DNA.

2. MATERIALS AND METHODS

2.1. Samples collection

A total of 102 chicken samples were collected from different farms in different governorates including Qalubia (62), Behera (10), Cairo (12), Assuit (9) and Menofia (9). All samples were collected under aseptic conditions. The surface of each organ was seared by hot spatula, and then sterile loopful was introduced through the seared portion. Loopfuls were taken from liver, spleen, lungs, intestine, kidneys and heart blood then inoculated on nutrient broth and incubated at 37°C for 24 hours.

2.2. Bacteriological examination

2.2.1. Isolation of E. coli:

It was performed according to Quinn et al., (2002) A loopful from each sample was streaked onto MacConkey's agar and Eosin Methylene Blue agar(EMB) and incubated at 37°C for 24 hours. Suspected colonies of *E. coli* were purified and kept for further identification.

2.2.2. Microscopic examination of suspected E. coli colonies:

It was performed by Gram's stain according to Cruickshank et al., (1975).

2.2.3. Biochemical identification of E. coli isolates:

It was performed according to Quinn et al., (2002) by application of oxidase test, sugar fermentation test, indole production test, methyl red test, voges proskauer test, citrate utilization test, H_2S production test, urea hydrolysis test and catalase test.

2.2.4. Serological identification of E. coli isolates:

It was performed according to Edwards and Ewing (1972). Serotyping of *E. coli* isolates were done at the Reference Laboratory of Veterinary Quality Control on Poultry Production, Dokki, Egypt using commercially available kits (*Escherichia coli* Antisera set 1 for O antigen, DENKA SEIKEN, Tokyo, Japan) which consists of 8 polyvalent sera and 43 monovalent sera.

2.3. Initial screening test for ESBL production

It was performed by disc diffusion method with various cephalosporins according to Giriyapur et al. (2011) and CLSI standers (2012). Isolates showed an inhibition zone size of ≤22 mm with ceftazidime (30 µg), ≤25 mm with ceftriaxone (30 µg) and ≤27 mm with cefotaxime (30 µg) were identified as potential ESBL producers and were short listed for confirmation of ESBL production.

2.4. Double disc synergy test (DDST)

It was performed according to Giriyapur et al. (2011). Synergy was determined between a disc of amoxicillin-clavulanate (20 µg/10 µg) (augmentin) and a 30-µg disc of each third-generation cephalosporin test antibiotic placed at a distance of 20 mm from center to center on a Mueller-Hinton Agar (MHA) plate swabbed with the test isolate. Clear extension of the edge of the inhibition zone of cephalosporin toward the augmentin disc was interpreted as positive for ESBL production.

2.5. Detection of blaTEM and blaSHV genes by Polymerase chain reaction

Primers with specific sequences were used to amplify specific product as shown in Table (1).

2.5.1. Genomic DNA extraction

QIAamp DNA Mini Kit was used according to manufacturer's instructions.

2.5.2. Plasmid DNA extraction

QIAprep Spin Miniprep Kit (Qiagen) was used according to manufacturer's instructions.

2.5.3. Amplification and cycling protocol for conventional PCR

Temperature and time conditions of the two primers during PCR were used according to specific author Colom et al. (2003) and Emerald Amp GT PCR master mix (Takara) kit Code No. RR310A.

2.5.4. Detection of PCR products:

It was performed according to Sambrooket al., (1989). Twenty µl of each PCR product samples,

negative control and positive control obtained from the Reference Laboratory of Veterinary Quality Control on Poultry Production, Dokki, Egypt were subjected to electrophoresis in 1.5% agarose gel.

3. RESULTS

3.1. Incidence of E. coli infection in chicken

A total of 55 *E. coli* isolates were recovered from 102 chicken samples from five different governorates (Qalubia 31, Behera 6, Cairo 8, Assuit 5, Menofia 5). Table (2).

3.2. Incidence of E. coli infection in internal organs

Internal organs from each chicken were bacteriologically examined to reveal the incidence of *E. coli* in different organs. *E. coli* was recovered from different internal organs as the following; 38.2 % from liver, 32.3 %intestine, 17.6 % spleen, 16.6% heart blood, 9.8 % Kidneys and 7.7% lungs. Table (3)

3.3. Serotyping of E. coli isolates recovered from chicken samples

The serological examination of 32 *E. coli* isolates resulted in detection of different serogroups including O_{158}, O_{125}, O_{111}, O_{27}, O_{20}, O_6, O_{25}, O_{26}, O_{145}, and O_{159}, while 12 strains were untyped (Table4).

