Molecular characterization of Quinolones and β-Lactams Resistant Salmonella Serovars Determinants in Diarrheic Calves, lambs and goats-kids in the Middle of Nile Delta, Egypt

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ABSTRACT

A total of (236 swabs) fecal samples from El-Menofiya and El-Kalubia Governorates, as sporadic cases of were subjected to bacteriological, biochemical, serotyping, sensitivity testing and PCR detection of resistance genes for β-Lactames and Quinolones. Ten isolates of Salmonella species were identified, as 7 isolates (4.7%) from calves (Salmonella Typhimurium, Salmonella Enteritidis, Salmonella saintpaul, Salmonella Langeveld and Salmonella Havana), 2 isolates (3.6%) from lambs (Salmonella Typhimurium, Salmonella Bardo) and one isolate (3.3%) from goat-kids (Salmonella Enteritidis) Salmonella isolates sensitivity to β-lacatmes was 0.00% for ampicillin, penicillin G, piperacillin, cephalexin, cefoxitin, ampicillin-sulbactam, ceftazidime, 10% for ceftriaxone and 20% for amoxicillin-clavulanic acid, increased to 60% and 100% for aztreonam, imipenem respectively. Susceptibility of isolates to quinolones were 10% for ciprofluxacin, 20% to nalidixic acid, and 100% for each of norfloxacin and levofloxacin. PCR study showed beta-lactamase encoding gene, *bla* (TEM-1), was identified in 90% and the extended-spectrum beta-lactamase, (CIT) in 20% while *bla* (SHV) and acc in 80% of isolates. The detection was 50%, 40%, and 0.00% for FOX, MOX, and *bla* (OXA-1) genes, respectively. Plasmid-mediated quinolone resistance, *qnr*B, *qnr*S were detected in 80% while aac*(6')-Ib-cr*, was only in 50% of the isolates. Results showed a high incidence of β-lacatmase than quinolones resistance genes and higher isolate susceptibility to quinolone than to β-lacatmes which indicates higher efficacy and validity of quinolones. Results indicates quinolones LEV and NOR in addition to β-lacatmes imipenem are the drugs of choice for suspected salmonella cases.

Key words: Diarrhea, Calves, Salmonella, β-lacatmes, Quinolones

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1. INTRODUCTION

Diarrhea is an important cause of morbidity and mortality in young calves (Constable, 2004). Especial attention was poorly undertaken to study the national epidemiology and risk factors of specific etiology of neonatal calf diarrhea in Egypt (El-Khodery and Osman, 2008). Calf salmonella infection can cause a considerable economic loss due to mortality and poor growth of infected animals in addition to risk of causative microorganism transmission to humans either through food chain or direct animal contact (Mohamed *et al.,* 2011). Mortality rates attributable to Salmonella infection are particularly high in young animals, which also generally require the greatest amount of treatment (Hoelzer et al., 2011). The distribution of *Salmonella* serotypes among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestation, and production systems (Animal and Plant Health Inspection Service 2006). *Salmonellosis* is a major cause of human foodborne illness in Egypt and its infections concern is to be increased

