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Full Length Research Paper

Antibacterial and antioxidant activities of crude aerial part extracts of *Satureja Punctata* (Benth) Briq

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This study evaluates the antimicrobial and antioxidant activities of the crude extract of an aerial part of *Satureja punctata* (Benth.) Briq. The dried aerial part of *S. punctata* (Benth.) Briq was extracted separately using aqueous, ethanol and n-hexane. The antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was assessed using disc diffusion method, and the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) of the extracts were determined by the agar dilution method. The radical scavenging activity of the extracts was evaluated by DPPH assay. The result indicated that the aqueous and ethanol extracts exhibited significant antibacterial activities on the test microorganisms. The antibacterial activity of 600 mg/ml aqueous extract as measured by inhibition zones against *S. aureus* was 10.33±0.25 mm, *E. coli* 9.83±0.19 mm and *P. aeruginosa* 8.53±0.12 mm. The 600 mg/ml ethanol extract inhibited through zone of inhibitions *S. aureus* (10.33±0.41 mm), *E. coli* (9.37±0.18 mm) and *P. aeruginosa* (10.17±0.46 mm). Furthermore, the MIC values for both aqueous and ethanol extracts were from 75 to 100 mg/ml; whereas the MBC values for same extracts were from 100 to 150 mg/ml. The *in vitro* free radical scavenging activity showed that the aqueous, ethanol and n-hexane extracts showed maximum antioxidant activity of 87.89, 78.68 and 47.18 % at 25 mg/ml concentration, respectively. The results showed that the aerial part of *S. punctata* contains components that have antibacterial and antioxidant properties that substantiate the medicinal importance of the plant.

Key words: *Satureja punctata*, antibacterial activity, antioxidant activity, zone of inhibition.

INTRODUCTION

The overuse and abuse of antibiotics in the treatment of bacterial infections has led to the emergence of multiple drug resistant bacteria (MDR) and has become a major cause of failure in the treatment of infectious diseases

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(Basak et al., 2015). Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Reuter et al., 2010). Natural antioxidants have been studied extensively in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage.

Ethnobotanical studies revealed that a wider range of Ethiopian plants are being used in the treatment of various diseases in the traditional health care system of the country (Dawit, 2001; Tilahun and Mirutse, 2010).

Satureja punctata (Benth) Briq (Lamiaceae), locally known as “Lomishet” in the Amharic language, is an erect perennial herb having purple or violet flowers with a pleasant fragrance and grows in different parts of Ethiopia (Sebsebe, 1993). In the traditional health care system of Ethiopia, the aerial parts of *S.punctata* (Benth.) Briq are used for the treatment of diseases such as diabetes mellitus, various other ailments (Tsegaye et al., 2010) and liver disorders (Wolde et al., 2010). The essential oil composition of the leaves of *S. punctata* has been recorded (Chagonda and Chalchat, 2005; Tariku et al., 2010). *In vitro* propagation protocol for *S. punctata* has also been developed, showing the importance of this plant (Teshome et al., 2016).

The preliminary phytochemical analysis of the methanol fraction of S. *punctata* showed the presence of flavonoids, alkaloids, tannins and polyphenols (Wolde et al., 2010). In this study the antibacterial and antioxidant activities of the crude extract of the aerial parts of *S. punctata* was evaluated.

MATERIALS AND METHODS

Chemicals

Ethanol (Reagent chemical Services Ltd., United Kingdom), n-Hexane (Fisher Scientific UK Limited, UK), Sulfuric Acid (SDFCL Fine Chemical Ltd., Mumbai, India), Muller Hinton Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient Broth (Oxoid Ltd, Basingstoke, Hampshire, England), Mannitol Salt Agar (Oxoid Ltd, Basingstoke, Hampshire, England), MacConkey Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Barium Chloride (Griffin, UK), NaCl (Labmerk chemicals (India) PVT Ltd), Tetracycline 30 µg/disc (Oxoid Ltd. Basingstoke, Hampshire England), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) Barium Chloride Dehydrate (BaCl2. 2H2O) (BDH Chemicals Ltd. Poole, England), Methanol (Reagent chemical Services Ltd, United Kingdom) were used for the study

