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Prevalence of multi-drug resistant *Escherichia coli* in diarrheic ruminants

Ashraf Abd El-Tawab[1], Fatma El-Hofy[1], Ahmed El Hamalawy[2],Amged Abo-Ela[3], Wafaa El-Shazly[3], Manar E. El-khayat[1]

1 Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

2Physics Department, Faculty of Science, Menofyia University, Egypt.

3Animal Production Research Institute, Agriculture Research Center (ARC),Egypt.

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ABSTRACT

Escherichia coli *(E. coli)* is the most significant cause of neonatal diarrhea in ruminants. The current study was designed to monitor the prevalence of multi-drug (MDR) resistant *E. coli* in diarrheic ruminants in Egypt. Rectal swab samples (n=150) were collected between November 2018 and April 2019 from diarrheic calves (n=35), lambs (n=35) and goat kids (n=80) up to 3 months from Gimmeza animal production researches station, Agriculture Research Centre (ARC), Egypt. Samples were submitted for isolation and identification of *E. coli* by conventional culture methods. From the examined samples , 82 (54.67%) were positive for *E. coli*. Among the *E. coli* isolates, 48 were identified as pathogenic *E. coli* by cultivation on Congo Red Agar and were submitted for antibiogram. Sensitivity tests revealed that 10/48 (20.83%) isolates were MDR. The 10 MDR *E. coli* isolates were serologically identified as O_{157}:H_7 (n=4; two isolated from calves and two from goat kids), O_{125} (n=3; two isolated from calves and one from lambs), O_{44} (n=3; two isolated from goat kids and one from lambs).

1. INTRODUCTION

Diarrhea is the principal cause of mortality and high morbidity in young animals causing massive economic and productive losses to livestock industry globally (Zahra *et al.*, 2019). About 57% of weaning calf mortalities were due to diarrhea according to National Animal Health Monitoring System (Cho and Yoon, 2014). In Egypt, Neonatal calf diarrhea is considered the major reason of calf mortality. It represents about 27.4-55.0% of the total deaths in young calves (El Seedy *et al.*, 2016).

Diarrhea has multifactorial causes including infectious as well as non-infectious factors related to the animal incorrect management, feeding, immunological status and animal breed (Hosein, 2019). Infectious diarrhea is the most significant cause of mortality in neonatal ruminants. It can be caused by many pathogens including viruses (coronavirus and rotavirus), protozoa (*C*ryptosporidium parvum) and bacteria (enterotoxigenic *Escherichia coli* (ETEC) and Salmonellae are the most economically important pathogens) (Izzo *et al.*, 2011).

Entero-toxigenic *E. coli* is considered the most common cause of neonatal diarrhea. It produces different virulence factors including, colonization in small intestine, avoiding the immune response and stimulating the deleterious inflammatory response (Muluken *et al.*, 2017). Escherichia coli causes calf coli septicemia, diarrhea in lambs and early-weaned piglets (Yu *et al*., 2011) and hemorrhagic colitis, hemorrhagic uremic syndrome and thrombotic thrombocytopenic purpura in humans (Pearce *et al.*, 2004).

Escherichia coli is a Gram negative, rod shaped flagellated, nonsporulating and facultative anaerobic bacterium belongs to family *Enterobacteriaceae*. This bacterium is classified into several categories based on its virulence factors such as ETEC, attaching and effacing *Escherichia coli* (AEEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and shiga toxin producing *E. coli* (STEC) (Wang *et al.*, 2010).

Antibiotics are widely used in veterinary medicine to control bacterial infections. During treatment of dairy cows, the milk used to feed calves could be contaminated with antimicrobial residues (Deng *et al.*, 2017; Leão *et al.*, 2017). Bacteria can develop antimicrobial resistance at sub-minimum inhibitory concentrations, (Francisco *et al.*, 2019). Antibiotic resistant bacteria carried by animals can enter the human food chain through the consumption of meat or other animal products, through farm run-off water and by other pathways (Collignon *et al.*, 2005).

The present study was designed to monitor the prevalence of pathogenic *E. coli* in diarrheic calves, lambs and goat kids younger than 3 months with determination of the resistance profile of the pathogenic *E. coli* to different antibiotics

* Corresponding author: Manar E. El-khayat, Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

2. MATERIAL AND METHODS

2.1. Samples

Rectal swabs (n=150) were collected between November 2018 and April 2019 from diarrheic cases of calves (n=35), lambs (n=35) and goat kids (n=80) up to 3months from Gimmeza animal production researches station, Agriculture Research Center , Egypt. Each swab was inoculated into 5 ml of sterile buffered peptone water then tightly closed, labeled and transported immediately to the laboratory in an ice container.

