Antimicrobial resistance and molecular characterization of pathogenic *E. coli* isolated from chickens

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ABSTRACT

Forty eight clinically diseased broiler chickens from different (n=18) farms from Beni Suef and El-Fayoum Governorates were subjected to euthanasia and post mortem examination. Lesions include airsaculitis, pericarditis and perihepatitis. Bacteriological examination showed that 22.9% of isolates were *E. coli* positive. Serogrouping of isolates revealed O125, O112, O91, O157, O115 and O25 % of each serogroup with an incidence rate of 18.2%, 9.1%, 9.1%, 9.1%, 9.1% and 9.1%, respectively and four strains were untyped. Antimicrobial sensitivity tests against 17 antimicrobials showed that the most common resistance patterns were against penicillin, lincomycin, oxytetracycline, clindamycin, amoxycillin and erythromycin followed by nalidix acid and trimethoprim. On the other hand, the most potent antimicrobials were colistin sulphate, gentamycin, doxycycline and ceftriaxone followed by enrofloxacin, ciprofloxacin, norfloxacin, chloramphenicol and lastly ampicillin. PCR showed that all isolates had B-lactam resistant gene (*bla*TEM) and tetracycline resistance gene A (*tet*A) but only 18 % have quinolones resistance gene A (*qnr*A).

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1. Introduction

*E. coli* is a member of the family *Enterobacteriaceae*. It is a normal inhabitant in the digestive system of chickens. Stress factors like cold weather, dry dusty conditions, contaminated environment, over crowdedness, feed/water restriction, temperature extremes, poor ventilation or other stresses lead to *E. coli* infection associated with high mortality, reduced weight gain and consequently condemnation of birds at the slaughter, Kaul et al., (1992). *E. coli* strains were classified by Russo and Johnson, (2000) into three major groups: commensal strains, intestinal pathogenic strains, and extra-intestinal pathogenic *E. coli* (ExPEC) strains.

*E. coli* is recognized as a major pathogen associated with public health
problems in developing countries and represents a leading etiological agent of diarrhea. Several classes of enterovirulent \textit{E. coli}, namely enterotoxigenic \textit{E. coli} (ETEC), enteropathogenic \textit{E. coli} (EPEC), enterohaemorrhagic \textit{E. coli} (EHEC), enteroinvasive \textit{E. coli} (EIEC), enteroaggregative \textit{E. coli} (EAEC), diarrhea-associated hemolytic \textit{E. coli} and cytolethal distending toxin (CLDT)-producing \textit{E. coli} have been recognized Nataro and Kaper, (1998).

Some strains of \textit{E. coli} produce a toxin called Shiga toxin which damages the lining of the intestine. The strains of \textit{E. coli} that make this toxin are sometimes called Shiga toxin-producing \textit{E. coli} (STEC). O\textsubscript{157:H7} \textit{E. coli} strain can induce dangerous diseases in humans. It causes abdominal cramps, vomiting, and bloody diarrhea. It is the leading cause of acute kidney failure in children. It can also cause life-threatening manifestations such as adult kidney failure, bleeding, confusion, and seizures (Wasey and Salen, 2018). Although antimicrobials are considered a very important tool for the treatment of clinical disease and maintaining birds' health and productivity, antimicrobial uses have been implicated as a risk factor in the dissemination and development of drug resistance (Gosh and LaPara, 2007). Increasing antimicrobial resistance is an important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. \textit{In-vitro} antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy (Radwan et al., 2016). Moreover, it is very useful to detect the multidrug resistant isolates. Therefore, the appropriate antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination.

Since the introduction of antibiotics, there has been a tremendous increase in the resistance in diverse bacterial pathogens (Gold and Moellering, 1996). The resistance of \textit{E. coli} species to antimicrobials is widespread and of concern to poultry veterinarians. This increasing resistance has received considerable national and international attention. Plasmids were the major vector in the dissemination of resistance genes through the bacterial population (Smalla et al., 2015; San Millan, 2018). There is a wide variety of \textit{E. coli} being resistant to more than one antimicrobial agent, so we can use polymerase chain reaction (PCR) to detect antimicrobial resistance genes in \textit{E. coli} isolates.

The aim of the present work was directed to study the antimicrobial resistance and molecular characterization of pathogenic \textit{E. coli} isolated from chickens.