Plant material collection and authentication

The aerial part of S. punctata was collected from a nearby hilly place called Entoto in Addis Ababa, which is located at an altitude of 2720 m in December, 2014. The plant was authenticated by a botanist and a representative sample with the specimen number MM01/14 was kept at the Natural Herbarium of Addis Ababa University (AAU), Addis Ababa, Ethiopia

Preparation of the plant material

The collected aerial part was washed thoroughly with tap water to remove dust particles, spread over clean cloth and then kept in an open shady area for 14 days in order to avoid loss of volatile compounds by direct sun light.

After complete dryness, plant sample was ground using a coffee grinder to a fine powder and then sieved using a mesh of 0.5 mm size. The powder was stored in an airtight closed bottle for further use.

Preparation of the extracts

The crude extracts were prepared by cold maceration technique (O’Neill et al., 1985). The dried powder (100 gm) of S. punctata was extracted by, soaking separately 1 L each of ethanol, n-hexane and aqueous (1/10 w/v) in conical flasks. The flasks were plugged with cotton wool and wrapped with aluminum foil and put on orbital shaker at 120 rpm for 72 h at room temperature. The extracts were filtered through a cotton plug followed by a qualitative filter paper.

After filtration, the n-hexane and ethanol extracts were subjected to partial concentration using rotary evaporator, attached to a vacuum pump set in a water bath at 45°C. The partially concentrated extracts in screw capped bottle were placed in an oven at 40°C for complete drying. The aqueous extracts were placed in deep-freeze at -20°C for 24 h and allowed for lyophilization to obtain fine crude extract. The dry residues of n-hexane, ethanol and aqueous extracts were weighed and, the yield of the extracted samples was calculated using the following formula:

Percent of yield of the extract = final weight (gm)/initial weight (gm) x 100

All extracts were reconstituted with their respective solvent for antibacterial test and were dissolved in methanol for antioxidant activity test.

Test microorganisms

Standard bacteria culture of Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 25922) were obtained from the Microbiology Department, Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

Preparation of inoculums

All test strains were transferred into sterile nutrient broth and incubated at 37°C for 24 h, then streaked on 90 mm Petri dishes using sterilized loop, which contained sterilized selective media and incubated at 37°C for 24 h. Four to five well-isolated colony of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 10 ml of 0.85% sterile normal saline to dilute the suspension.

The turbidity of the 0.5 McFarland standard was prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulfuric acid (Lalitha, 2009). The turbidity of the actively growing bacteria suspension was adjusted with sterile saline to

obtain turbidity comparable to that of the 0.5 McFarland standards. This resulted in a suspension containing approximately 1.5 x 108 CFU/ml.

Determination of antibacterial activity

The antibacterial activity of the ethanol and aqueous extracts was evaluated using disc diffusion method (NCCLS, 1997). Sterilized Muller Hinton Agar (25 ml) was poured in 90 mm petri dishes which were allowed to solidify. The plates were seeded with suspension of test bacteria adjusted to 10^8cells/ml using sterile cotton swab and allowed to settle for 10 min. Sterile, 6 mm diameter filter paper discs were soaked each in plant extracts at different concentrations (300, 400 and 600 mg/ml) and placed on the surface of the inoculated media agar plates using sterile forceps, gently pressed down onto the agar surface.

Disk soaked with solvents and tetracycline 30 µg/disc was used as negative and positive controls, respectively. All plates were incubated at 35 to 37°C for 24 h. Clear inhibition zones around the discs, indicated the presence of antibacterial activity. Diameter of inhibition zones was measured in millimeters. Each experiment was carried out in triplicates and the mean diameter of the inhibition zones was recorded for each test organism.

