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Risk Factors, Antibiotic Profile, and Molecular Detection of Virulence and Antibiotic Resistance Genes of Enteric Bacteria in Diarrheic Calves in Egypt

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ABSTRACT

Diarrhea caused by different bacterial infections in calves is a serious issue. In the present study, 215 diarrheic animals, including 175 buffalo and 40 cattle calves were examined. The incidence varied amongst farms, ranging from 0 to 27.9%. Among the affected calves, 37.2% were 1 to 7 days old, 55.8% were 7 days to 3 months of age, while 6.9% were more than 3 months old. Bacteriological, antibiogram, and PCR-based detection of specific virulence and antimicrobial resistance genes were performed. *E. coli* (85.5%), *C. perfringens* (8.8%), and *Salmonella* (3.7%) were bacterial infections recovered from affected calves. Various *E. coli* pathotypes, such as Shiga-toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), extraintestinal *E. coli* (ExPEC), and enterotoxigenic *E. coli* (ETEC), caused diarrhea in these calves. The most prevalent virulence genes in *E. coli* were the *sta* and *eaeA* genes (60%). All *Salmonella* strains were found positive for the *inv*A gene, while all *C. perfringens* strains were tested positive for the *cpa* gene. Most of the identified strains were resistant to clindamycin, erythromycin, and oxytetracycline. The isolated strains harbored bla_{TEM}, *qnr*A and *tet*A. Approximately 96.2% of *E. coli* and 62.5% of *Salmonella* isolates were MDR to different antimicrobial classes. Moreover, 10.5% of *C. perfringens* isolates were extensive drug-resistant (XDR) to seven antimicrobial classes, while 84.2% of them were MDR to various antimicrobial classes. The findings of this study provide a better understanding regarding the epidemiological aspects of bacterial illnesses and for the development of prevention techniques for this problem. The antibiograms recorded in this study highlight the dangers of indiscriminate antibiotic usage in diarrheic calves.

Key words: Diarrhea, Calves, MDR, *E.coli*, *Salmonella*, *C. perfringens.*

INTRODUCTION

Calf diarrhea is a multifactorial disease with significant economic consequences in dairy and beef herds. This is one of the most serious diseases worldwide affecting newborn and young calves <1-month old (Wei et al. 2021). In Egypt, cattle and buffaloes are the major source of meat, milk and milk products. Calves of these species have a compromised immune system, rendering them more susceptible to infection (Sobhy et al. 2020). It has been estimated that acute diarrhea in the pre-weaning period causes 75% of early calf mortality in dairy herds (Uhde et al. 2008). More than half of all cases of diarrhea in newborn calves are still caused by bacterial infections (Malik et al. 2012). During the first two months of post-natal period, the most common pathogens in calves were observed to be *Salmonella* species and *E. coli* (Acha et al. 2004). Throughout the first three weeks of a calf's life, *E. coli* is routinely isolated from the intestinal material (Malik et al. 2012; Mandouh et al. 2020).

E. coli is a rod-shaped, gram-negative, motile, non-sporulating and facultatively anaerobic microorganism that belongs to the Enterobacteriaceae family. Shiga toxin-producing *Escherichia coli* (STEC) Enterotoxigenic *Escherichia coli* (ETEC) and Enterohemorrhagic *Escherichia coli* (EHEC) are disease-causing *E. coli* strains that can cause diarrhea (Mohammed et al. 2019). In ETEC, there are two types of virulence factors, enterotoxins and colonization, with enterotoxins being the most common in developed countries (Rojas-Lopez et al.

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2018). Enterotoxins are classified into two types: heat-stable (*ST*) and heat-labile (*LT*) (Arenas-Hernandez et al. 2012). *Stx1* and *Stx2* can prevent protein synthesis, inducing apoptosis (Melton-Celsa 2012). Even though it does not yield enterotoxins or Shiga toxin, an intimin protein encoded by the *eae*A gene of EPEC can form A/E lesions in intestinal cells (Shahrani et al. 2014). Many *Escherichia coli* strains contain the *astA* gene, which codes for the enteroaggregative *E. coli heat-stable enterotoxin* (*EAST1*) (Maluta et al. 2017). Though the aerobactin receptor (*Iut*A protein) is an important virulence factor in extra-intestinal infections, little research has been carried out as a protective antigen (Tokano et al. 2008).

