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https://dx.doi.org/10.4314/nvj.v43i2.1 **ORIGINAL ARTICLE**

CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF ESCHERICHIA COLI ISOLATED FROM SELECTED INTERNAL ORGANS OF DONKEYS SLAUGHTERED IN ABATTOIRS IN KADUNA STATE, NIGERIA

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SUMMARY

Escherichia coli (*E. coli*) is a natural inhabitant of the gastrointestinal tract of both humans and animals. Several strains exist some are harmless while some are pathogenic causing various fatal conditions in both humans and animals. To characterize and determine the antibiotic susceptibility patterns of *E. coli* isolated from liver, spleen and intestine of donkeys slaughtered in Maraban Idah, Kaduna State, Nigeria, a total of 384 samples were collected from 128 donkeys from April to August 2018. The samples were processed using standard methods of cultural enrichment, growth on selective media, biochemical and antibiotics susceptibility test. The overall isolation rate was 5.2% with organ distribution of 4.7%. 4.7% and 6.3% for intestine, liver and spleen, respectively. *Escherichia coli* was slightly higher in the spleen than in the liver and intestine though there was no significant association ($p = 0.810$) between the presence of *E. coli* and the organs sampled. Most of the isolates were susceptible to ciprofloxacin (90%), enrofloxacin (80%) and colistin (70%) while some were highly resistant to common antibiotics such as penicillin (95%), oxytetracycline (90%) and amoxycilline (75%). Most of the isolates (75%) displayed resistance to two (2) different classes of antimicrobials with a total of 5 resistance patterns. Therefore, there is a need for education and public awareness regarding hygienic processing and consumption of offals of slaughtered donkeys. Also, further studies should be done to ascertain the genes responsible for resistance to antimicrobials observed in the *E. coli* isolates from the slaughtered donkeys.

Key words: Abattoir, Antibiotics susceptibility test, Biochemical test, Donkey, *Escherichia coli*.

INTRODUCTION

Escherichia coli (*E. coli*) are gram-negative, rod-shaped, flagellated, nonsporulating, facultative anaerobic bacterium which belongs to the family *Enterobacteriaceae* (Yu *et al*., 2014). *E. coli* are natural inhabitant of the gut of humans, birds and other warm-blooded animals and are widely accepted as an indicator for faecal contamination (Touchon *et al*., 2009). This bacterium is genetically highly adaptable to environmental stresses, and has the ability to survive and multiply in the environment (Van Elsas *et al*., 2010). The harmless strains are part of the normal microbiota of the gastrointestinal tract. They benefit their hosts by producing vitamin K_2 (Bentley and Meganathan, 1982). The bacterium has been known to prevent colonization of the intestine with pathogenic bacteria in a symbiotic relationship (Hudault *et al*., 2001; Reid *et al*., 2001). *E. coli* are expelled into the environment within faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for 3 days after which its numbers starts declining slowly. Under favorable conditions the generation time of this bacterium is usually 20 minutes (Russell and Jarvis, 2001). *E. coli* are regarded to be among a group of intestinal microflora in human beings and animals that are usually harmless (Greenland *et al*., 2009) while many of the *E. coli* gastrointestinal microflora are innocuous. Their build-up is usually due to gut stasis, disruptions of the intestinal activities or a sudden change of diet (Kaper *et al*., 2004). It is recognized as an important food borne pathogen (Gyles, 2007). The presence of *E. coli* in the environment is a cause for concern because its relationship with humans is not entirely benign. Indeed, *E. coli* are a major cause of diarrhoeal diseases, peritonitis, colitis, bacteremia, infant mortality, and urinary tract infections (Kaper *et al*., 2004). Some strains may even cause cancer (Arthur *et al*., 2012).

Some opportunistic *E. coli* infections are caused by normally harmless or beneficial strains when introduced to sick hosts or to parts of a host's body outside of the gut (Kaper *et al*., 2004). However, there are also pathogenic strains that produce virulence factors and can cause illness in even the healthiest host.

These strains are classified by where and how they cause disease into groups called pathotypes which include enteroaggregative, enterohaemorrhagic, enteropathogenic, enterotoxigenic, uropathogenic, meningitis-associated, and septicemic-associated *E. coli* (Leimbach *et al*., 2013).