Determination of minimum inhibitory concentration (MIC) values

The MIC of the ethanol and aqueous extracts of plant was determined by agar dilution technique (NCCLS, 2002). Series of two folds dilution of ethanol and aqueous extracts (300 to 37.5 mg/ml) each was mixed with 19 ml of sterilized and molten nutrient agar medium and 1 ml each concentration was poured into pre-labeled sterile petri dishes.

Plates were dried at 35°C for 30 min prior to spot inoculation with bacterial suspension (adjusted to 0.5 McFarland standards), containing approximately $1.5x10^8$ CFU/spot using a sterilized inoculating loop. Nutrient agar with solvent was used as a positive control. The inocula spots were allowed to dry at room temperature and plates were incubated at 35 to 37°C for 24 h. Each test was done in triplicate. Growth inhibition was judged by comparison with growth in the control plates.

Determination of MBC (Minimum bactericidal concentration) values

The MBC of the extracts on the test isolates was determined according to Mathur (2013). Fresh nutrient agar medium was poured into petri dishes and allowed to solidify. Inocula from the different plates of MIC experiment that did not show any growth was subcultured on freshly prepared plates. The lowest concentration at which test bacteria did not recover on fresh medium was considered to be MBC.

***In vitro* antioxidant activity**

The radical scavenging activity of plant extracts was determined on the basis of the radical scavenging effect of DPPH (Blois, 1958), which is a known compound for test. Dose dependent concentrations of 1.56, 3.12, 6.25, 12.5 and 25 mg/ml; 1 ml each of the extract and the standard control ascorbic acid was mixed with 2 ml each of 0.1 mM solution DPPH in methanol in labeled tubes.

The tubes were incubated in dark for 30 min at room temperature and then the absorbance was measured at 517 nm using UV-Vis spectrophotometer 201215 (Single Beam, India). The control was prepared by mixing 2 ml of DPPH solution with 2 ml methanol. Experiment was done in triplicates. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

%DPPH radical scavenging capacity = A control – A sample/A control x 100

Where;

A control is the absorbance of DPPH radical + methanol reaction
A sample is the absorbance of DPPH radical + sample extract /standard
The antioxidant activity of the different extract was expressed as % inhibition.

Data analysis

The data obtained for antibacterial and antioxidant tests were analyzed with Microsoft office Excel 2007. Results were expressed as mean ± SEM. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *Post Hoc* Multiple Comparison Tests using statistical software (SPSS) package version 20.0 for windows. P-value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Yield of extraction

The yields in grams and percentage (w/w) of n-hexane, ethanol and aqueous extracts of the aerial parts of *S. punctata* were 2.11 (4.63), 6.28 (12.56) and 5.10 gm (10.19%), respectively. The ethanol extracts give relatively higher yield.

Antibacterial activity

The aqueous and ethanol extracts showed dose dependent antibacterial activity against *S. aureus*, *E.coli* and *P. aeruginosa* at concentrations 600, 400 and 300 mg/ml (Table 1). For example the inhibition zones at the highest concentration of 600 mg/ml were 10.33±0.41mm for *S. aureus*, 9.37±0.18 mm for *E. coli* and 10.17±0.46 mm for *P. aeruginosa*. The standard drug tetracycline (Tet) showed inhibition zone of 25.00±0.00, 22.33±1.4 and 10.17±0.17 mm for *S aureus, E. coli* and *P. aeruginosa*, respectively.

Inhibition of test bacteria by tetracycline is 2 to 3 times higher when compared to ethanol and aqueous extracts as shown in Table 1. The n-hexane extract of *S. punctata*