Salmonellae are gram-negative rod-shaped facultative anaerobic bacteria in the Enterobacteriaceae, which cause infections accompanied by various clinical symptoms such as diarrhea in humans and animals, septicemia, extra-intestinal localized infections affecting multiple organs, and even death in severe cases (Berge et al. 2008; Huang et al. 2018). The *inv*A is considered a gold standard gene in the genetic diagnosis of *Salmonella* species because it is only found in *Salmonella* species (O'Regan et al. 2008). The *Omp*A gene is essential in the ability of *Salmonella* to adapt to environmental stress (Krishnan and Prasadarao 2012). *Salmonella* enterotoxin *stn* causes gastroenteritis with severe symptoms (Huehn et al. 2010).

Clostridium is a gram-positive rod that is motile, spore-forming, and fermentative that appears as normal commensals in the gastrointestinal tracts of several animals and humans (Brynestad and Granum 2002). Calf enterotoxemia caused primarily by *C. perfringens* is distinguished by a high mortality rate, sudden death, and hemorrhagic enteritis lesions in the small intestine. *C. perfringens* species are classified into five types (A, B, C, D, E), depending on toxins production: alpha (α), beta (β), epsilon (€) and iota (I), which have been linked to the disease (Bendary et al. 2022). The plasmid-mediated *cpa* gene encodes the alpha-toxin, which is found in all *C. perfringens* serotypes, and enterotoxin can be produced by any serotype with the *cpe* gene (CPE) (Bendary et al. 2022).

MDR patterns have grown globally in the recent decade. Pathogenic microorganisms are frequently linked to antimicrobial resistance, which may result from widespread and inappropriate antibiotic use. More specifically, MDR has been linked to numerous genes, including *bla*-$_{TEM}$ (-lactamase genes), *tet*A (tetracycline resistance gene), and *qnr*A (Fluoroquinolone resistance gene) (Algammal et al. 2020b; Algammal et al. 2022).

The current study was conducted to detect the prevalence of *E. coli*, *Salmonella* and *C. perfringens* in calf diarrhea, with a focus on *stx1*, *stx2*, *sta*, *iut*A and *ast*A, that control their pathogenesis and virulence genes; *eae*A, *Salmonella* virulence genes (*inv*A, *omp*A, and *stn*) and *C. perfringens* alpha and enterotoxins (*cpa* and *cpe*). Furthermore, antibiogram and antimicrobial resistance genes such as *qnr*A, *tet*A, and *bla*-$_{TEM}$ were screened to select the antibiotics of choice against these isolates.

MATERIALS AND METHODS

Ethics statement

Experienced veterinarians in the Faculty of Veterinary Medicine, Suez Canal University, Egypt, collected the rectal swabs without anesthesia or pain relief, following standard protocol. After obtaining the owners' permission, the rectal swabs were transported to the laboratory for diagnosis.

Sampling protocol

Dairy farms (F1 to F5) in Sharkia, Egypt, were selected for this study. These farms were organized enough to collect study data and samples. At the selected farms, pregnant dams were not vaccinated against the clostridial disease before calving. A total of 215 diarrheic buffalo and cattle calves, aged <1 week to one year were selected. On the basis of their age, experimental calves were divided into three groups: less than one week (n=80), one week to three months (n=120) and 3-12 months (n=15). The observed clinical signs were diarrhea, ranging from mild to profuse watery feces; its color varied from whitish-yellow to greenish and, sometimes, tinged with blood or mucous. Sometimes the calved suffered from dehydration, weakness, standing inability, and rise in body temperature. The calves (buffalo-cattle) that did not meet these criteria were considered healthy or non-diarrheic and were not included in the study. There were 175 and 40 rectal swabs taken from diarrheic buffalo and cow calves, respectively. The sampling was collected on four different times. All samples were taken before the administration of any antibiotics.

Bacterial examination

For the isolation of *E. coli* and *Salmonella*, samples were streaked on MacConkey agar (Merck, Germany) and incubated at 37°C for 24 hours to distinguish lactose fermenters from non-lactose fermenters for bacteriological examination. One colony of each lactose fermenter sample was suspended in sterile distilled water, streaked on eosin methylene blue agar (Merck, Germany) and incubated at 37°C. Non-lactose fermenters were streaked on the XLD surface (xylose-lysine-deoxycholate agar). Bacterial identification was accomplished using biochemical tests (Mahon et al. 2015). A cooked meat medium (Becton, Dickinson and Company, USA) was used to isolate *C. perfringens* anaerobically at 37°C for 24 hours in an anaerobic jar with GasPak^{TM} (Oxoid Limited, Thermo Fisher Scientific Inc., UK). Then these were streaked on the surface of 5-10% sheep blood agar that contained 200g/mL neomycin (Koneman et al. 1992).