The donkey or ass, scientifically known as *Equus africanus asinus*, is a domesticated member of the horse family, Equidae (Grubb, 2005). Donkeys have been used as a work animal for at least 5000 years. They are mostly found in underdeveloped countries where they are used principally as draught or pack animals. In developing countries donkeys are valued in particular for their ability to survive under harsh conditions (Swai and Bwanga, 2008). Interestingly, donkey faeces is sometimes used in rural communities to rub/coat the inner walls of mud buildings where human beings live, and this creates a strong potential for contamination and/or infection of those persons who perform this work and people who live in such houses and touch these surfaces (Pritchard *et al*., 2009). Furthermore, it has been established that donkeys shed *E. coli* (Jesse *et al*., 2015) which poses a great risk to the people handling these animals directly or indirectly, it could also expose these people to diseases caused by this organism. In developing countries,

including Nigeria, Ethiopia, etc animals are commonly slaughtered and processed under unhygienic conditions and this further compromises the microbiological quality and safety of the meat obtained from the animals (Bello *et al*., 2015; Dulo *et al*., 2015). Since *E. coli* commonly resides in the intestines of warm-blooded animals, it is subjected to frequent encounters with antibiotics, providing it with high selection pressure leading to resistance against antibiotics consumed by its host (Looft *et al*., 2012). This led to the hypothesis that the antibiotic resistance patterns of *E. coli* strains from different hosts could be used to track host origin (Harwood *et al*., 2014). While this method was subsequently shown not to be useful for its intended purpose, specific *E. coli* phylogenetic groups were shown to exhibit different resistance levels to antibiotics, regardless of the acquisition of resistance, indicating that the genetic background of *E. coli* also affects its antibiotic resistance pattern (Tenaillon *et al*., 2010; Brisse *et al*., 2012). Thus, the aim of this study was to isolate, characterize and determine the antimicrobial susceptibility of *E. coli* from liver, spleen and small intestine of slaughtered donkeys in Maraban Idah, Kaduna State, Nigeria

MATERIALS AND METHODS

2.1 Study area

This study was carried out in Kaduna State. Kaduna State is located between latitudes (10°35″N) and longitude (7°19 ″E). Agricultural activity within the study area can be divided into two types: rain-fed (from May to October) and irrigation farming in the dry season (from November to April) (Agbogu *et al*., 2006). Animals commonly reared within the study area include cattle, sheep, goats and poultry (Chuo, 2009).

2.2 Study design

This was a cross sectional study in which tissue samples were aseptically collected from apparently healthy slaughtered donkeys at Maraban Idah, Kagarko Local Government Area using convenience sampling method. Samples were also collected from animal owners who consented to the study. For each donkey sampled, sections of the liver, spleen and intestine were collected at the point of evisceration in the abattoir. A total of 384 samples were collected from 128 donkeys from April to August 2018.

2.3 Sample collection

Ten gram (10g) of the tissue sample was aseptically collected from the whole organ of each sampled donkey immediately after slaughter and placed in sterile polythene bags, properly labeled and kept in a cooling box containing ice packs and transported within an hour to the Kaduna Poultry Laboratory, Barnawa, Kaduna for analysis. The samples were taken once every week, usually on the market day of the week when many donkeys are being slaughtered.

2.4 Laboratory procedures

2.4.1 Culture and Identification of Escherichia coli isolates

The isolation of *E. coli* was done using the method described by Campbell *et al*. (2008). Each of the tissue samples were macerated. One gram (1g) of the macerated tissue sample

was added into a sample bottle containing 9 ml of buffered peptone water (Oxoid® UK) and incubated at 37℃ for 24 hours. After 24 hours of incubation, a loop full of the inoculum was streaked on Eosin Methylene Blue (EMB) Agar (Oxoid® UK) and incubated at 37℃ for 24 hours. This process was repeated for all the tissue samples from the different organs separately. The isolates were further subcultured on EMB to obtain a pure culture. The isolates showing greenish metallic sheen colonies on the media were subjected to further examination by carrying out biochemical tests such as Triple Sugar Iron (TSI) Agar, Urease, Citrate, Indole, Methyl Red, Voges Proskauer and Motility agar.