Table 1. Antibacterial activity of *S. punctata* at different concentrations

Plants	Types of solvent/extracts	Concentration (mg/ml)	Zone of inhibition (mm)		
			S. aureus	***E. coli***	***P. aeruginosa***
Satureja punctata	Aqueous	300	8.01±0.29[c]	7.79±0.23[c]	7.27±0.27[c]
		400	9.07±0.13[b]	8.67±0.11[b]	7.83±0.15[b]
		600	10.33±0.25[a]	9.83±0.19[a]	8.53±0.12[a]
	Ethanol	300	8.17±0.39[c]	7.93±0.17[c]	7.73±0.32[c]
		400	9.04±0.22[b]	8.54±0.29[b]	8.43±0.28[b]
		600	10.33±0.41[a]	9.37±0.18[a]	10.17±0.46[e]
	n-hexane	300	-	-	-
		400	-	-	-
		600	-	-	-
+ve control (Tet)	-	30 µg/disc	25.00±0.00[d]	22.33±1.45[f]	10.17±0.17[e]
-ve Control	-	-	-	-	-

Data is represented as mean ± SEM (N=3). Values followed by different letters indicate statistical significance (-) in the table which showed no

Table 2. MIC and MBC (mg/ml) of ethanol and aqueous extract of *S. punctate*

Test organisms	Ethanol Extract (mg/ml)		Aqueous Extract(mg/ml)	
	MIC	**MBC**	**MIC**	**MBC**
S. aureus	100	150	75	100
E. coli	75	100	75	100
P. aeruginosa	100	150	100	150

did not show any antibacterial activity against the tested pathogenic microorganisms at the given doses.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values of the different bacteria are shown in Table 2. The ethanol extract of *S. punctata* was 100, 75, 100 and 150, 100 and 150 mg/ml, against *S. aureus, E. coli* and *P. aeruginosa,* respectively (Table 2). Other workers have reported the antibacterial effect of *Satureja*. The methanol and hexane extracts of aerial part of *Satureja hortensis* L. was tested against *E. coli* (Sahin et al., 2003). The authors found that, the MIC values of the methanol extract were 0.25 to 0.5 mg /ml but the hexane extract did not show any inhibition on *E. coli*, *P. aeruginosa* and *S. aureus* (Sahin et al., 2003). This agrees with the present study, in which n-hexane extract of aerial part *S. punctata* did not show any zone of inhibition against the above microorganisms.

Furthermore, the ethanol and aqueous extracts of *Satureja bachtiarica* at 40 mg/ml has shown zones of inhibitions of 8.8±0.28 and 6.4±0.5 mm against *P. aeruginosa*, respectively (Sureshjani et al., 2013). The results of Sureshjani and co-workers (2013) showed better activity at a lower concentration than results obtained in the present study. The difference could be due to the species difference, extraction procedure of the plant parts or any other factor that contributes to the plant biology. In addition, other workers tested the antimciorbial activity of the methanol extract of the aerial part of *S. kitaibelii* Wierzb Ex heuff (Stanojkovic et al., 2013). Therefore, the present study is an important addition to the antibacterial property of genus Satujera.

The antioxidant test

The result of the antioxidant activity is presented in Figure 1. Data showed that, all extracts demonstrated