3.4. Initial screening test for ESBL production

Thirty two *E. coli* isolates were tested by disc diffusion method with various cephalosporins to detect antimicrobial sensitivity. The results showed that the highest rate of resistance was against cefotaxime (43.75%), followed by ceftriaxone (40.62%) and ceftazidime (34.37%) (Table 5).

3.5. Double disc synergy test (DDST) for ESBL production detection:

By double disc synergy test, ESBL were detected in 19 *E.coli* isolates from a total of 32 *E. coli* isolates in a percentage of 59.37% (Figure 1).

3.6. PCR for detection of blaTEM and blaSHV in genomic and plasmid DNA:

PCR was used for detection of two genes that may play an important role in resistance of *E. coli* against cephalosporins in both genomic DNA and Plasmid DNA. These genes were *bla*TEM and *bla*SHV which give PCR products of 516 and 392 bp, respectively.

3.6.1. PCR for detection of blaTEM and blaSHV in genomic DNA:

PCR results showed that *bla*TEMgene was detected in the genomic DNA of 32 tested *E. coli* isolates, while *bla*SHV was detected in the genomic DNA of 12 isolates (Table 6 and Figures 2&3).

3.6.2. PCR for detection of blaTEM and blaSHV in plasmid DNA:

PCR results showed that *bla*TEM gene was detected in plasmid DNA of 18 isolates from 32 tested *E. coli* isolates. while *bla*SHV was detected in plasmid DNA of 9 isolates (Table 6, Figures 4&5).

3.6.3. PCR detection of blaTEM and blaSHV genes in both plasmid and genomic DNA:

Amplification of both genomic and plasmid DNA increase the positivity of detection in comparison with amplification of each of DNAs alone (Table 7).

4. DISCUSSION

Escherichia coli infections are responsible for significant losses in the poultry industry all over the world (Janßen*et al*., 2001). It is one of the most prevalent pathogenic agents causing number of disease syndromes and mortalities in poultry (Janssen *et al*., 2003, Barnes, *et al*., 2008 and Satyajit, et al., 2013). Consequences of Avian Pathogenic *E. coli* infection in poultry include colisepticemia, Coligranuloma, airsaculitis, pericarditis, perihepatitis, omphalitis, cellulitis, salpingitis, swollen head syndrome, reduced growth rate and increased condemnation rate at the abattoirs (Dho-Moulin *et al.,* 1999, Saif 2003, Gomis *et al.,* 2003 and Satyajit et al., 2013). As a result of increased *E. coli* infection, the use of antimicrobial therapy had become an important tool in reducing the incidence and mortality associated with *E. coli* infection in poultry (Blanco et al., 1997 and Hafez 2008).

Wide spread use of broad spectrum beta-lactams as antimicrobial therapy resulted in induction of ESBLs production in *E. coli* which resulted from mutations in the genes for common plasmid mediates beta-lactams specially TEM and SHV resulting in alteration in the enzyme configuration and increased affinity and hydrolytic ability of beta lactamase (Emery and Weymouth, 1997, Chaudhary and Aggarwal, 2004, and Giriyapur*et al.,* 2011). Therefore, the present study was planned for the isolation of *E. coli* from different governorates in Egypt with detection of ESBL producers and detection of two of the genes

involved in ESBL production in both genomic and plasmid DNA.

The results of *E. coli* isolation showed that out of 102 chicken samples, 55 samples were positive for *E. coli* isolation in a percentage 54%. These results agreed with that of Saha et al. (2003) who isolated *E. coli* in a percentage of 58.18% and Hashem et al. (2012) who isolated *E. coli* in a percentage of 54.55%. Higher percentages were reported by El-sukhonetal. (2002) who isolated 88.2%, Abd El.Aziz etal. (2007) who isolated 90% and Barros et al. (2013) who isolated *E. coli* in a percentage of 82.5%. Lower percentages were reported by Barbour et al. (1985) who isolated 45.4%, Gomis et al. (2001) who isolated 34.6% and Roshdy et al. (2012) who isolated *E. coli* in a percentage of 43.1%. The differences in rates of isolation may be attributed to many reasons such as immune status of the bird, vaccination programs used, use of medication and hygienic status

Table (1): Oligonucleotide primers sequences:

Primer	Sequence	Amplified product	Reference
*bla*TEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003
*bla*SHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	

Table (2): Percentages of *E. coli* isolated from the chicken samples in different governorates

Government	Number of samples	Number of positive samples	Percentage of positive samples*
Qalubia	61	31	50.8%
Behera	10	6	60%
Cairo	12	8	66.6%
Assuit	9	5	55.5%
Menofia	10	5	50%
Total (average)	102	55	(53.9%)

* Percentage of positive samples in relation to number of samples collected from each governorate.