2.4.2 Biochemical Tests

Biochemical tests were performed as described by Cheesbrough (2002). The media were prepared according to the manufacturer's directive. A colony from each plate was taken from the pure culture on EMB and inoculated into the various biochemical media (Triple Sugar Iron (TSI) Agar, Urease, Citrate, Indole, Methyl Red, Voges Proskauer and Motility agar) per sample. The tubes were incubated at 37℃ for 24 hours and results were interpreted based on a laboratory guide by Cheesbrough (2002).

2.4.3 Antibiotic Susceptibility Testing

Kirby-Bauer antibiotics disk diffusion procedure was used with Mueller Hinton Agar (Oxoid® UK), plates and antibiotics impregnated disk. The antibiotics impregnated disks used in this study and their respective concentrations were Oxoid®; enrofloxacin (5 µg), doxycycline (30 µg). oxytetracycline (30 µg), ciprofloxacin (5 µg), amoxicilline (30 µg), gentamicin (10 µg), penicillin (10 µg), colistin(10 µg) and neomycin (30 µg). Two colonies were picked and uniformly spread using a sterile loop over the entire surface of the Mueller Hinton agar media in a petri dish. The antibiotics discs were then placed on the inoculated media using a sterile forcep and incubated at 37℃ for 24 hours after which a ruler was used tomeasure the zone of inhibition in millimeters and recorded. The diameter for each drug was compared with Clinical and Laboratory Standards Institute Standards (2016) and the interpretation chart was used to interpret the zone sizes of inhibition and the results were recorded as susceptible, intermediate or resistant based on the size of the zone of inhibition of each antibiotics disc used in this study (Andrews *et al*., 2005; CLSI, 2016).

2.5 Data analysis

Data collected was analyzed using SPSS software (version 20). Chi-square was used to test for association between *E. coli* and organs sampled from each donkey slaughtered. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

From the 384 tissue samples collected from donkeys at the slaughter slab from the different selected internal organs, 20 were positive for *E. coli* with an isolation rate of 5.2 %. The isolation rate of *E. coli* varies among the different internal organs: 4.7% in the intestine, 4.7 % in liver and 6.3 % in spleen. The highest rate of isolation was recovered from the spleen but statistical analysis of data obtained showed that there was no significant association ($p = 0.810$) between the occurrence of *E. coli* and the type of organ sampled as shown in Table I.

TABLE I: *Escherichia coli* isolation rate from slaughtered Donkeys in Maraban Idah, Kagarko Local Government Area of Kaduna State, Nigeria

Organ/Sample	Number examined	Number Positive	Isolation rate (%)
Liver	128	6	4.7
Spleen	128	8	6.3
Intestine	128	6	4.7
Total	384	20	5.2

(χ^2= 0.422; p= 0.810)

Most of the isolates showed high level of antibiotics resistance to oxytetracycline (90%) and penicillin (95%) followed by amoxycyline (75%). It was also observed that some of the isolates showed intermediate resistance to neomycin (35%), gentamicin (45%) and doxycycline (50%) while most isolates were susceptible to enrofloxacin (80%) and ciprofloxacin (90%) as shown in Table II.

TABLE II: Antibiotics Profiles of *Escherichia coli* isolates from slaughtered donkeys in Maraban Idah, Kagarko Local Government Area of Kaduna State, Nigeria

Antibiotics (µg)	Resistance (%)	Intermediate (%)	Sensitive (%)
Oxytetracycline (30)	18(90)	1(5)	1(5)
Penicillin (10)	19(95)	0(0)	1(5)
Gentamycin (10)	9(45)	2(10)	9(45)
Neomycin (30)	7(35)	1(5)	12(60)
Amoxiciline (30)	15(75)	2(10)	3(15)
Doxycycline (30)	10(50)	4(20)	6(30)
Enrofloxacin (5)	2(10)	2(10)	16(80)
Ciprofloxacin (5)	1(5)	1(5)	18(90)
Colistin (10)	6(30)	0(0)	14(70)

Most of the *E. coli* isolates (75%) displayed resistance to two (2) different classes of antimicrobials, with a total of 5 resistance patterns as shown in Table III.