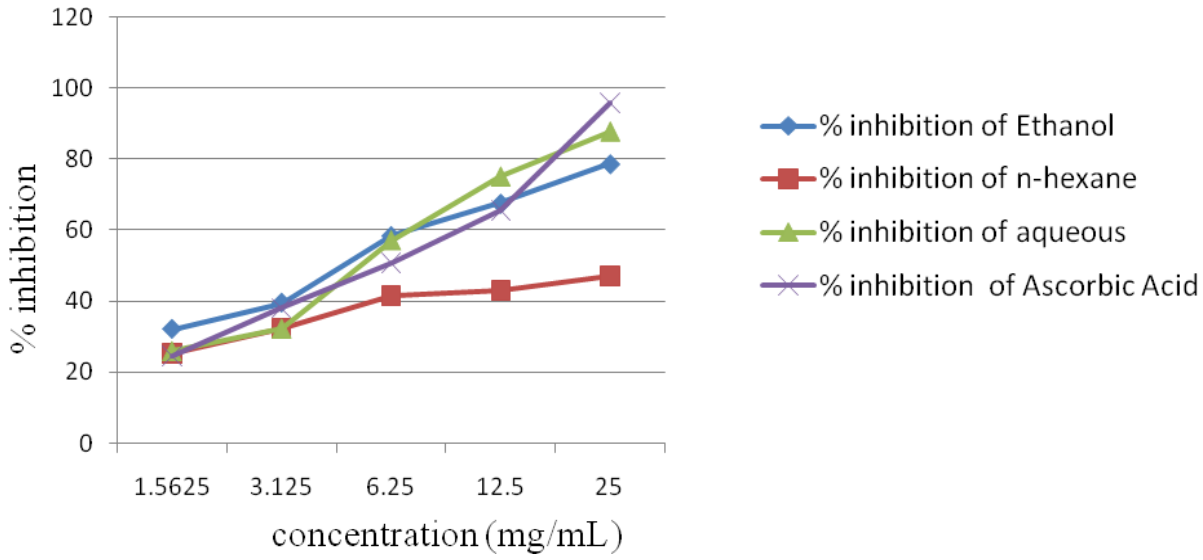

Figure 1. Antioxidant activity of aqueous,ethanol and n-hexane extract of *S.punctata*

dose dependent percentage inhibition. For example, at a concentration of 1.5625 and 25 mg/ml the percentage inhibition was 26.05 and 87.89%, respectively.

However, significant inhibitions are only observed for aqueous and ethanol extracts. The n-hexane extract exhibited limited inhibition (Figure 1). Highest radical scavenging effect was found in aqueous extract, followed by ethanol extract and the lowest by n-hexane at concentration 25 mg/ml is 87.89, 78.67 and 47.18%, respectively. A similar result was reported by Wolde and co-workers (2010), substantiating the antioxidant activity of *S. punctata*.

Conclusion

The results of the present study confirmed that, *S. punctata* aerial part has an antimicrobial and antioxidant constituents. Further fractionation of the crude extract and purification of the active compounds is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Basak S, Singh P, Rajurkar M (2016). Multidrug resistant and extensively drug resistant bacteria: A study. Journal of Pathogens 2016:1-5.

Blois MS (1958). Antioxidant determination by the use of a stable free radical. Nature 181(4617):1199-1200.

Chagonda LS, Chalchat JC (2005). The composition of the leaf essential oil of Satureja punctata (Benth.) Briq. from Zimbabwe. Flavour and Fragrance Journal 20(3):316-317.

Dawit A (2001). The Role of Medicinal Plants in Healthcare Coverage of Ethiopia, the possible integration. In: Proceeding of the National workshop on Biodiversity Conservation and Sustainable Use of Medicinal Plants in Ethiopia. Medhin Z, Abebe D (eds.). IBCR, Addis Ababa. pp. 6-21. Available at: http://agris.fao.org/agris-search/search.do?recordID=ET2003000174

Lalitha MK (2009). Manual on Antimicrobial Susceptibility Testing. (Under the Auspices of Indian Association of Medical Microbiologists). American Society for Microbiology, Washington DC. Available at: http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1220937

Mathur R (2013). Phytochemical and antimicrobial evaluation of plant extracts of Enicostemma hyssopifolium. Journal of Pharmacognosy and Phytochemistry 2(4):30-36.

National Committee for Clinical Laboratory Standards (NCCLS) (1997). Performance standards for antimicrobial disk susceptibility tests: approved standard: NCCLS document M2-A6. Wayne, Penn. Available at: http://www.worldcat.org/title/performance-standards-for-antimicrobial-disk-susceptibility-tests-approved-standard-nccls-document-m2-a6/oclc/440346709

National Committee for Clinical Laboratory Standards (NCCLS) (2002).

Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Available at: http://demo.nextlab.ir/getattachment/45f0bc90-98b5-4705-a4ad-83c4723c6310/CLSI-M31-A2.aspx

Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010). Oxidative stress, inflammation, and cancer: how are they linked?. Free Radical Biology and Medicine 49(11):1603-1616.