Table (3): Incidence of *E. coli* in different internal organs of examined chickens.

Examined organ	Number of Examined organs	Number of Positive	Percentage of Positive *E. coli*%
Liver	102	39	38.2
Lung	102	8	7.7
Spleen	102	18	17.6
Intestine	102	33	32.3
Kidney	102	10	9.8
Heart blood	102	17	16.6
Total (average)	612	125	(20.4)

Table (4): Percentages of different *E.coli* serotypes from 32 randomly selected *E. coli* isolates representing different organs.

	Isolates serogroups										
	E.coli O158	*E.coli O125*	*E.coli O111*	*E.coli O27*	*E.coli O20*	*E.coli O6*	*E.coli O25*	*E.coli O26*	*E.coli O146*	*E.coli O159*	Untyped
No. of isolates	6	5	2	1	1	1	1	1	1	1	12
%	18.7	15.6	6.25	3.1	3.1	3.1	3.1	3.1	3.1	3.1	37.5

Table (5): Results of antimicrobial resistance of *E. coli* against cephalosporin antibiotics by disc diffusion method

Antibiotic	Concentration	Sensitive	Intermediate	Resistant	Percent of total resistance*
Ceftriaxone	30 µg	15	4	13	40.62
Cefotaxime	30 µg	15	3	14	43.75
Ceftazidime	30 µg	17	4	11	34.37

*Percentage of total resistance in relation to 32 *E. coli* isolates subjected to the test

Table (6): The results of PCR of *bla*TEM and *bla*SHV in both chromosomal and plasmid DNA

Serial Number of *E.coli* isolates	Isolates code	Results			
		blaTEM		*blaSHV*	
		Plasmid DNA	Chromosomal DNA	Plasmid DNA	Chromosomal DNA
1	8	-	+	-	+
2	22G	+	+	-	-
3	102	+	+	-	-
4	62	-	+	-	+
5	76	-	+	-	+
6	30	+	+	+	+
7	7G	+	+	+	-
8	79	+	+	-	+
9	82	+	+	-	+
10	83	-	+	-	+
11	2’	+	+	-	-
12	89	-	+	-	-
13	24	-	+	-	+
14	56	+	+	-	-
15	66	+	+	+	+
16	7	-	+	-	-
17	29	+	+	+	+
18	71	+	+	+	-
19	61	+	+	+	-
20	57	+	+	-	-
21	9	+	+	-	-
22	72	+	+	+	-
23	90	+	+	+	-
24	12	+	+	+	-
25	8G	-	+	-	-
26	3’	-	+	-	-
27	85	-	+	-	+
28	91	-	+	-	+
29	25G	-	+	-	-
30	65	-	+	-	-
31	23	+	+	-	-
32	31	-	+	-	-

Table (7): PCR detection of *bla*TEM and *bla*SHV genes in both plasmid and genomic DNA:

Organisms (n=32)	TEM			SHV		
	Plasmid DNA	Genomic DNA	Plasmid+ Genomic DNA	Plasmid DNA	Genomic DNA	Plasmid+ Genomic DNA
Number	18	32	32	9	12	18
Percentage*	56.25	100	100	28.12	37.5	56.25

* Percentage in relation to 32 *E. coli* isolates subjected to PCR.

Figure (1): DDST showing extension of the inhibition zone of cephalosporins toward amoxicillin clavulanic acid disc which indicate positive ESBL production.