Antibiogram test

All isolates were examined against 13 antimicrobial agents of nine different classes by the disk-diffusion protocol suggested by Clinical & Laboratory Standards Institute (CLSI). Interpretation of results was also made following CLSI guidelines. The tested isolates were divided into multiple drug resistant (MDR) and extensive drug resistant (XDR) categories, as described previously (Magiorakos *et al.* 2012). Multiple antibiotic resistance index (MARI) was computed following the method described earlier by Krumperman (1983).

Virulence and antimicrobial resistance genes factors

Five isolated representative strains were used for extraction. Centrifugation was used to pellet bacteria grown overnight in 2mL of trypticase-soy broth. The

QIAamp DNA Mini Kit was used for extraction of bacterial DNA from purified bacterial cells (Invitrogen, USA). A Nanodrop (Nanodrop 1000, Thermo Scientific, Loughborough, UK) was used to measure the amount of DNA templates recovered.

To detect the virulence and antimicrobial resistance genes in the *E. coli*, *Salmonella* and *C. perfringens,* representative strains were obtained, and PCR was carried out with appropriate primer sets from Metabin, Germany. Shiga-toxins genes, *stx*1 and *stx*2 (Dhanashree and Mallya 2008), enterotoxins genes, *sta* (Lee et al. 2008), ExPEC, *iut*A (Yaguchi et al. 2007) and *E. coli* heat-stable enterotoxin, *ast*A (Piva et al. 2003) that regulate their pathogenesis, and the virulence genes; *eae*A (Bisi-Johnson et al. 2011), *Salmonella* virulence genes including invA (Oliveira et al. 2003), *omp*A (Kataria et al. 2013) and *stn* (Murugkar et al. 2003,) and *C. perfringens* alpha-toxin, *cpa* (Yoo et al. 1997) and enterotoxins, *cpe* (Kaneko et al. 2011) were studied. Furthermore, antibiogram and antimicrobial resistance genes were inline with previous findings: such as *qnr*A, fluoroquinolone-resistance gene (Robicsek et al. 2006), *tet*A, tetracycline-resistance gene (Randall et al. 2004), and *bla*-$_{TEM}$, extended-lactamase gene (Colom et al. 2003). Go Taq®Green Master Mix 2X (Promega, Wisconsin, USA), 12.5µL of each primer, 5µL of DNA extract, and PCR-grade water to 25µL make up PCR reaction. Negative controls (no DNA template) and positive controls (previously isolated) were provided by AHRI, Dokki, Egypt. A 1.5% agarose gel electrophoresis (Applichem GmbH, Darmstadt, Germany) was used to screen amplified fragments. A 100-bp ladder (Thermo Scientific, Germany) was applied for the experimentation.

Statistical analysis

Potential risk factors related to isolated strains: frequency according to age of calves, species, and sampling season, as well as the total number of collected samples (n=215), were determined. The Chi-square test was applied for analyzing data and testing the null hypothesis for various antibiotics. All analyses were conducted using SPSS® (version 25, United States) software.

RESULTS

Two hundred fifteen calves were reared at five farms throughout the survey period. Almost all cases occurred during one of the four seasons, and they all showed typical symptoms of diarrhea. Combining the data for both species, the incidence varied from 0 to 27.9% among farms. The farms were classified as "high incidence farms", which included F3 (n=60) and F4 (n=60) (Table 1). There was statistically significant difference among farms, and there was also significant difference among farms with buffalo and cattle calves. The incidence of diarrhea varied from 7.4 to 62.7% during different seasons (Table 2). The highest prevalence of diarrhea was recorded in winter (62.79%), while the lowest (7.4%) was in summer. There is a statistically significant difference among four seasons for buffalo and cattle calves (Table 2).

Occurrence of bacterial infections among calves

Fecal samples were collected from 175 buffalo and 40 cattle calves showing signs of diarrhea. The occurrence of diarrhea differed significantly between cattle and buffalo calves and among age groups of calves (Table 3).

