Prevalence of multi-drug resistant Escherichia coli in diarrheic ruminants
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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 O124:H1 (n=4; two isolated from calves and two from goat kids), O12 (n=3; two isolated from calves and one from lambs), O41 (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., 2017). 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 (Cryptosporidium parvum) and bacteria (enterotoxigenic Escherichia coli (ETEC) and Salmonellae are the most economically important pathogens) (Izzo et al., 2011). Enterotoxigenic 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 (Muluk et al., 2017). Escherichia coli causes calf colic septicemia, diarrhea in lambs and early-weaned piglets (Yu et al., 2011) and hemorrhagic colitis, hemorrhagic urogenic 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 subminimum 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.
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 3 months from Gimmeza animal production research 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 (Oxoid) and incubated at 37 °C for 18-24 hrs., followed by sub-culturing onto MacConkey’s agar (Oxoid) and incubated at 37 °C for 24 hrs. Lactose fermenter colonies were picked up and streaked onto EMB agar (Oxoid). 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, H₂S 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 (Oxoid, UK) that used for sensitivity testing were Amoxicillin + Clavulanic acid (30 μg), Cefotaxime (30 μg), Ciprofloxacin (5μg), Gentamicin (10 μg), Erythromycin (15μg), Ampicillin (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 Amoxicillin 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).

| Animal | No. of fecal samples | No. of pathogenic E. coli isolates | No. of non-pathogenic E. coli isolates | Total No. of 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 fecal samples of each animal species.

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-Sheldi 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 the 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,

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

* Percentage in relation to the total number of E. coli isolates submitted to the antibiotic sensitivity test.

| Animal | Isolated serogroup | No. of isolates |
|--------|---------------------|-----------------|
| Calves | 2 O157:B2 O111      | 4               |
| Goat kids | 2 O111:12 O14     | 4               |
| Lambs  | 1 O123/1 O131      | 2               |

| Total  | 10                 |
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 Aasmie 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 was 100% 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, Anmar 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 10 isolates were done and the results showed that: 4/10 isolates were (O1:K12) which agreed with Mosaad et al. (2008), Hakim et al. (2017), and Safaa et al. (2019). 2/10 isolates were (O4:K12) that agreed with Mohamed et al. (2018) and Safaa et al. (2019). 4/10 isolates were (O17:K17), which agreed with Mosaad et al. (2008), Dastmalchi and Ayremliou (2012), Shahrani et al. (2014) and Maryam and Ali (2018).

### 5. CONCLUSION

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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