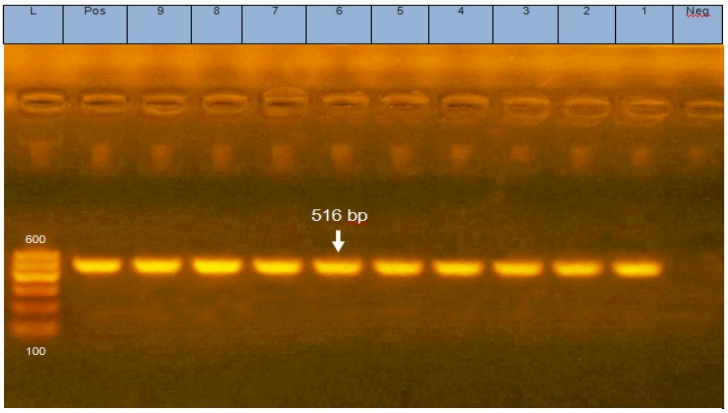

Figure (2): Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect blaTEM gene in genomic DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Post: Positive control Lane: 1, 2, 3, 4, 5, 6, 7, 8 and 9 were positive to *bla*TEM gene

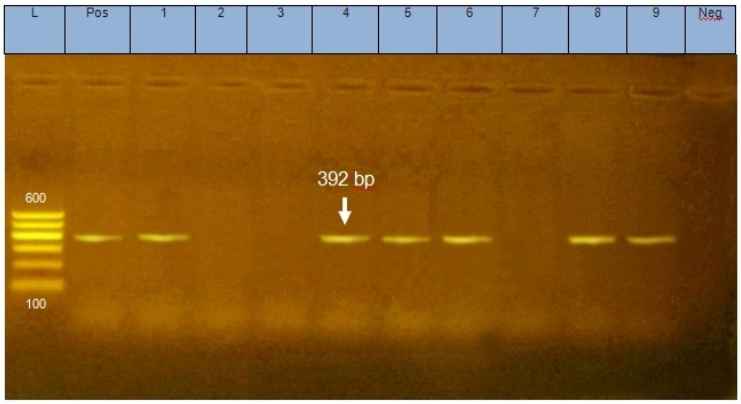

Figure (3): Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect blaSHV gene in genomic DNA. A: Lane L: 100-600 bp DNA ladder. Neg: Negative control, Post: Positive control. Lane: 1, 4, 5, 6, 8 and 9 were positive to *bla*SHV gene. Lane: 2, 3 and 7 were negative to *bla*SHV gene

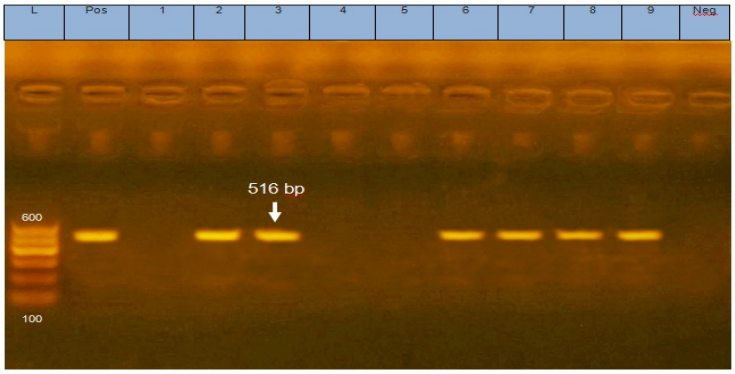

Figure (4): Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect blaTEM gene in Plasmid DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Post: Positive control. Lane: 2, 3,6,7,8 and 9 were positive to *bla*TEM gene. Lane: 1, 4 and 5 were negative to *bla*TEM gene

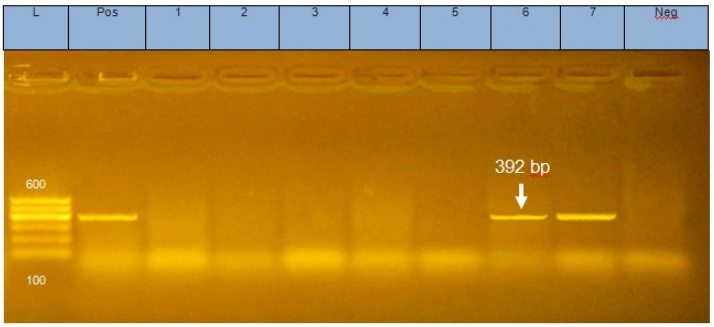

Figure (5): Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect blaSHV gene in Plasmid DNA. Lane L: 100-600 bp DNA ladder. Neg: Negative control, Post: Positive control Lane: 6 and 7 were positive to *bla*SHV gene. Lane: 1,2,3,4 and 5 were negative to *bla*SHV gene