Based on microscopy, colonial characteristics on MacConkey's and eosin-methylene-blue agar, and biochemical assays, the prevalence of *E. coli* was found to be 88.8% in diarrheic buffalo calves feces swabs (Table 3). There were statistically significant differences in the prevalence of *E. coli* between buffalo and cattle and calves of three age groups.

Based on microscopy, colonial characteristics on MacConkey's and XLD agar, and biochemical tests, the overall prevalence of *Salmonella* was 3.7%. However, there was significant difference in the prevalence of *Salmonella* between buffalo and cattle or among three age groups.

Based on microscopy, colony characteristics, and biochemical testing, the overall prevalence of *C. perfringens* was 8.8% (Table 3). There were statistically significant differences in the prevalence of *C. perfringens* between buffalo and cattle calves and among different age groups.

Table 1: Prevalence of calves with diarrhea and number of samples from each farm

Farm	No. of calves with diarrhea (%)	Buffalo No. (%)	Cattle No. (%)
Farm 1	40(18.6)	35(20)	5(12.5)
Farm 2	25(11.6)	25(14.3)	0(0)
Farm 3	60(27.9)	40(22.9)	20(50)
Farm 4	60(27.9)	50(28.6)	10(25)
Farm 5	30(13.9)	25(14.3)	5(12.5)
Total	215	175	40
Chi-square	25.116	12.857	28.75
P-value	P<0.001	P<0.05	P<0.001

Table 2: Prevalence of calves with diarrhea among different seasons

Season	Buffalo calf	Cow-calf	Total	%
Winter	110	25	135	62.70
Spring	30	3	33	15.34
Summer	15	1	16	7.40
Autumn	20	11	33	14.40
Chi square	136.43	35.60		
P value	P<0.01	P<0.01		

Antimicrobial resistance profiles and genes of isolates

The bacterial isolates were subjected to antimicrobial susceptibility testing (Table 4). Results revealed that all tested isolates exhibited statistically significant (P<0.0001) difference in their resistance to various antimicrobial drugs. The isolated strains of bacteria that harbour antibiotic resistance genes are described as follows: 5/5(100%) of the tested *E. coli* strains harbored the *tet*A, 4/5(80%) *bla*$_{TEM}$ and 3/5(60%) harbored *qnr*A resistance gene. Moreover, 4/5(80%) of the tested *Salmonella* strains harbored the *qnr*A and *bla*$_{TEM}$, and 3/5(60%) harbored *tet*A resistance gene. There was a non-significant difference in isolated strains among antimicrobial resistance genes.

Virulence genes of isolates

Regarding the virulence genes among the isolated strains, for *E. coli*, genes encoding virulence to

enterotoxins associated with toxigenic ETEC (*st*, 60%; 3/5), genes encoding virulence to enterotoxins associated with *shiga* toxin STEC (*stx1*, 20%; 1/5 and *stx2*, 20%; 1/5), gene that encodes the EAST-1 toxin associated with diarrheagenic EAEC (*ast*A, 40%; 2/5), gene encoding outer membrane protein called intimin with pathogenic EPEC (*eaeA*, 60%; 3/5) and gene encoding the aerobactin siderophore receptor associated with extraintestinal pathogenic ExPEC (*iut*A, 60%; 3/5) were recorded. The distribution of *E. coli* pathotypes showed that ETEC (*sta*) ExPEC (*iut*A) and EPEC (*eae*A) was the highest pathotype among recovered *E. coli* strains with 60% value. For *Salmonella,* genes encoding virulence to outer membrane protein (*omp*A, 60%; 3/5), gene that codes for protein in the inner bacterial membrane (*inv*A, 100%; 5/5) and *Salmonella* enterotoxin (*stn*, 80%; 4/5) were observed. For *C. perfringens*, genes encoding alpha toxin (*cpa*, 100%; 5/5) and enterotoxin (*cpe*, 60%; 3/5) were recorded. There was non-significant difference in isolated strains among virulence genes.

Resistance profiles of isolated strains

Approximately 96.2% of *E. coli* isolates were MDR to numerous antimicrobial agents, 62.5% of *Salmonella* isolates were MDR to different antimicrobial classes, and 10.5% of *C. perfringens* were extensive drug-resistant (XDR) to seven antimicrobial classes, and 84.2% of *C. perfringens* were MDR to various antimicrobial classes (Table 5).