considering the emergence and increased prevalence of multiantibiotic resistant strains (Davis et al., 1999). *Salmonella* is a primary etiological agent of infectious diarrhea and represents an important zoonotic pathogen worldwide (Hoelzer *et al.,* 2010). A considerable number of serotypes frequently isolated from humans have been isolated from sick or clinically healthy cattle (Hoelzer et al., 2011). The efficacy of many antimicrobials drugs for treating clinical cases is decreasing as more antimicrobial resistant *Salmonella* subtypes emerge (Angulo et al., 2000 and Winokur et al., 2000). The emergence and spread of antimicrobial resistant *Salmonella* strains, particularly those that are resistant to multiple antimicrobials (multi-drug resistant [MDR] *Salmonella*) is thus a major public health concern (CDC, 2009). In its report at 2013 CDC showed that Drug resistance in Salmonella Typhi has jumped significantly from about 20% in 1999 to more than 70% in 2011 (CDC, 2013). The use of antimicrobial agents for growth promotion and feed efficiency is not well monitored and thus in most cases it undergoes miss-use in Egypt. Farmers normally use antimicrobials to treat sick animals with high doses and using their own experience without veterinary prescription, supervision and laboratory diagnosis. This imposes a selective pressure for the emergence and dissemination of antimicrobial-resistant bacteria including animal and humans pathogens (Van and Stobberingh 1999). There are geographical and time-different regimes of antibiotic use for growth promotion, prophylactic or treatment in different region in Egypt. Given the increasing prevalence of *Salmonella* isolates resistant to antibiotics, necessitates the investigation over different geographical region and continuous periodical search for the prevalence of newly emerged drug-resistant salmonella strains and their MDR determinants. This study aimed to characterize the prevalence, serotypes of Quinolones and β-Lactames resistant salmonella serovars determinants in diarrheic calves, lambs and goat kids in the Middle of Nile Delta, Egypt.

2. MATERIAL AND METHODS

2.1. Samples Collection:

A Total of 236 fecal swabs were collected from diarrheic calves (150 cases), lamb (55cases) goat kids (31cases) as sporadic cases presented in Governmental Veterinary Clinics in El-Menofiya and El-Kalubia Governorates, Middle-Delta, Egypt

2.2. Isolation and Identification of salmonella:

The procedures for isolation of Salmonella from animal faces were according to (ISO-6579: 2002 standard). Swabs were weighed and suspended in buffered peptone water (1:10 dilution) then incubated at 37°C (16-20 hours). Then Buffered peptone water (0.1 ml) was transferred with a pipette into a tube containing (10 ml) of Rappaport-Vassiliadis soy peptone (RVS) broth and incubated at 42°C (20-24 hours). After that, a loopfull of RVS broth was inoculated and streaked separately onto selective agar plates as Xylose Lysine Desoxycholate (XLD) agar , Brilliant Green agar (BGA) , MacConkey's agar and Salmonella-Shigella (S-S) agar plates then incubated at 37°C for 24-48h. Typical colonies of Salmonella on XLD agar were pale pink with black center. On Brilliant Green agar appear as red colonies with a reddening of the media, on Salmonella-Shigella (S-S) agar salmonella colonies appear pale color with or without black centers and on MacConkey's agar appear pale, colorless smooth and transparent. Smears from the suspected *Salmonella* colonies were stained with Gram's stain and microscopically examined. Suspected colonies were identified as Salmonella spp. based on their colony morphology on selective media, and the biochemical testing using TSI agar, Urea agar, L-lysine decarboxylase, Voges Proskauer, Methyl red tests**,** Simmons citrate and Indole tests (Edwards and Ewing, 1986). Also, Salmonella spp. were

confirmed biochemically by using API 20E system (BioMérieux, Marcy-l'Étoile, France). Finally Salmonella isolates were serotyped based on slide agglutination for O and H antigens according to Kauffmann-White (1974) and using the antisera from (Mast Salmonella diagnostic antisera) (UK).

2.3. Antimicrobial sensitivity:

The antimicrobial sensitivity phenotypes of Salmonella were determined by agar disc diffusion method as described by Finegold and Martin (1982), and according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2014), using antibiotic disc from Oxoid (Thermo Fisher Scientific, Inc. (NYSE: TMO, UK). The discs used were ampicillin (AM) (10µg), penicillin G (P) (10µg), piperacillin PRL (100µg), cephalexin CL (30µg), cefoxitin FOX (30µg), ceftazidime CAZ (30 µg), ceftriaxone CRO (30 µg), aztreonam ACM (30 µg), imipenem IPm (10 µg), amoxicillin-clavulanic acid AMC (30 µg), ampicillin-sulbactam SAM (20 µg), nalidixic acid NA (30µg), norfloxacin NOR (10µg), ciprofloxacin CIP (5µg), levofloxacin LEV (5µg) (Oxid,UK).