2. Material and Methods

2.1. Chicken samples

A total of 48 clinical samples were collected aseptically from air sacs, liver and pericardium from chickens of different ages (2-5 weeks) from flocks suspected to be infected with \textit{E. coli}.

2.2. Bacteriological examination

MacConkey's broth, MacConkey bile salt lactose agar medium, eosine methylene blue, tryptone soya agar and semi-solid agar were used for bacteriological examination.

2.3 Microscopical examination

It was carried out using Gram's stain as described by Quinn et al., (2002) for morphological study.

2.4. Biochemical identification of the bacterial isolates

It was performed according to Quinn et al. (2002) including oxidase test, catalase test, indole production, methyl red test, Voges Proskauer test, citrate utilization test, sugar fermentation test, hydrogen sulphide (H\textsubscript{2}S)
production on TSI medium and urea hydrolysis test.

2.5. Serological identification of E. coli isolates

It was performed according to Edwards and Ewing, (1972). Isolates were serotyped using Polyvalent and monovalent diagnostic E. coli antisera.

2.6. Antibacterial sensitivity testing:

Disk diffusion method was applied according to Quinn et al., (2002). All E. coli isolates were tested for their susceptibility to 17 different antimicrobial agents including; ampicillin (10µg), penicillin (10µg), amoxicillin (10µg), chloramphenicol (30µg), colistin sulphate (25µg), ciprofloxacin (5µg), doxycycline HCl (30µg), erythromycin (15µg), gentamicin (10µg), nalidixic acid (30µg), norfloxacin (10µg), lincomycin (10µg), ceftriaxone (30µg), oxytetracycline (30µg), enrofloxacin (5µg), clindamycin (2µg) and trimethoprim (5µg) (Oxoid, Basing Stoke, UK). The interpretation of inhibition zones of tested culture was done according to NCCLS, (2002).

2.7. Detection of antimicrobial resistant genes of E. coli by PCR

DNA Extraction was performed using QIA amp DNA mini kit according to the manufacturer’s instruction. DNA was used for PCR (Sambrook et al., 1989) for detection of the target genes (blaTEM, tetA and qnrA). PCR products were separated by gel electrophoresis and visualized using U.V illuminator.

| Primer | Primer sequence (5’-3’) | Amplified product | Annealing temperature |
|--------|-------------------------|-------------------|----------------------|
| qnrA   | F ATTTCACCGCCAGGATT    | 516 bp            | 55°C/40sec.          |
|        | R GATCGGCAAGGTTAGGTCA  |                   |                      |
| tetA(A) | F GGTTCACCTCAACGACGTC | 576 bp            | 50°C/40sec.          |
|        | R CTGTCCGACAAGTTGCA    |                   |                      |
| blaTEM | F ATCAGCAATAAACCCAGC   | 516 bp            | 54°C/40sec.          |
|        | R CCCGAAGAACCCTTTTC    |                   |                      |

3. Results

3.1. Prevalence of E. coli in the examined broiler chickens

Out of 48 samples taken from diseased broiler chickens from 18 different broiler farms from Beni-Suef and El-Fayoum Governorates, 11 E. coli isolates were recovered with a prevalence rate of 22.9%.

3.2. Results of serogrouping of E. coli isolates recovered from diseased broiler chickens

Serogrouping of E. coli isolates showed that 7 isolates (63.6%) were belonged to 6 serogroups including O_{125} (n=2), O_{112} (n=1), O_{91} (n=1), O_{157} (n=1), O_{115} (n=1), and O_{25} (n=1) with incidence rate of 18.2%, 9.1%, 9.1%, 9.1%, 9.1%, and 9.1%, respectively. Meanwhile, four isolates (36.4%) were untyped by the available antisera and were considered as untyped (Table 2).
Table 2. Results of serogrouping of *E. coli* isolated from broiler chickens

| Serogroup of *E. coli* | No. | %  |
|------------------------|-----|----|
| O₁₂₅                   | 2   | 18.2 |
| O₁₁₂                   | 1   | 9.1  |
| O₉₁                    | 1   | 9.1  |
| O₁₅₇                   | 1   | 9.1  |
| O₁₁₅                   | 1   | 9.1  |
| O₂₅                    | 1   | 9.1  |
| **Total serotypes**    | 7   | 63.6 |
| Untyped                | 4   | 36.4 |
| **Total**              | 11  | 100  |

%: was calculated according to the total number of the tested isolates.