DISCUSSION

Neonatal calf diarrhea has a significant influence on the global economic sustainability of cowherds. This study intended to investigate the prevalence of diarrhea in neonatal calves of buffaloes and cows. Results of the present research on 215 diarrheic neonatal calves showed variable degrees of diarrhea. The prevalence rate of diarrhea was higher in buffalo calves (81.3%) than in cow calves (18.6%). However, Malik et al. (2012) reported that the overall prevalence of calf diarrhea was 53.66%.

The highest incidence of diarrhea was observed on Farm 3 and Farm 4 (27.9% each). On the other hand, the lowest incidence of 11.6% was observed in Farm 2. This seems to be due to different hygienic management conditions associated with seasonal variations on each farm. The incidence of neonatal calf diarrhea was higher during the winter season. It may be due to lower ambient temperature and higher humidity, which support the survival of infectious agents for extended periods. Similar findings were obtained by El-Naker et al. (2008**),** who reported a higher prevalence of calf diarrhea in winter (40%). Concerning the age of examined calves, the highest prevalence was observed in the 2nd age group (55.8%), while the lowest rate was in the 4th age group (6.9%), which is supported by the findings of Sobhy et al. (2020). However, El-Naker et al. (2008**)** reported that the highest rate of diarrhea was observed in the 1st week of age. The differences recorded in the prevalence rate recorded in different studies might be related to sanitation and area management variations.

Table 3: Prevalence of *E. coli*, *Salmonella,* and *C. perfringens* among animal species and different age groups

Animal	*E. coli*	*Salmonella*	*C. perfringens*
Overall prevalence among the two animals			
Buffalo (Number)	167	6	16
Prevalence (%)	88.8	75	84.3
Cattle (Number)	21	2	3
Prevalence (%)	11.1	25	15.7
Chi square	107.46	2	8.8947
P-value	P<0.001	0.1573^{NS}	P<0.01
Overall prevalence among different age categories			
1-7 days	78	0	3
Prevalence (%)	41.4	0	15.7
7 days to 3 months	100	8	14
Prevalence (%)	53.1	100	73.7
> 3 months	10	0	2
Prevalence (%)	5.3	0	10.5
Chi square	70.255	16	2
P-value	P<0.001	P<0.001	P<0.001

Multiple enteropathogens have been linked to diarrhea in new born calves. Etiologic diagnosis of infectious agents helps in selecting preventative and control measures. In our study, 85.5% of diarrheic calves were found to have infection with *E. coli*, which is in line with previous studies in Egypt (Younis et al. 2009; El-Seedy et al. 2016). However, *E. coli* infection in calves

Table 4: The Antimicrobial Resistance Profiles among the retrieved strains of *E. coli, Salmonella* and *C. perfringens*

Antibiotic class	Antibiotic	*E. coli* (n=188)			Salmonella (n=8)			*C. perfringens* (n=19)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Penicillin	AMX	100(53.1)	60(31.9)	24(13.7)	6(75)	0(0)	2(25)	13(68.42)	4(21.05)	2(10.5)
	AM	80(42.5)	50(26.5)	54(28.7)	4(50)	1(12.5)	3(37.5))	11(57.89)	6(31.5)	2(10.5)
Polymixin	CT	120(63.8)	59(31.38)	5(2.65)	7(87.5)	1(12.5)	0(0)	0(0)	0(0)	19(100)
Fluroquinolones	MC	160(85.1)	18(9.57)	6(3.19)	8(100)	0(0)	0(0)	3(15.7)	7(36.8)	9(47.3)
Tetracycline	OX	80(42.5))	55(29.2)	49(26.06)	5(62.5)	0(0)	3(37.5)	12(36.1)	5(26.3)	2(10.5)
Aminoglycoside	CN	130(69.1)	40(21.7)	14(7.4)	7(87.5)	1(12.5)	0(0)	3(15.7)	2(10.5)	14(73.68)
	N	100(53.1)	53(28.19)	31(16.4)	6(75)	1(12.5)	1(12.5)	2(10.5)	1(5.5)	16(84.2)
	S	120(63.8)	45(23.9)	19(10.1)	6(75)	1(12.5)	1(12.5)	1(5.2)	8(42.1)	10(52.6)
	AK	150(79.7)	25(13.29)	9(4.78)	7(87.5)	1(12.5)	0(0)	6(31.5)	3(15.7)	10(52.6)
Phenicol	C	70(37.2)	80(42.5)	34(18.08)	7(87.5)	1(12.5)	0(0)	16(84.2)	1(5.5)	2(10.5)
Sulfonamide	SXT	115(61.1)	40(21.27)	29(15.4)	2(25)	4(50)	2(25)	11(57.89)	4(21.05)	4(21.5)
Macrolides	E	2(1.06)	2(1.06)	180(95.7)	0(0)	1(12.5)	7(87.5)	2(10.5)	5(26.3)	12(63.1)
Lincomycin	CLI	0	0	188(100)	0(0)	0(0)	8(100)	15(78.94)	2(10.5)	2(10.5)
Chi square		306.4	163.53	909.84	16.957	14	40.889	55.389	19.708	55.25
P value		P<0.0001*	P<0.0001*	P<0.0001*	NS	NS	P<0.0001*	P<0.0001*	NS	P<0.0001*