TABLE III: Antibiotics Resistance Patterns of *Escherichia coli* isolates from slaughtered donkeys at Maraban Idah, Kagarko Local Government Area of Kaduna state, Nigeria

S/No	Resistance Pattern	Number (%) Of *E. coli* Isolates
1	**OT-DO-P-AML**	8(40)
2	**CT-P-AML**	4(20)
3	**OT-DO-GEN-N**	1(5)
4	**P-AML-GEN-N**	1(5)
5	**P-AML-GEN-N-CT**	1(5)

KEY; OT- Oxytetracycline (30µg)
P- Penicillin (10µg)
GEN- Gentamycin (10µg)
N- Neomycin (30µg)
AML- Amoxiciline (30µg)
DO- Doxycycline (30µg)

DISCUSSION

The isolation rate of *E. coli* recovered in donkeys from this study is of public health significance because some strains may be pathogenic. The high isolation rate from the spleen in this study could be as a result of an ongoing clinical infection or may be associated with product contamination during processing which has important implication from the point of view of both meat and public health (Adanech and Temesgen, 2018). The spleen being the organ with the highest isolation differs from the result obtained in a study carried out in India on ducks where the highest isolation was recovered from the intestine (53.19 %) followed by the liver (37.14 %), spleen (30.23 %) (Hui and Das, 2000). Also, in the study carried out in fish from Zeway Lake in Ethiopia, the intestine had the highest isolation of 9% followed by the spleen with 2% and the liver with the lowest (1%) (Adanech and Temesgen, 2018). This disparity may be due to variation in the host sampled and point of sampling. In this study the *E. coli* isolates recovered showed the highest resistance to penicillin (95 %). This finding is similar with the study carried out by Ali *et al*. (2014) who determined the antibiotic resistance pattern of different *E. coli* phylogenetic groups from human urinary tract infection and avian colibacillosis. This could be due to the outer membrane covering surrounding the cell wall of *E. coli* which gives it the ability to create a barrier to certain antibiotics like penicillin such that *E. coli* are not damaged by penicillin (Tortora, 2010). Also, a high resistance to oxytetracycline (90 %) was observed which could probably be due to indiscriminate use and abuse of this antibiotic. Based on this study, 75% of the isolates showed resistance to

two different classes of antimicrobials, with a total of 5 resistance patterns. This could be as a result of frequent use of antibiotics for therapy in donkeys as well as for prophylaxis and growth promotion of food producing animals. Also, inappropriate selection and abuse of antibiotics may lead to resistance in various bacteria which makes the treatment of bacterial infections more difficult (Sabaté *et al*., 2008). Resistance in bacterial populations may spread from one ecosystem to another (Johnson *et al*., 2007) and resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract and also has the ability to acquire resistance from other organisms (Österblad *et al*., 2000). This has made antibiotics resistance to be recognized as an emerging worldwide problem in human and veterinary medicine (Cohen, 2000) both in developed and developing countries. This widespread use of antibiotics in agriculture and medicine has been accepted as a major selective force in the high incidence of antibiotic resistance among gram-negative bacteria (McKeon *et al*., 1995). A variety of foods and environmental sources have been known to harbor bacteria that are resistant to one or more antibiotics used in human or Veterinary Medicine and in food-animal production (Anderson, 2003) and the meat gotten from donkeys based on this study could possibly contribute to multi-drug resistant bacteria in the area of study.

CONCLUSION

The overall isolation rate of *E coli* in slaughtered donkeys in Maraban Idah, Kagarko Local Government Area in Kaduna, Nigeria was 5.2 %. The distribution of *E coli* in slaughtered donkeys based on organ sampled was 4.7 % in intestine, 4.7 % in liver and 6.3 % in spleen**.** Almost all (95%) of the isolate were resistant to penicillin while 90 % were susceptible to ciprofloxacin. A total of 5 different antibiotics resistance patterns were recorded. We therefore recommend that further studies should be done to ascertain the genes responsible for the resistance to antimicrobials observed in the *E. coli* isolates from the slaughtered donkeys in the study. Due to the high antibiotic resistance observed in this study which is of great public health importance, the abuse and indiscriminate use of antibiotics in animals should be discouraged.

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