2.2. Isolation and identification of E. coli:

It was performed according to Quinn *et al.* (2002). Swabs were inoculated into 5 ml nutrient broth (Oxiod) and incubated at 37 °C for 18-24 hrs., followed by sub-culturing onto MacConkey's agar (Oxiod) and incubated at 37 ºC for 24 hrs. Lactose fermenter colonies were picked up and streaked onto EMB agar (Oxiod). Suspected *E. coli* isolates were indicated by the appearance of the characteristic green metallic sheen colonies.

2.3. Biochemical identification of E. coli isolates:

It was performed according to Quinn *et al.* (2002) by oxidase, lactose fermentation, indole production methyl red, Vogues Proskauer, citrate utilization, H_2S production, urea hydrolysis and catalase tests.

2.4. Detection of pathogenicity:

It was performed according to Ruchi *et al.* (2015) by cultivation of *E. coli* isolates on Congo Red Agar medium (Berkoff and Vinal, 1986).

2.5. In vitro antibiotic sensitivity of E. coli isolates:

It was performed by disc diffusion method according to Finegold and Martin (1982) and CLSI (2016). The antimicrobial discs (Oxiod, UK) that used for sensitivity testing of *E. coli* were Amoxicillin + Clavulinic acid (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5µg), Gentamicin (10 µg), Erythromycin (15µg), Ampcillin (30 µg), Chloramphenicol (10 µg), and Oxytetracycline (30 µg).

2.6. Serotyping of MDR pathogenic E. coli isolates:

Ten multi-drugs resistant pathogenic *E. coli* isolates were sero-grouped in Animal Health Research Institute, Dokki, Egypt according to Kok *et al.* (1996) by using the commercially available rapid diagnostic *E.coli* antisera (*E. coli* antisera set 1 for O antigen- *E. coli* antisera set 2 for H-antigen) (DENKA SEIKEN Co., Japan).

3. RESULTS

From the examined samples (n=150), 82 (54.67%) were positive for *E. coli*. Among the 82 *E. coli* isolates, 48 were identified as pathogenic *E .coli* by cultivation on Congo Red Agar as shown in table (1).

To assess the resistance profile, the 48 pathogenic *E. coli* isolates were submitted for antibiogram sensitivity tests, showed that 85% were resistant to Oxytetracycline followed by Ampicillin 83%, Chloramphenicol 60% and cefotaxime 20% but no resistance to Amoxicillin + clavulanic acid, Ciprofloxacin, Gentamicin, and Erythromycin. Among *E. coli* isolates, 10 isolates (20.83%) were found to be multi-drug resistant to three or more antibiotic groups.

The 10 MDR *E. coli* isolates were serologically identified as described in table (3).

Table 1 Prevalence of pathogenic *E. coli* isolated from fecal samples

Animal	No of fecal samples	No. pathogenic *E. coli* isolates	No. non-pathogenic *E. coli* isolates	Total No. *E.coli* isolates	%*
Calves	35	16	12	28	80%
Goat kids	80	23	16	39	48.7 %
lambs	35	9	6	15	42.8 %
Total	150	48	34	82	54.6 %

* Percentage in relation to the total number of faecal samples of each animal species.

Table 2 Antibiotic sensitivity for the 48 pathogenic *E. coli* isolates by disc diffusion method

Antimicrobial agent	Conc.	Sensitive	Intermediate	Resistant	% of resistance *
Amoxicillin+ Clavulanic acid	30 µg	17	31	-	0%
Ampicillin	10 µg	2	6	40	83%
Cefotaxime	30 µg	18	20	10	20%
Chloramphenicol	30 µg	3	16	29	60 %
Ciprofloxacin	5 µg	22	26	-	0%
Erythromycin	15 µg	28	20	-	0%
Gentamicin	10 µg	34	14	-	0%
Oxytetracycline	30 µg	-	6	41	85%

* Percentage in relation to the 48 pathogenic *E. coli* isolates submitted to the antibiotic sensitivity test.

Table 3 Serotyping of MDR pathogenic *E. coli* isolates

Animal	Isolated serogroup	No. of isolates
Calves	2 $O_{157:H7}$/ 2 O_{125}	4
Goat kids	2$O_{157:H7}$/2 O_{44}	4
Lambs	1 O_{44} /1 O_{125}	2
Total		10

4. DISCUSSION

Diarrhea is the principal cause of mortality in young animals causing massive economic and productivity losses globally (Zahra *et al.,* 2019). In developing countries, diarrheagenic *E. coli* is the cause of large proportion of diarrhea (Clarke, 2001). In the present study, *E. coli* was isolated in prevalence of 80% from Calves (n=28/35) that agreed with Shahrani *et al.* (2014), El-Seedy *et al.* (2016) as 76.45% and 75.6%, respectively. But, Abu El-Ella *et al.* (2013) 57.1%, Islam *et al.* (2015) 57%, Olagun *et al.* (2016) 63.2 % and Aref *et al.* (2018) 58.5%. In contrast, Zahra *et al.* (2019), who isolated *E. coli* from calves with higher percentage 100%, and Izzo *et al.* (2011), Masud *et al.* (2012), El-Shehdi *et al.* (2013), Hakim *et al.* (2017) and Safaa *et al.* (2019), who isolated *E. coli* from calves with lower percentage as 17.4%, 44% , 35.8% , 24.1%, 46.4%, respectively.