3.3. Antimicrobial sensitivity testing of *E. coli* isolated from broiler chickens

Results of in-vitro antimicrobial sensitivity testing of *E. coli* isolates are demonstrated in table (3).

*E. coli* isolates were completely resistant (100%) to penicillin, amoxicillin, erythromycin, lincomycin, oxytetracycline, and clindamycin. Meanwhile, they were highly resistant to ampicillin, nalidixic acid, and trimethoprim (90.9% for each) as well as chloramphenicol, ciprofloxacin, norfloxacin, and enrofloxacin (81.8% for each). On the other hand, moderate sensitivity was observed against colistin sulphate (63.6%).

MDR was detected in all *E. coli* isolates (100%). All isolates were MDR to at least 8 antimicrobials up to 16 antibacterial agents.

Table 3. Antibiogram patterns for *E. coli* recovered from cases of colibacillosis in broiler chickens

| Antibacterial agents | Disc content(µg) | Susceptible | Intermediate | Resistant |
|----------------------|------------------|-------------|--------------|-----------|
|                      |                  | No. | %  | No. | %  | No. | %  |
| Penicillin           | -                | 0   | 0  | 0   | 0  | 11  | 100 |
| Ampicillin           | 10µg             | 1   | 9.1| 0   | 0  | 10  | 90.9|
| Amoxicillin          | 10µg             | 0   | 0  | 0   | 0  | 11  | 100 |
| Ceftriaxone          | 30µg             | 0   | 0  | 6   | 54.5| 5   | 45.5|
| Nalidixic acid       | 30µg             | 0   | 0  | 1   | 9.1| 10  | 90.9|
| Enrofloxacin         | 5µg              | 2   | 18.2| 0  | 0  | 9   | 81.8|
| Ciprofloxacin        | 5µg              | 2   | 18.2| 0  | 0  | 9   | 81.8|
| Norfloxacin          | 10µg             | 2   | 18.2| 0  | 0  | 9   | 81.8|
| Doxycycline          | 30µg             | 1   | 9.1| 6   | 54.5| 4   | 36.4|
| Oxytetracycline      | 30µg             | 0   | 0  | 0   | 0  | 11  | 100 |
| Colistin sulphate    | 25µg             | 7   | 63.6| 0  | 0  | 4   | 36.4|
| Gentamycin           | 10µg             | 5   | 45.5| 1  | 9.1| 5   | 45.5|
| Lincomycin           | 10µg             | 0   | 0  | 0   | 0  | 11  | 100 |
| Clindamycin          | 2µg              | 0   | 0  | 0   | 0  | 11  | 100 |
| Chloramphenicol      | 30µg             | 2   | 18.2| 0  | 0  | 9   | 81.8|
| Erythromycin         | 15 µg            | 0   | 0  | 0   | 0  | 11  | 100 |
| Trimethoprim         | 5µg              | 0   | 0  | 1   | 9.1| 10  | 910.9|

No.: Number of positive cases.

%: was calculated according to the total number of *E. coli* tested isolates (n=11).
3.4. Antimicrobial resistance genes detection by PCR

PCR was applied on 11 isolates of MDR E. coli isolates to detect three antimicrobial resistant genes including β-lactam resistance gene \( bla_{TEM} \), quinolones resistance gene A \( qnrA \) and tetracycline resistance gene A \( tetA \). The results illustrated in the table (4) revealed that 100 % of the tested E. coli isolates harbored both \( bla_{TEM} \) and \( tetA \) genes while only two isolates (18.2%) harbored \( qnrA \) gene.

| Gene | \( bla_{TEM} \) | \( qnrA \) | \( tetA \) |
|------|----------------|---------|---------|
| Sample |                |         |         |
| 1     | +              | -       | +       |
| 2     | +              | -       | +       |
| 3     | +              | -       | +       |
| 4     | +              | -       | +       |
| 5     | +              | +       | +       |
| 6     | +              | -       | +       |
| 7     | +              | +       | +       |
| 8     | +              | -       | +       |
| 9     | +              | -       | +       |
| 10    | +              | -       | +       |
| 11    | +              |         |         |
| Total | 11 (100%)      | 2 (18.2%) | 11 (100%) |

+: Positive detection of the target gene by PCR.
- : Negative detection of the target gene by PCR.