*a significant difference P<0.0001. AMX= Amoxicillin (10µg), AM= Ampicillin (10 µg), CT= Clostin (15 µg), MC= Marbofloxacin (10 µg), OX= Oxytetracycline (30 µg), CN= Gentamicin (10 µg), Streptomycin=S (10 µg), Neomycin =N (10 µg), AK= Amikacin (10 µg), C= Florophenicol (30 µg), SXT= Trimethoprim-sulfamethoxazole (25 µg), E= Erythromycin (15 µg) and CLI=Clindamycin (2 µg).

Table 5: The frequency of the phenotypic multidrug resistance among the retrieved strains of *E. coli, Salmonella* and *C. perfringens*.

Type of bacteria	No. of strains	%	Type of resistance	Phenotypic multidrug resistance	*MARI*
E. coli	54	29.3	MDR	AM, E and CLI	0.23
	49	26.6	MDR	OX, E and CLI	0.23
	31	16.8	MDR	E, CLI, N and C	0.38
	24	13	MDR	AMX, E CLI and SXT	0.38
	14	7.6	MDR	E, CLI, CN and S	0.38
	5	2.7	MDR	E, CLI, CT, AK, S, SXT and MC	O.53
	4	2.2	DR	CLI, and AK	0.15
	3	1.6	DR	C	0.076
Salmonella	3	37.5	MDR	AM, OX, E and CLI	0.38
	2	25	MDR	AMX, SXT, E and CLI	0.38
	2	25	DR	E and CLI	0.15
	1	12.5	DR	CLI and N, S	0.23
C. perfringens	10	52.6	MDR	COL, EN, CN, S and AK	0.46
	4	21.1	MDR	CT, N, CN SXT and MAR	0.38
	2	10.5	XDR	AM, AMX, CT, E, N, OX, CLI and MC	0.61
	2	10.5	MDR	CT,C and MC	0.23
	1	5.2	DR	CT and MC	0.15

**Characteristics of multidrug resistance (MDR), extensively drug-resistance (XDR).* AMX= Amoxicillin, AM= Ampicillin, CT= Clostin, MC= Marbofloxacin, OX= Oxytetracyclinee, CN= Gentamicin, Streptomycin=S, Neomycin =N, AK= Amikacin, C= Florophenicol, SXT= Trimethoprim-sulfamethoxazole, E= Erythromycin and, CLI=Clindamycin.

recorded in this study is higher than 58.7% recorded by Sobhy et al. (2020) and 28.8% reported by Algammal et al. (2020a). This indicates that *E. coli* infections are less prevalent in developed nations, which may be related to better agricultural, hygienic, and managerial practices in these countries (Cho et al. 2013). *Salmonella* prevalence of 3.7% observed in our study is close to 4.09% reported by Younis et al. (2009), but lower than 18.1% recorded in Egypt by El-Seedy et al. (2016). The low incidence of pathogens such as *Salmonella* recorded in diarrheic calves in the present study may suggest that opportunistic bacteria have a major role in the diarrhea of calves. Prevalence of *C. perfringens* (8.8%) recorded in this study is similar to 10.5% previously reported by El-Naker et al. (2008**),** 12.06% observed by Ferrarezi et al. (2008**)** and 12.4% found by Ngeleka et al. (2019). Minor differences in isolation rates of *C. perfringens* may be attributed to differences in management conditions.