2.4. Bacterial DNA preparation for PCR:

Bacterial culture DNA was extracted using the (QIAamp DNA extraction mini kit) (QIAGEN. Duesseldorf, Germany, (Egypt branch) according to the mini kit instructions (200 µl) of an overnight bacterial culture was mixed with 200 µl buffer plus 20 µl QIAGEN protease in 1.5 ml tube, mixture was incubated at 56˚C for 10 min, then 200 µl ethanol (96%) were added, vortexing for 15 seconds, applied to the QIAamp mini spin column, centrifugated at 8000 rpm for 1 min, washed two time with washing buffer and DNA was eluted from the column with 150 µl buffer AE elution buffer.

2.5. Amplification of Antibiotic Resistant genes in salmonella serovars:

PCR mixture was prepared according to Emerald Amp GT PCR mastermix (Takara, Co., Japan). Code No. RR310A kit: In PCR tube a 6µl bacterial DNA template, 12.5µl Emerald Amp GT PCR master mix (2x premix),1µl of forward and reverse primers (20 pmol) and 4.5 µl PCR grade water to bring total volume to 25 µl. The amplification condition was primary denaturation cycle at 94˚C for 5 min followed by 35 cycle of 94˚C for 30 sec, 53˚C for 45 sec and 72˚C for 45 sec followed by one final extension cycle at 72˚C for 10 min using the primers sequence and amplification conditions listed in table (1) for quinolone resistance genes and table (2) for β-lactamase.

3. 3. RESULTS

A total of 236 fecal samples from ruminants (150 from calves, 55 from lambs and 31 from goats-kids) were examined for the presence of Salmonella species. Cultivation, isolation and identification procedures identified 10 isolates of Salmonella species as 7 isolates (4.7%) from calves (Salmonella Typhimurium, Salmonella Enteritidis, Salmonella Saintpaul, Salmonella Langeveld and Salmonella Havana) , 2 isolates (3.6%) from lambs (Salmonella Typhimurium, Salmonella Bardo) and one isolate (3.3%) from goat-kids(Salmonella Enteritidis) (Table 3, 4). All Salmonella isolates showed (100%) resistance to ampicillin, pencillin G; cephalexin, cefoxitin, ampicillin-sulbactam, ceftazidime, and 100% sensitivity to imipenem (Table 5).

None of the tested Salmonella isolates were positive for OXA β-lactamase resistance gene, While they have some of other β-lactamase resistance gene (Table 6). All isolates were sensitive to norfloxacin and levofloxacin and negative for *qnr*A gene but positive for some or all other quinolone resistance genes (Table 7, 8).

Table (1) primers used for amplification of quinolone resistance genes

Primer	Sequence	Product size	Reference
*qnr*A	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516 bp	Robicsek *et al*., 2006
*qnr*B	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469 bp	
*qnr*S	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417 bp	
aac(6')-Ib-cr	CCCGCTTTCTCGTAGCA TTAGGCATCACTGCGTCTTC	113 bp	Lunn *et al*., 2010

Table (2) primers used for amplification of β-lactamase

Primer	Sequence	Product size	Reference
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003
bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	
bla_{OXA-1}	TCAACTTTCAAGATCGCA GTGTGTTTAGAATGGTGA	609 bp	
MOX	GCT GCT CAA GGA GCA CAG GAT CAC ATT GAC ATA GGT GTG GTG C	520 bp	Pérez-Pérez and Hanson, 2002
CIT	TGG CCA GAA CTG ACA GGC AAA TTT CTC CTG AAC GTG GCT GGC	462 bp	
Acc	AAC AGC CTC AGC AGC CGG TTA TTC GCC GCA ATC ATC CCT AGC	346 bp	
FOX	AAC ATG GGG TAT CAG GGA GATG CAA AGC GCG TAA CCG GAT TGG	190 bp	