Escherichia coli was isolated in prevalence of 48.7% from Goat kids and 42.8% from lambs that didn't agree with Fuente *et al.* (2002), who isolated *E. coli* in diarrheic animals at prevalence of 66.7%, 100% from (goat kids, lambs) and from calves, respectively, which were higher than those found in healthy animals (33.3–40.6%) from (goat kids, lambs) and calves, respectively. On other hand, Orden *et al.* (2002) isolated *E. coli* in lower percentage 24.4% and 16.2% from healthy lambs and goat kids, respectively. It was detected in 3.1% and 5.9% of the diarrheic lambs and goat kids, respectively. Osman *et al.* (2013) recorded that *E. coli* prevalence rate in the diarrheic animals was 63.6% in calves,

27.3% in goat and 9.1% in sheep. This variation in the prevalence of *E. coli* may be attributed to difference in geographical distribution, age of calves, weather, managements and hygiene measurements.

Antibiotics are widely used for control bacterial infections in human and veterinary medicine and also used as growth promoter (Sarmah *et al.,* 2006). Antimicrobial resistance occurs when bacteria are exposed to antimicrobial drugs. It may be irreversible, even if organism is no longer exposed to antimicrobial drug (Sundsfjord *et al.,* 2004).

In this study, antibiotic sensitivity for 48 pathogenic *E. coli* isolates showed that 85% were resistant to Oxytetracycline followed by Ampicillin 83%, Chloramphenicol 60% and cefotaxime 20% but no resistance to Gentamicin, Ciprofloxacin, Amoxicillin + clavulanic acid and Erythromycin. Among those *E. coli* isolates,10 isolates were found to be MDR to 3 or more antibiotic groups. This may be due to miss use of antibiotics or change in microbial genetic structure and metabolism (Boskovic *et al.,* 2013). About 85% of *E .coli* isolates were resistant to Oxytetracycline, this result is slightly lower than that Karzmarczyk *et al.* (2011) and Masud *et al.* (2012), who obtained 99% and 100%, respectively. Moreover, the results were higher than Srivani *et al.* (2017), Kohansal and Aasd *et al.* (2018) and Aasmae *et al.* (2019), who obtained 63.2 %, 65% and 32%, respectively.

In our study, resistant to Ampicillin was 83% that agreed with Karzmarczyk *et al.* (2011), Kohansal and Aasd *et al.* (2018) and Mohamed *et al.* (2018), who detected 82%, 73%, and 83% resistance to Ampicillin, respectively. While Srivani *et al.* (2017) and Gupta *et al.* (2018), obtained lower resistance 11% and 55%, respectively. In contrast, sensitivity to Gentamicin was100% which agreed with Srivani *et al.* (2017) who detected 96% sensitivity and disagreed with Sun *et al.* (2012) and Badi *et al.* (2018), who reported 51.8 and 75% resistance to Gentamicin, respectively.

Sensitivity to Ciprofloxacin was 100% that agreed with Gupta *et al.* (2018), who reported 100% and slightly lower than Masud *et al.* (2012), who reported that sensitivity % was 91. Sensitivity to Amoxicillin + clavulanic acid was 100%. On other hand, Ammar *et al.* (2017) reported that 100 % of *E. coli* was resistance to Amoxicillin + clavulanic acid.

Serologically, *E. coli* is divided into serogroups and serotypes on basis of their antigenic structures (Griffin and Tauxe, 1991). In this study, Serotyping of ten MDR pathogenic *E.coli* isolates were done and the results showed that: 4/10 isolates were (O_{125}) which agreed with Mosaad *et al*. (2008), Hakim *et al.* (2017), and Safaa *et al.* (2019). 2/10 isolates were (O_{44}) that agreed with Mohamed *et al.* (2018) and Safaa *et al.* (2019). 4/10 isolates were ($O_{157:H7}$), which agreed with Mosaad *et al*. (2008), Dastmalchi and Ayremlou (2012), Shahrani *et al. (*2014) and Maryam and Ali (2018).

5. CONCULSION

Neonatal diarrhea caused by pathogenic *E. coli* is a perilous problem for young animals including calves, lambs and goat kids younger than 3 months of age specially with the emergence of multidrug resistance strains

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