The development of MDR species is an important global problem, which develops due to abuse antimicrobial medication in animal or human medicine. Drug resistance can be spread to bacteria that have not been exposed to the drug (Algammal et al. 2020b; Algammal et al. 2022). For the detection of antibiotic resistance, antibiograms are believed to be more reliable than genotypic resistance gene identification (Scaria et al. 2010). Our study indicated a significant prevalence of resistance to penicillin, tetracyclines, and aminoglycosides, presumably due to the widespread use of these broad-spectrum antibiotics by healthcare workers and farmers (Sobhy et al. 2020). The incidence of resistance to trimethoprim-sulfamethoxazole may be related to the widespread usage of this drug by Egyptian paramedics. Antibiotics applied in animal feedstuff as growth promoters are the critical causes of bacterial evolution, specifically *C. perfringens* resistance patterns, as the bacteria become adapted due to recurrent antibiotic use (Bendary et al. 2022).

Virulence genes in *E. coli* were identified using polymerase chain reaction (PCR) in our study. This study identified a low percentage of STEC, which may be attributable to the extensive use of a commercial vaccination in Egypt. Vaccination against other pathogens remains a challenge. Three ETEC isolates were tested positive for ST. Strains that carry *Eae* but not the *Stx1* or *Stx2* variants are classified as EPEC, whereas strains that carry *eae* but have either the *Stx1* or *Stx2* variants are classified as STEC (Ishii et al. 2007**).** According to Wani et al. (2003**),** the *Stx2* gene was more prevalent than *Stx1,* and both these genes were associated with the *Eae* gene in STEC strains. The *iut*A gene encodes the aerobactin siderophore ferric receptor protein, mediating siderophore uptake (Ikeda et al. 2021). Enteric illnesses caused by *E. coli* pathotypes that cause diarrhea are suspected to involve EAST1. This protein is detected in diarrhoea-causing *E. coli* (Maluta et al. 2016). In addition, results of the current research showed that *stn* gene occurred in 80% of isolates. *Salmonella* enterotoxins induce gastroenteritis, which is characterized by nausea, vomiting, abdominal cramping, fever, and diarrhea (Huehn et al. 2010). The *stn* gene was found in all *Salmonella* strains isolated, as reported previously (Murugkar et al. 2003; Zou et al. 2012). The *omp*A gene helps *Salmonella* adapt to environmental challenges and cause illness or death by adhering to, invading, and damaging host tissue or evading host defense (Krishnan and Prasadarao 2012). The *omp*A gene was found in 60% of the isolates studied compared to Kataria et al. (2013), who found that *omp*A gene was present in all 68 tested *Salmonella* serovars. According to our results, all of the *Salmonella* serovars examined had the *inv*A gene. A gold standard for the genetic diagnosis of *Salmonella* species has been identified as the *inv*A gene, which is only found in *Salmonella* species (O'Regan et al. 2008; El-Gresly et al. 2021). Among the major toxins, type A strain of *C. perfringens* produces only alpha-toxin (Goossens et al. 2017). This gene (*cpa*) is chromosomally encoded and placed near the bacterial chromosome's origin of replication, one of the most stable areas (Canard and Cole 1989). The current investigation found *cpe* in only three (60%) of all examined isolates, highlighting the fact that these calves were infected by food poisoning strains which cause sudden death in neonatal calves (Khan and Zaman 2007; Goossens et al. 2017).

Conclusion

The current study revealed that the most common bacterial pathogen implicated in calf diarrhea was *E. coli*. Surveillance of antimicrobial sensitivity is required to pick the antibiotic of choice due to the continuous emergence of MDR bacteria. Colistin and gentamycin were the most efficient antimicrobials against the pathogens *E. coli* and *Salmonella* (Enterobacteriaceae) but they had no effect on *Clostridium*. Penicillins were the most effective antimicrobials against *Clostridium*, while they did not affect Enterobacteriaceae. Use of both phenotypic and genotypic studies is more effective diagnostic tool for identifying the etiologic agent and may aid in treating diarrhea. Combining conventional and genotypic analysis is a good tool for identifying bacterial infections that cause diarrhea.

Conflict of Interest

The authors state that they have no financial or personal conflicts of interest that may have impacted their decision to prepare this publication.

Authors' Contribution

All authors have made remarkable contribution to this work.

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