Table (3) Prevalence of Salmonella species in diarrheic calves, lambs and goats kids

Animals species	*Number of cases*	*Salmonella positive cases*	*Percentage*
Calves	150	7	4.7%
Lambs	55	2	3.6%
Goat kids	31	1	3.3%

Table (4) Serotypes of Salmonella isolates from diarrheic calves, lambs and goats kids

Animal species	*Isolate number*	*Serotype*	*Serotype % in all S. isolates from Calves*	*Serotype % in all S. isolates*
Calves	99	*S. Typhimurium*	14.3%	20 %
	103	*S. Enteritidis*	14.3%	20 %
	62	*S. Saintpaul*		
	73	*S. Saintpaul*	28.6%	20 %
	67	*S.Langeveld*		
	96	*S. Langeveld*	28.6%	20 %
	133	*S. Havana*	14.3%	10 %
			Serotype % in all S. isolates. from Ovine	
Lambs	10	*S. Typhimurium*	33%	
	27	*S. Bardo*	33%	10 %
Goats kids	25	*S. Enteritidis*	33%	

Table (5) Sensitivity of the Salmonella Serotypes isolated from diarrheic calves, lambs and goats kids to β-Lactams

S. Serovars	Zone of inhibition due to Antibiotic discs in each Salmonella Serovars										
	Am	P	PRL	CL	FOX	AMC	SAM	CAZ	CRO	ATM	IPM
S. Typhimurium	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(21) I	(25)S	(30)S
S. Enteritidis	(9)R	(-)R	(18)I	(8)R	(-)R	(19) S	(-)R	(-)R	(24)S	(26)S	(32)S
S. Saintpaul	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(27)S
S. Saintpaul	(-)R	(-)R	(-)R	(-)R	(-)R	(10) R	(-)R	(-)R	(-)R	(-)R	(30)S
S.Langeveld	(-)R	(-)R	(-)R	(-)R	(-)R	(15) R	(-)R	(-)R	(-)R	(-)R	(30)S
S. Langeveld	(-)R	(-)R	(18) I	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(30)S
S. Havana	(-)R	(-)R	(-)R	(-)R	(-)R	(9)R	(-)R	(-)R	(20)I	(22)S	(27)S
S. Typhimurium	(-)R	(-)R	(-)R	(-)R	(-)R	(11) R	(-)R	(-)R	(17)R	(21)S	(30)S
S. Bardo	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(-)R	(22)I	(24)S	(29)S
S. Enteritidis	(6)R	(-)R	(17) R	(6)R	(-)R	(19) S	(-)R	(7)R	(21)I	(25)S	(30)S
Number of resistant serovars	10	10	8	10	10	8	10	10	5	4	0.00
Percentage of resistant Serovars	100 %	100 %	80%	100 %	100%	80%	100%	100 %	50%	40%	0.00

-Am, ampicillin; P, pencillin G; PRL, piperacillin; CL, cephalexin; FOX, cefoxitin; AMC, amoxicillin-clavulanic acid; SAM, ampicillin- sulbactam; CAZ, ceftazidime; CRO, ceftriaxone; ATM, aztreonam; IPM, imipenem. -Number in brackets: size of inhibition zone with mm according to (CLSI 2014). -R: resistant, S: sensitive, I: intermediate.

Table (6) Incidence of B-Lactamase genes in Salmonella isolates detected by PCR

S. Serovars	Genes						
	bla_{TEM}	bla_{SHV}	bla_{OXA-1}	MOX	CIT	Acc	FOX
S. Typhimurium	+	-	-	-	-	+	-
S. Enteritidis	+	+	-	+	-	+	+
S. Saintpaul	+	+	-	-	+	-	+
S. Saintpaul	+	+	-	-	-	+	-
S.Langeveld	+	+	-	-	-	+	-
S. Langeveld	+	+	-	-	-	+	-
S. Havana	+	+	-	+	+	-	+
S. Typhimurium	-	-	-	-	-	+	-
S. Bardo	+	+	-	+	-	+	+
S. Enteritidis	+	+	-	+	-	+	+