of the farm. The results of *E. coli* isolation from different internal organs revealed that isolation of *E. coli* from liver (38.2 %), intestine (32.3 %), spleen (17.6 %), heart blood (16.6%), Kidneys (9.8 %) and lungs (7.7%) as shown in Table (3) which indicated the involvement of multiple lesions in different organs as liver, lungs, kidneys and heart in *E. coli* infection resulted from acute nature of the disease (Hassanain 1977, Mukhopadhyaya and Mishra 1992, Blanco et al. 1997 and Ewers et al. 2003). Also, higher rates of isolation of *E. coli* from liver are agreed with Ogunleye et al., (2008), sharada et al. (2010) and Roshdy et al. (2012) who isolated higher percentages of *E. coli* from liver. Biochemical identification of different *E. coli* isolates revealed that all isolates exhibit the same biochemical profile including fermentation of different sugars, positive methyl red and indol production tests, inability to utilize citrate in simmons citrate agar nor to breakdown urea in urea hydrolysis test. Negative H_2S production with yellow acid and butt on TSI slant. These reactions are similar to those previously reported by Gomis et al. (2001), Quinn et al. (2002) and Vandekerchove et al. (2004). Systems for classification of *E. coli* by serotyping are based on the O (Somatic antigens), K (capsular) and H (flagellar filament) antigens. (Whitfield and Roberts 1999 and Compos et al., 2004). A lot of *E. coli* serotypes have been reported but not all of them are implicated in poultry diseases (Cloud et al., 1985).

In this study, a total of 32 *E. coli* isolates randomly selected were serologically examined. The results showed that out of 32 *E.coli* isolates, 6 strains were serogrouped as O_{158} (18.7%), 5 strains O_{125} (15.6%), 2 strains O_{111} (6.25%), one strain O_{27}, O_{20}, O_6, O_{25}, O_{26}, O_{145}, O_{159} (3.1% for each serogroup) and 12 strains were untyped (37.5%). Similar *E. coli* serogroupes were isolated in previous studies in Egypt as reported by Rezk et al. (2010), Roshdy et al. (2012), Ashraf et al. (2014), Asmaa (2015) and Ashraf et al. (2015) who isolated different serotypes with prevalence of O_{158}, O_{125}, O_{111}, O_{145}, O_{26}, O_{20} and O_6 serotypes. At the same time, other serotypes were identified in other countries with O_1, O_2 and O_{78} being the most prevalent avian pathogenic serotypes, in addition to other serotypes as O_{36}, O_{153}, O_5, O_8, O_9, O_{11}, O_{12}, O_{14}, O_{15}, O_{17}, O_{18}, O_{35}, O_{36}, O_{45}, O_{53}, O_{81}, O_{83}, O_{88}, O_{102}, O_{103}, O_{115}, O_{116}, O_{132}(Vandemaele et al.,2003 and Kobayashi et al., 2010). These variations indicated that *E. coli* serotypes differed from one country to another which must be taken in the consideration when dealing with bacterin as it must be specific to serotypes prevalent in the locality. The percentage of untyped isolates was 37.5% which is agreed with Asmaa (2015) and Ashraf et al. (2015) who also reported high percentages of untyped strains and this may be attributed to limited range of diagnostic antisera.

Thirty-Two *E. coli* isolates were tested by Disc diffusion method with various cephalosporins to detect antimicrobial sensitivity. The results showed that highest rate of resistance was against cefotaxime (43.75%), followed by ceftriaxone (40.62%) and ceftazidime (34.37%). (Table5). Nearly similar percentage of resistance was observed by Radu etal. (2001) who reported 37% resistance to ceftazidime. Lower percentages of resistance were observed by Randall et al. (2011) who reported 0.6% and 2% resistance to cefotaxime and ceftazidime respectively, Tapan et al. (2012) who reported 22.96% and 8.75% resistance to cefotaxime and ceftriaxone respectively and Ibrahem et al. (2005) who reported 14.3% resistance against cefotaxime. This increase in resistance percentage may be attributed to over use of these antibiotics which resulted in emergence of resistance bacrteria that is capable of overcoming antibiotic by various resistant mechanisms.