Fig. 1 Agarose gel electrophoresis of E. coli showing the amplification of \( bla_{TEM} \) with amplicon of 516 bp expected product. DNA Ladder (L). Lanes (1–11): tested E. coli isolates. Lane (+ve): positive control. Lane (-ve): negative control.

Fig. 2 Agarose gel electrophoresis of E. coli showing the amplification of \( qnrA \) with amplicon of 516 bp expected product. DNA Ladder (L). Lanes (1–11): tested E. coli isolates. Lane (+ve): positive control. Lane (-ve): negative control.
Fig. 3 Agarose gel electrophoresis of *E. coli* showing the amplification of *tetA* with amplicon of 516 bp expected product. DNA Ladder (L). Lanes (1–11): tested *E. coli* isolates. Lane (+ve): positive control. Lane (-ve): negative control.

4. Discussion

In this study the prevalence of *E. coli* in 18 different broiler farms from Beni Suef and Fayoum governorates during the year 2017 was about 22.9%. Nearly similar incidence (23.5%) in El-Sharkia and (30%) in Alexandria were reported by Roshdy et al., (2012). Higher prevalences (91.8%) were detected by Derakhshanfar and Ghanbarpour, (2002); (82%) by Jakaria et al., (2012) and (58%) by Akond et al., (2009). The differences in the prevalence rates of *E. coli* may be due to the difference in the season (Ashraf et al., 2015), difference in biosecurity or hygienic conditions from area to area and from farm to farm as well as the pathogenicity, and virulence of the strains and the immunological status of the flock.

In the current work, 7 out of 11 *E. coli* isolates recovered from chickens could be serologically identified. They belonged to 6 different serogroups which are (O125, O112, O91, O157, O115, O25). These results to some extent are similar to those obtained by Roshdy et al., (2012) and Abd El Tawab et al., (2015).

Enterohemorrhagic *E. coli* EHEC (verotoxigenic *E. coli* VTEC or Shiga toxigenic *E. coli* STEC) causes hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TP) in humans. They synthesize shiga toxins (verotoxins) which are potent cytotoxic substances, adherence factors and enterohaemolysin. EHEC is responsible for many outbreaks of bloody diarrhea caused by contaminated foods. The most important serogroups among EHEC are O26, O111 and O157, being O157:H7 the most relevant serotype in foodborne outbreaks (González, 2002).

In the present study, the percent of untyped *E. coli* was 36% which to some extent agrees with that obtained by Allan et al., (1993) 39% while differs from that obtained by Amer et al., (2018) 15%.

Antimicrobials are usually used for treating infected animals and humans and also for prophylaxis and growth promotion of food producing animals. So inadequate selection and abuse of antimicrobials are the main causes of the emergence of resistance among various bacteria and this makes the treatment of bacterial infections more difficult (Aarestrup et al., 2008).

As commensal bacteria, *E. coli* is considered a reservoir of resistance genes for pathogenic bacteria; their resistance level is considered a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens (Murray, 1992). Resistant *E. coli* strains can transfer antibiotic resistance determinants not only to other *E. coli* strains, but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms (Österblad et al., 2000).
Resistance to nalidixic acid was 90.9%. Other higher resistance 100% was obtained by Yang et al., (2004); Salehi et al., (2006), 98% Zakeri and Kashefi, (2012), 97.7% Rahimi, (2013) and 96.7 Messaï et al., (2013). On the other hand lower results 82.3% was obtained by Dou et al., (2016) and the lower resistance 13% was obtained by Radu et al., (2001).

Resistance to Enrofloxacin was high (81.8%) which to some extent goes hand with those obtained by Salehi and Bonab, 76% (2006); 76% by Saberfar et al., (2008) and 75% by Amer et al., (2018). Other results 72.2% was obtained by Messaï et al., (2013); 60% by Zakeri and Kashefi, (2012); 43% by Gregova et al., (2012) and 37.74% by Talebiyan et al., (2014). On the other hand high susceptibility to enrofloxacin 91.8% was reported by Sarkar et al., (2013).