Sahin F, Araman T, Gulluce M, Ogutcu H, Sengul M, Adiguzel A, Ozturk S, Kotan R (2003). Evaluation of Antimicrobial Activities of *Satureja hortensis* L. Journal of Ethnopharmacology 87(1):61-65.

Sebsebe DA (1993). Description of some essential oil bearing plants in Ethiopia and their indigenous uses. Journal of Essential Oil Research 5(5):465-479.

Stanojkovic T, Kolundzija B, Ciric A, Sokovic M, Nikolic D, kundakovic T (2013). Cytotoxicity and Antimicrobial Activity of *Satureja kitaibelii* WIERZB. EX HEUFF (Lamiaceae). Digest Journal of Nanomaterials and Biostructures 8(2):845-854.

Sureshjani MH, Yazdi TF, Mortaza A, Shahidi F, Behbahani BA (2013). Antibacterial effect of *Satureja bachtiarica* extracts aqueous, ethanol methanol and glycerin on *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* Scientific Journal of Microbiology 2(2):53-60.

Tariku Y, Hymete A, Hailu A, Rohloff J (2010). Essential-oil composition, antileishmanial, and toxicity study of *Artemisia abyssinica* and *Satureja punctata* ssp. punctata from Ethiopia. Chemistry and Biodiversity 7(4):1009-1018.

Teshome I, Teshome S, Soromessa T, Feyissa, T (2016). Development of an efficient in vitro propagation protocol for *Satureja punctata* – A rare aromatic and medicinal plant. Taiwania 61(1):41-48.

Tilahun T, Mirutse G (2010). Ethnobotanical study of wild edible plants of Kara and Kwego semi-pastoralist people in Lower Omo River Valley, Debub Omo Zone, SNNPR, Ethiopia. Journal of Ethnobiology and Ethnomedicine 6(1):23.

Tsegaye W, Urga K, Asres K (2010). Hypoglycaemic activity of extracts of the aerial part of *Satureja punctata* Benth. Briq in streptozotocin-induced diabetic mice. Ethiopian Journal of Biological Sciences 9(2):143-152.

Wolde T, Engidawork E, Asres K, Eregete W (2010). Evaluation of hepatoprotective activities of Satureja punctata Benth Briq and Solanecio angulatus Vahl Jeffrey in ferric nitrillotriacetate induced hepatotoxicity in rats. Pharmacology Journal 28:63-74.

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Full Length Research Paper

In vitro assessment of *Leuconostoc mesenteroides* zinc nanoparticles against *Salmonella* serovars recovered from broilers chickens

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Salmonella **serovars are responsible for a variety of acute and chronic diseases in poultry.** ***Leuconostoc mesenteroides*** **probiotic can exert antimicrobial activity by producing diverse fermentative metabolites with bactericidal or bacteriostatic activities such as lactic and acetic acids, fatty acids, hydrogen peroxide or diacetyl and antimicrobial proteins such as bacteriocins and peptidoglycan hydrolase enzymes. This trial aimed at adding novel therapy against virulent and multidrug resistance avian salmonellosis. The incidence of salmonellae in this study was 20% with high recovery rate from liver followed by yolk sac and the most common serovars were** ***Salmonella*** **Kentukey,** ***Salmonella*** **Infantis and** ***Salmonella*** **Enteritidis whose antibiogram showed high resistance to ampicillin, nalidexic acid, sulphamethoxasole + trimethoprim and tetracyclines. Selected virulent and multidrug resistant** ***Salmonella*** **serovars were exposed to probiotic mixture consisting of** ***L. mesenteroides*** **and zinc nanoparticles in different concentration to detect the antibacterial effect against different** ***Salmonella*** **serovars as novel therapy for avian salmonellosis. This study revealed that green synthesis of zinc nanoparticles by using** ***L. mesenteroides*** **biodegradation 100 nm in size and 10 µg/ml in concentration has potent inhibitory effect against broad range of** ***Salmonella*** **serovars but its salmonicidal effect occurred only at 2000 µg/ml.**

Key words: Broilers chicken, *Salmonella*, *Leuconostoc mesenteroides*, zinc nanoparticles.