Table (7) Sensitivity of the Salmonella Serotypes isolated from diarrheic calves, lambs and goat's kids to quinolones

S. Serovars	Antibiotic discs			
	NA	NOR	CIP	LEV
S. Typhimurium	(-) R	(31)S	(35)S	(30)S
S. Enteritidis	(7) R	(21)S	(25)I	(23)S
S. Saintpaul	(20) S	(25)S	(30)I	(28)S
S. Saintpaul	(-) R	(25)S	(23)I	(20)S
S.Langeveld	(-) R	(22)S	(22)I	(20)S
S. Langeveld	(19) S	(27)S	(24)I	(20)S
S. Havana	(-) R	(22)S	(20)R	(18)S
S. Typhimurium	(17) I	(21)S	(21)I	(19)S
S. Bardo	(-)R	(22)S	(26)I	(24)S
S. Enteritidis	(-)R	(24)S	(27)I	(24)S
Number of resistant serovars	7	0.00	1	0-00
Percentage of resistant Serovars	70%	0.00	10%	0.00

-NA, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; LEV, levofloxacin. -Number in brackets: size of inhibition zone with mm. according to (CLSI 2014). -R: resistant, S: sensitive, I: intermediate

Table (8) Incidence of quinolone resistance genes in Salmonella isolates detected by PCR

S. Serovars	Genes			
	*qnr*A	*qnr*B	*qnr*S	*aac(6') -Ib-cr*
S. Typhimurium	-	+	+	+
S. Enteritidis	-	-	+	-
S. Saintpaul	-	+	+	+
S. Saintpaul	-	+	+	+
S.Langeveld	-	+	+	+
S. Langeveld	-	-	+	+
S. Havana	-	-	-	-
S. Typhimurium	-	+	-	-
S. Bardo	-	+	+	-
S. Enteritidis	-	+	+	-