Resistance to ciprofloxacin was 81.8% of tested isolates. This result goes hand with those obtained by Akond et al., (2009), 82% and to some extent with that 79% obtained by Yang et al., (2004). Lower resistance 67% was reported by Salehi and Bonab, (2006); 30% by Islam et al., (2008) and 19%, as rarely used in poultry farming in Kenya, by Adelade et al., (2008). On the other hand, high sensitivity to ciprofloxacin as 100% was reported by Saidi et al., (2012) and 74.29% by Sahoo et al., (2012). Also, Jakaria et al., (2012) found that E. coli isolates were highly sensitive to ciprofloxacin in his study.

Resistance to norfloxacin was 81.8% which differs from that recorded by Salehi and Bonab, (2006) 68%; 55% by Zakeri and Kashefi, (2012); 36.9% by Younis et al., (2017). In contrast, high norfloxacin sensitivity (86%) was obtained by Akond et al., (2009).

In this study, Enrofloxacin, ciprofloxacin and norfloxacin gave the same results of resistance and sensitivity on the same isolates.

Resistance to fluoroquinolones was high which goes hand with the previous study of Rahimi, (2013), also (Li et al., 2005) reported that resistance to fluoroquinolones ranged from 57.1% to 66.7%.

Resistance to chloramphenicol was 81.8% of tested isolates. Other higher result of resistance 100% was obtained by Islam et al., (2008) and 91.6% by Rahimi, (2013). Lower result of resistance as 67% found by Salehi and Bonab, (2006); 65% by Amer et al., (2018); 52.9% by Li et al., (2005); 52% by Saberfar et al., (2008); 49% by Zakeri and Kashefi, (2012); 45.6% by Messaï et al., (2013); 30% by Younis et al., (2017); 20.75% by Talebiyan et al., (2014); 20% by Akond et al., (2009); 13.2% by Adelade et al., (2008) and 10% by both Radu et al., (2001) and Gregova et al., (2012). On the other hand high rate of the sensitivity of 82.67% was recorded by Sahoo et al., (2012) while 50% by Rahman et al., (2008).

In this study, all isolates (100%) were resistant to lincomycin, this result run hand to hand with that obtained by Salehi and Bonab, (2006) and close 97.5% to that obtained by Zakeri and Kashefi, (2012). Meanwhile, resistance to lincospectin (lincomycin plus spectinomycin) was low as reported by Salehi and Bonab, (2006); 49.4% by Rahimi, (2013) and 79% by Saberfar et al., (2008).

All isolates tested for clindamycin were resistant, also (Amer et al., 2018) found that 80% of isolates were resistant to clindamycin.

Also, all isolates tested for erythromycin resistance were resistant 100%. This result goes hand with that were resistant found by Saberfar et al., (2008) ≥ 99%; 97% by Salehi and Bonab, (2006) and Zakeri and Kashefi, (2012) and 96.1% by Rahimi, (2013). Other lower resistance rates were reported 71.1% by Talebiyan et al., (2014), 66.6% by Islam et al., (2008), 64% by Akond et al., (2009), 63.3 by Abd El Tawab et al., (2015), 35% by Amer et al., (2018) and 30% by Radu et al., (2001).

Moreover, all isolates were resistant to penicillin 100%. This result agreed 100% with that obtained by Radu et al., (2001); Islam et al., (2008) and Younis et al., (2017). Relatively
lower resistance was found as 88 % by Akond et al., (2009) and 82.86 % by Sahoo et al., (2012).

In the current study, 90.9 % of isolates were resistant to ampicillin. This result is close to that found by Saidi et al., (2012) 94.1 %; 89 % by Grgova et al., (2012) and 87.5 % obtained by Rahman et al., (2008). A higher resistance 100 % was obtained by Tabatabaei et al., (2011). Other lower resistance 84.5 % was reported by Messaï et al., (2013); 81.1 % by Dou et al., (2016); 80 % by Amer et al., (2018); 79% by Yang et al., (2004); 76.2 % by Li et al., (2005); 74.29 % by Sahoo et al., (2012); 58 % by Akond et al., (2009); 57 % by Radu et al., (2001); 49 % by Saberfar et al., (2008); 47 % by Salehi and Bonab, (2006); 39 % by Adelaide et al., (2008) and 24.1 % by Tadesse et al., (2012). On the other hand, Aggad et al., (2010) mentioned that all strains were susceptible to ampicillin, also Gregova et al., (2012) found that resistance lowered from 89 % to only 6 % on using ampicillin plus sulbactam.