Wide spread use of broad spectrum beta-lactams as antimicrobial therapy resulted in induction of ESBLs production in *E. coli*. So, the importance of ESBL detection has increased to prevent dissemination of ESBL producers. Initial screening was done on 32 *E. coli* isolates and isolates showing an inhibition zone size of ≤22 mm with ceftazidime (30 µg), ≤25 mm with ceftriaxone (30 µg) and ≤27 mm with cefotaxime (30 µg) were identified as potential ESBL producers.

By double disc synergy test, ESBL were detected in 19 *E. coli* isolates from a total of 32 *E. coli* isolates in a percentage of 59.37%. This method of detection matched with HO et al., (2000) who reported that sensitivity of DDST can reach 97.9% when the interdisc width is reduced to 20 mm, Vercauteren et al. (1997) who reported 96.9% specificity of DDST. and Garrec et al. (2011) who reported that the combination of routine screening method followed by specific detection test might achieve almost 100% sensitivity for ESBL detection. The percentage of ESBL producing *E. coli* in this study was 59.37% which is close to Giriyapur et al. (2011) who reported that 62.19% of from 82 *E. coli* isolates were ESBL producers. Higher percentages were reported by Bali et al. (2010) reported that 42 (84%) of 50 *E. coli* were confirmed as potentially ESBL producers and Overdevest et al. (2011) found that out of 89 chicken samples, 68 (76.8%) samples contained ESBL-producing *E. coli*. Lower percentages were reported by Drugdová and Kmeť (2013) who reported that extended spectrum β-lactamases (ESBLs) were present in 24 strains (16%) from 151 *E. coli* strains isolated from chickens over a two-year period. These variations in percentages of ESBL production detected may be attributed to difference in using rate of different cephalosporins as medication from locality to another, dissemination of resistance through plasmids and co administration of beta lactamase inhibitors with beta-lactam drugs.

Increased resistance to broad-spectrum β lactams-mediated by extended-spectrum β-lactamases (ESBLs), is a concerning problem worldwide. This resistant to third generation cephalosporins arise from mutations in the genes for common plasmid-mediated β-lactamases, especially Temoniera (TEM) and sulfhydryl variable (SHV) enzymes Colom et al. (2003) and Giriyapur et al. (2011). In this study, two sets of primers for TEM and SHV genes were used for amplification of beta lactamases. PCR amplification of TEM and SHV was carried out on both genomic DNA and Plasmid DNA extracted from 32 *E. coli* isolates. PCR results showed that *bla*TEM gene was detected in the genomic DNA of all serogroups and in 18 serogroups in plasmid DNA. While *bla*SHV gene was detected in the genomic DNA of 12 serogroups and in plasmid DNA of 9 serogroups shown in Table (6). These results were agreed with Altalhi et al. (2010) who detected *bla*TEM gene in all isolates and Colom et al. (2003) who detected *bla*TEM in a percentage higher than *bla*SHV in 51 *E. coli* isolates. Detection of TEM and SHV in ESBL-negative strains that tested susceptible may be attributed to presence of ESBL genes that code for production of low amounts of enzyme or enzyme that has poor hydrolytic activity. These strains are categorized correctly as susceptible (CLSI, 2012).

At the same time, the results showed that amplification of both genomic and plasmid DNA increased the positivity of detection in comparison with amplification of either of DNAs alone as shown in table (7). This increased positivity is matched with results obtained by Sharma et al. (2010) who reported increasing in number of positive *bla*TEM with Plasmid and genomic DNA (13 *E. coli*) isolates as compared with 4 *E. coli* isolates with amplification of plasmid DNA only and 10 *E. coli* isolates with amplification of genomic DNA only and increasing in numbers of positive *bla*SHV with amplification of both plasmid and genomic DNA (12 positive *E. coli* isolates) as compared with 12 positive *E. coli* isolates with amplification of plasmid DNA only and no positive *E. coli* isolates with amplification of genomic DNA only. These results supports that the expression of B-lactamases is controlled by both genomic and plasmid DNA.

Finally, it could be concluded from results in the present study that *E. coli* is one of the most prevalent pathogenic agents in poultry and it is associated with a number of disease conditions with a high percentage of isolation (54%). As a result of the increasing percentage of ESBL production, ESBL detection become very important to prevent dissemination of ESBL producers so monitoring of ESBL by both screening method and double disc synergy test is needed for accurate results. Accordingly, for better understanding of ESBL production, PCR for amplification of TEM and SHV genes in both genomic and plasmid DNA is needed.

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