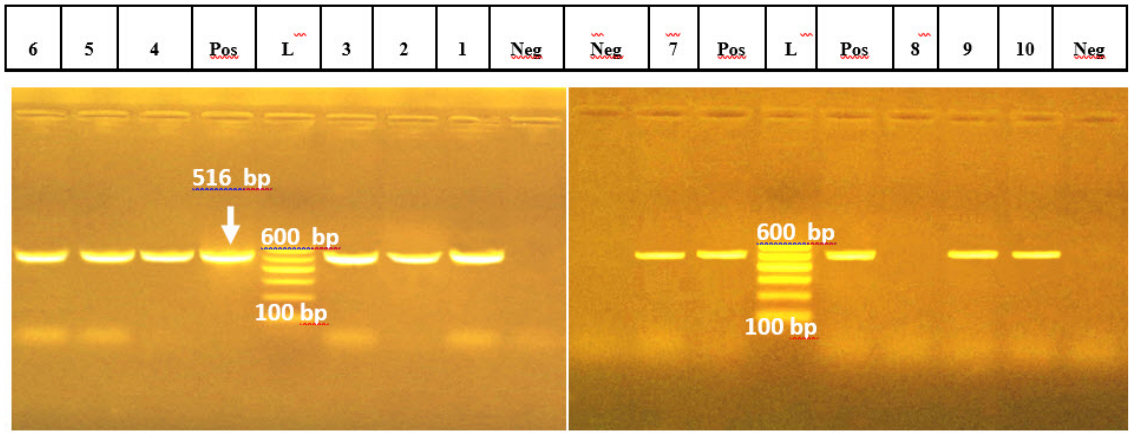
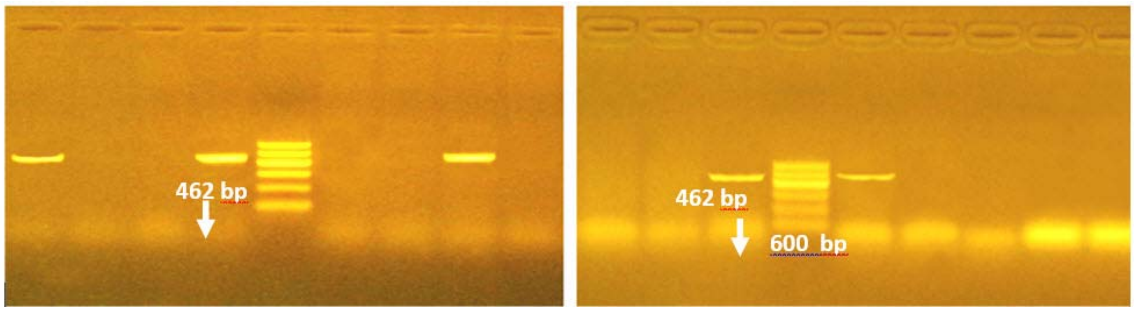
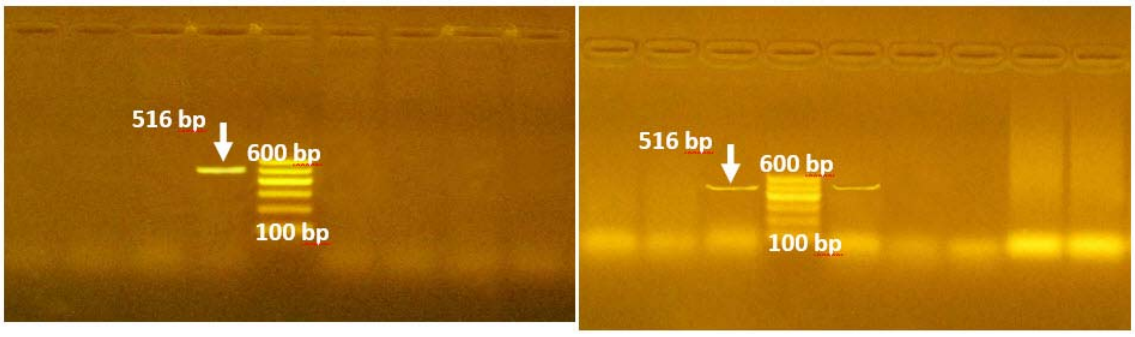


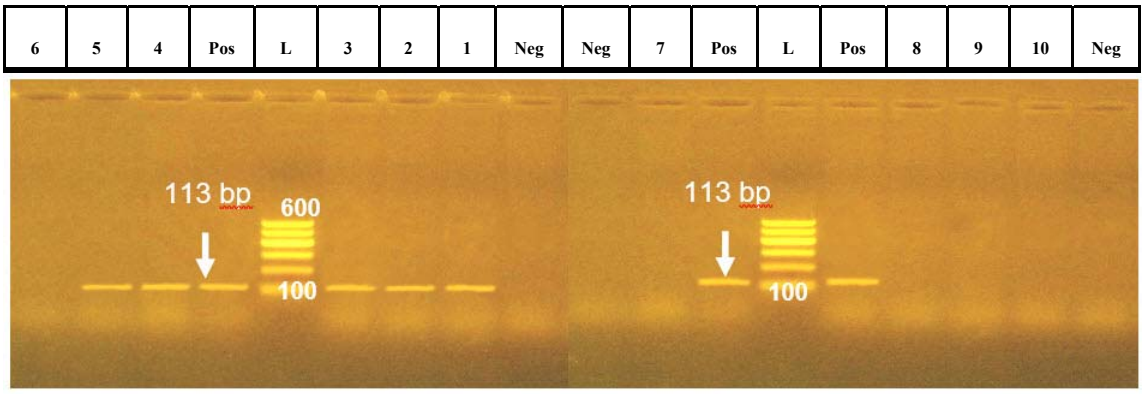
Figure (1):PCR detection of one of the β-Lactamase genes (*bla*$_{TEM}$) in different Salmonella serovars (Amplicons of *bla*$_{TEM}$ gene detected in 1, 2, 3, 4, 5, 6, 7, 9 and 10 at 516 bp)