All isolates tested for amoxicillin resistance were resistant 100 %. This result agreed with the 100 % resistance obtained by Tabatabaei et al., (2011). Other high results of resistance as 87.8 % was reported by Messaï et al., (2013); 74.3 % by Li et al., (2005); 71.43 % by Sahoo et al., (2012) and 53% by Salehi and Bonab, (2006). In contrast, resistance was only 5.6 % on using amoxicillin / clavulanic, Tadesse et al., (2012).

In this study, 45.5 % of isolates tested for ceftriaxone resistance were resistant. This result differs from that 2.4 % obtained by Tadesse et al., (2012). On the other hand, 91.43 % sensitivity was reported by Sahoo et al., (2012).

All isolates (100%) were resistant to oxytetracycline which to some extent goes hand with that 95 % found by Salehi and Bonab, (2006); 93 % by Saberfar et al., (2008) and 92 % by Zakeri and Kashefi, (2012). Other results of oxytetracycline resistance 85 % was reported by Amer et al., (2018) and 43.4 % by Talebiyan et al., (2014).

In this work, 36.4 % of isolates were resistant to doxycycline while 60 % were intermediate sensitive and only 10 % were highly sensitive. Lower rate of resistance 16.98 % was reported by Talebiyan et al., (2014) while higher rate of resistance 80 % was reported by Zakeri and Kashefi, (2012); 88 % by Salehi and Bonab, (2006) and 98.3 % by Messaï et al., (2013).

Colistin sulphate has the lowest rate of resistance (36.4 % of isolates were resistant). The highest rate of sensitivity was against this drug with 63.4 % sensitivity. This result goes hand with that found by Salehi and Bonab, (2006); Zakeri and Kashefi, (2012) both mentioned that resistance to colistin was low, also Messaï et al., (2013) found that 5.5 % of isolates were resistant to colistin. On the other hand Saberfar et al., (2008) found resistance in 99 % of isolates.

Meanwhile, 45.5 % of isolates were resistant to gentamycin. This result goes hand with the resistance of 46.6 % reported by Abd El Tawab et al., (2015) and to some extent with resistance of 50 % found by Islam et al., (2008) and 55 % obtained by Amer et al., (2018). Lower rate of resistance to gentamycin 39 % was published by Li et al., (2005); 14 % by Gregova et al., (2012); 12 % by Saberfar et al., (2008) and 5.5 % by Messaï et al., (2013). In this Study Gentamycin was the second potent drug after colistin against the E. coli isolates, There were many studies which discussed efficacy of Gentamycin on E. coli serotypes as sensitivity of 97.1 % found by Saidi et al., (2012); 85.72 % by Sahoo et al., (2012); 80 % by Akond et al., (2009) and also majority of isolates were sensitive to gentamycin as reported by Jakaria et al., (2012).

Resistance to trimethoprim was very high (90.9 %) which differs from that 78.2 % found by Dou et al., (2016), 25.3 % by Ozawa et al., (2008) and 20 % by Tabatabaei et al., (2011). In this study, the most common resistance patterns were against penicillin, lincomycin, oxytetracycline, clindamycin,
amoxyccillin and erythromycin followed by nalidix acid and trimethoprim. On the other hand, the most potent antimicrobials were colistin sulphate, gentamycin, doxycycline and ceftriaxone followed by enrofloxacin, ciprofloxacin, norfloxacin, chloramphenicol and lastly ampicillin.

Multidrug resistance in E. coli has become a worrying issue that is increasingly observed in human and also in veterinary medicine worldwide (Poirel et al., 2018). In this study, there was multiple drug resistance to at least 8 antimicrobial up to 16 antimicrobials out of a total of 17 tested antimicrobials. No isolate was resistant to all antimicrobials used but there were 2 isolates which were intermediate sensitive to only one antimicrobial which either doxycycline or ceftriaxone. Such bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer (Poirel et al., 2018).

Efflux proteins have been the best studied of the Tet determinants and tetA, tetB, tetC, tetD, tetE, tetG, tetH, tetK, tetL, tetA(P) and otrB genes have been identified. All of these genes code for energy-dependent membrane-associated proteins that export tetracycline out of the cell. The export of tetracycline reduces the intercellular concentration of tetracycline and thus protects the bacterial ribosomes. Efflux genes are found in both Gram-positive and Gram-negative species. The Gram-positive genes tetK, tetL and tetA(P) and the Gram-negative genes tetA, tetC, tetD, tetE, tetG, tetH code for efflux proteins which confer resistance to tetracycline but not minocycline. In contrast, the Gram-negative tetB gene codes for an efflux protein which confers resistance to both tetracycline and minocycline (Roberts, 1996). Guay et al., (1994) suggested that the TetB protein’s ability to confer minocycline resistance was not due to the TetB protein substrate specificity for minocycline, but rather TetB was a better pump when compared with other Tet efflux pumps. In the current study, all isolates have been found to carry the tetA(A)gene. β-lactam antibiotics are a class of antibiotics that contain a beta-lactam ring in their molecular structures. This includes penicillin derivatives and cephalosporins. Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. β-lactam antibiotics are bactericidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls.

If the bacteria produce β-lactamase or penicillinase enzyme, the enzyme will hydrolyze the β-lactam ring of the antibiotic and make the antibiotic ineffective. The genes encoding these enzymes may be inherently present on the bacterial chromosome or acquired via plasmid transfer (plasmid-mediated resistance), and β-lactamase gene expression may be induced by exposure to β-lactams.

Extended-spectrum β-lactamas (ESBLs) are often mediated by blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae and other Gram-negative bacteria.

β-lactamas from Gram-negative bacteria inactivate penicillins and cephalosporins by hydrolysis, and this is the predominant cause of resistance to these antibiotics. So far, more than 340 β–lactamases have been identified and on the basis of their amino acid sequences, substrate and inhibitor profiles. Gram-negative β-lactamases are divided into the four classes A to D. Class A enzymes, which include the plasmid-encoded broad-spectrum blaTEM and blaSHV families, and class C enzymes, which include the chromosomally encoded cephalosporinas, are the most frequently occurring enzymes (Monstein et al., (2007). In the current study, 100 % of isolates had blaTEM gene.

Since the plasmid-borne quinolone resistance gene qnr was reported in 1998, many additional qnr alleles have been discovered on plasmids or the bacterial chromosome. The
plasmid-borne qnr genes currently comprise three families, qnrA, qnrB, and qnrS.

Quinolone resistance in Enterobacteriaceae results mostly from chromosomal mutations in genes coding for DNA gyrase (topoisomerase II), for efflux and outer membrane proteins, or for their regulatory elements (Hooper 2001). DNA gyrase and topoisomerase IV are responsible for decatenation of interlinked chromosomes in the bacterial cells; the key event in quinolone action is reversible trapping of gyrase-DNA and topoisomerase IV-DNA complexes. Complex formation with gyrase is followed by a rapid, reversible inhibition of DNA synthesis, cessation of growth. The 218-amino-acid protein qnrA, which belongs to the pentapeptide repeat family, protects DNA gyrase and topoisomerase IV from the inhibitory activity of quinolones (Tran and Jacoby, 2002). qnrA confers resistance to nalidixic acid and increases MICs (Minimum inhibitory concentration) of fluoroquinolones up to 32-fold (Mammeri et al., 2005). In the current study, 18% of isolates have qnrA (a plasmid-mediated quinolone resistance determinant).

The large problem associated with E. coli, is the acquisition of genes coding for extended-spectrum β-lactamases which conferring resistance to broad-spectrum cephalosporins, carbapenemases which conferring resistance to carbapenems, 16S rRNA methylases which conferring pan-resistance to aminoglycosides, plasmid-mediated quinolone resistance (PMQR) genes which conferring resistance to fluoroquinolones, and mcr genes which conferring resistance to polymyxins. However, the spread of carbapenemase genes has been mainly recognized in the human sector but poorly recognized in animals (Poirel et al., 2018).

The past 20 years have witnessed major increases in the emergence and spread of multidrug-resistant bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins (Levy and Marshall, 2004).

5. Conclusion

Antimicrobial susceptibility testing showed that the most common resistance patterns were against penicillin, lincomycin, oxytetracycline, clindamycin, amoxicillin, and erythromycin followed by nalidixic acid and trimethoprim. On the other hand, the most potent antimicrobials were colistin sulphate, gentamycin, doxycycline and ceftriaxone followed by enrofloxacin, ciprofloxacin, norfloxacin, Chloramphenicol and lastly ampicillin.

Molecular examination for the presence of antimicrobial resistance genes showed that 100% of isolates have both tet A (A) and blaTEM and 18% of isolates have qnr.

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