Figure(2): PCR detection of one of B-lactamase gene (CIT) in different salmonella isolates. (Amplicons of CIT gene detected in 1 and 6 at 462 bp). L (DNA ladder), Neg (Negative control), Pos (Positive control), (1-7) Salmonella isolates from Diarrheic calves: 1,3 (*S. Saintpaul),* 2,4 (*S. Langeveld)) ,5 (S. Typhimurium)* ,6 (*S. Havana*), 7 (*S. Enteritidis),* (8, 10) Salmonella isolates from diarrheic lambs :*8 (S. Typhimurium), 10 (S. Bardo), 9 (S. Enteritidis)* from diarrheic goats kids.



Figure(3):PCR detection of one quinolone resistance genes (*qnr*A) in different salmonella isolates (all isolates negative for this gene).



Figure(4): PCR detection of one of the quinolone resistance gene (*aac6-Ib-cr*) in different Salmonella isolates (Amplicons of *aac(6') -Ib-cr* gene detected in 1, 2, 4 and 5 at 113 bp). L (DNA ladder), Neg (Negative control), Pos (Positive control), (1-7) Salmonella isolates from Diarrheic calves: 1,3 (*S. Saintpaul),* 2,4 (*S. Langeveld)) ,5 (S. Typhimurium)* ,6 (*S. Havana*), 7 (*S. Enteritidis),* (8, 10) Salmonella isolates from diarrheic lambs :*8 (S. Typhimurium), 10 (S. Bardo), 9 (S. Enteritidis)* from diarrheic goats kids.

4. DISCUSSION

Salmonella causes substantial economic loss resulting from mortality, morbidity and poor growth with hazard of transmitting food poisoning with gastroenteritis to human and represents a serious problem for the food industry (Khan et al., 2007). Salmonella infections continue to be a challenge in Egypt and it is thus important to study the resistance mechanisms to antimicrobial agents in species of this genus and their clinical impact in human and animals. The two main therapeutic alternatives against these organisms are both fluoroquinolones and new β-lactams, for their bactericidal activity and their excellent pharmacokinetic properties (Piddock 2002). This study aimed to study the prevalence of salmonella species in the sporadic cases of diarrheic calves, lambs and goat kids in the middle of Egypt and to identify their serotypes in addition to determination of their sensitivity or resistant to β-lactames and Quinolones and to characterizes the determinant of their resistance to these antibiotics.

The results showed the prevalence of salmonella species in sporadic cases of diarrheic calves is about 4.7% while its prevalence is 3.6% and 3.3% in Lamb and goats kids respectively. The result in diarrheic calves is somewhat higher than a previous results showing salmonella prevalence in Egypt a 4.09% (Younis et al 2009). The higher prevalence encountered in our study may be due to time or regional differences depending on the antibiotics regimes used. As the administration of therapeutic and sub-therapeutic antimicrobials to animals may determine the emergence and dissemination of antibiotic-resistant bacteria (Piddock 2000). Salmonella prevalence in lambs and goat kids in this study differs from that reported from a study conducted on lambs in Behera, Province, Egypt that was 5.26% (Mohammed et al., 2014). This difference may be regional dependent according to environmental condition and antibiotics regimes used in that region. Salmonella was also isolated in higher rate 15% of lamb diarrhea cases (Ahmed et al. (2010). This may be related to the relative small number of cases collected as multiple fecal samples are required for higher rates of salmonella isolation (Duijkeren et al., 1995).

Serotyping of salmonella isolates showed calves diarrhea was mostly caused by *S. Saintpaul* 28.6% and S. Langeveld 28.6% while the percentage was only 14.3% for *S. Typhimurium*, *S. Entritidis* and *S. Havana*.

This prevalence of salmonella serotypes that caused calves diarrhea is completely differs than that was found in diarrheic calves in other region in Egypt that was mainly *S. Typhimurium*, *S. Enteritidis* (Younis et al., 2009). This may be explained by the different antibiotics regimes in different region. Indeed, selective pressure favors the emergence of antimicrobial resistance pathogens such as Salmonella, which is frequently harbored in the animal intestinal tract (Aarestrup, 2000). This may necessitate different antibacterial systems in different region to treat salmonella infection based on screening of the isolates in each specified region.

Susceptibility of different salmonella to members of β-lactames antibiotics showed that most isolates are resistant to all β-lactames except 10%, 60% and 100% of the isolates were sensitive to ceftriaxone, aztreonam and Imipenem respectively. The wide range of resistant salmonella isolates to β-lactames in this study corresponds well to the diversity of β-lactamase genes detected by PCR in these isolates. The greatest variation in β-lactames resistance and β-lactamase genes was seen among diagnostic *E. coli* isolates from cattle and pigs, which according to DANMAP 2002 also are the animal groups that are treated with the most different β-lactames antibiotics (DANMAP 2003). However, the bla_{TEM-1} gene was detected in all isolates in current study while it was detected in about 81% in Denmark and 77% in Spain (Olesen et al., 2004). In this study the high rate of resistant isolates and high expression of the bla_{TEM-1} gene may reflect the uncontrolled and misuse of the β-lactames in Egypt that has been used in the past period and their invalidity for combating salmonella at least in the regions of sampling. Majority of isolates expressed bla_{SHV} and Acc genes whereas none of them produced bla_{OXA} β-lactamases, and few 20%, 40% and 50% produced CIT, MOX and FOX β-lactamases respectively. This may explain the sensitivity of some isolates to β-lactamases genes.

Mechanisms of resistance to quinolones in Salmonella include target gene mutations, active efflux, and decreased outer membrane permeability (Axel and Elisabeth, 2001). Quinolone resistant in Egyptian typhoid fever patient has been reported to be rising from 0.00% at 1993 (Abdel-Ghafar et al.,1994) to 15% in 2013 (Fatma et al.,2014) . In this study on diarrheic calves, lambs and goats kids we found 70% of the isolates were resistant to NA and 10% were resistant to CIP while 0.0 % were resistant to both LEV and NOR. In contrary to our results, it was found that 7.5% of salmonella isolates from Egyptian typhoid fever patient were resistant to both NAL and LEV, and 2.5% were resistant to all three quinolones NAL, NOR, and LEV (Fatma et al., 2014). This discrepancy could be species and/or regional dependent. It is worse mentioning here that the susceptibility of the isolates to CIP was 90% meanwhile it was only 30% to NA which indicates the difference between the two antibiotics efficacy. This may contradict the assumption considering nalidixic acid resistance as an indicator for reduced susceptibility to ciprofloxacin (Rahman et al., 2014). The absence of the *qnr*A and *aac(6') -Ib-cr* gene from 100% and 50% of the isolates respectively may be the key that renders all isolates susceptible to NA and NOR while the presence of the *qnr*B and *qnr*S in the majority of the isolates may be the cause of 70% and 10% of the isolates being resistant to NA and CIP respectively. However, the susceptibility of all isolates to quinolone is higher than β-lactames which make quinolone efficacy and validity for treatment of Salmonella infection is more acceptable than β-lactames in the region of sampling of this study.

5. CONCLUSION

Overall susceptibility of the isolates is higher to quinolone than to β-lactames.

This make quinolones efficacy and validity for treatment of Salmonella infection is more acceptable than β-lactames especially in the region of sampling of this study. These results also indicate quinolones especially NOR and LEV in addition to the β-lacatmes imipenem are drugs of choice for suspected salmonella cases a note to be considered by Veterinarians and animal breeder in the region of our study.

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