INTRODUCTION

Avian salmonellosis can develop as a result of infection with poultry-specific serovars, causing systemic illness in birds (Gast, 2003). Great attention has been paid to bacterial resistance to antibiotics for its adverse impacts on morbidity and mortality from diseases caused by resistant bacteria, economic costs of therapy and high risks of the spread of resistant strains among animals and humans (White et al., 2001). Probiotics are defined

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as viable microorganisms, which in sufficient numbers, alter the microbiota of a host body compartment and thereby exert beneficial health effects (Shida-Nanno, 2008). The use of probiotics in enhancing intestinal health has been proposed for many years through several postulated mechanism including competition for limited nutrients, inhibition of the epithelial and mucosal adherence of pathogens, inhibition of epithelial invasion by pathogens, the production of antimicrobial substances and/or the stimulation of mucosal immunity (Servin and Coconnier, 2003). Lactic acid bacteria (LAB) are regarded as a major group of probiotic bacteria.

They are usually described as Gram-positive bacteria, devoid of cytochromes and preferring anaerobic conditions, but are aerotolerant, fastidious, acid-tolerant and strictly fermentative, producing lactic acid as a main product. The most important genera are: *Lactobacillus, Lactococcus, Enterococcoccus, Streptococcus, Pediococcus, Leuconostoc* and *Bifidobacterium* (Vasiljevic and Shah, 2008; Perez et al., 2014). *Leuconostoc mesenteroides*, a member of the LAB occurs in several naturally fermented foods and known to produce biodegradable glucose polymer dextran that has wide range of applications in food, cosmetics, pharmaceutical and oil industries (Aman et al., 2012). *L. mesenteroides* is known to produce both water soluble and insoluble dextran (Shukla et al., 2011). While importance of zinc as an essential nutrient has been recognized for many years, only recently, researchers understood the full impact of this nutrient on animal health as they identified 200 zinc dependent enzymes in all the major biochemical pathways in the body (Case and Carlson, 2002).

Nanotechnology has opened the way for introduction of functional nanostructures which can be used as building blocks to create novel finding such as antimicrobial biodegradable materials that is effective against a variety of pathogens including Gram negative pathogens, so the current study aimed to investigate the inhibitory effect of *L. mesenteroides* Zn-NPs on broiler chicken *Salmonella* serovars.

MATERIALS AND METHODS

Isolation, identification, virulence and antibiotic resistance profile of *Salmonella* serovars from broiler chickens

Under complete sterile condition, broilers internal organs including liver, yolk sac, lung, caecum and spleen were examined for isolation and identification of Salmonellae according to (ISO, 2002); the recovered isolates were serotyped in the Central Laboratory for Quality Control on Poultry Production (CLQP) in Dokki, Giza, Egypt, according to Kauffmann and Das Kauffmann (2001). The antibiogram disk diffusion technique was adapted according to CLSI (2017).

Green synthesis of Zn-NPs using *L. mesenteroides* probiotic

L. mesenteroides NRRL B-1149 was propagated as stab in MRS agar medium at 30°C according to Goyal and Katiyar (1996) as used in biodegradation of zinc sulphate as a substrate for production of Zn-NPs sized 100 nm according to Otari et al. (2012) with slight modification and characterized in the central laboratory of elemental and isotopic analysis, nuclear research center, atomic energy authority, Egypt according to the technique of Mashrai et al. (2017).

In vitro assessment of *L. mesentroids* Zn-NPs against *Salmonella* serovars

Selected virulent and multidrug resistant *Salmonella* serovars were cultured in Tryptic soya broth and incubated at 30°C for 24 h. In order to examine the antibacterial activity of the Zn-NP on *Salmonella* serovars, ZnO nanoparticles were suspended in sterile normal saline and constantly stirring until a uniform colloidal suspension. Agar diffusion method was carried out according to Perez et al. (1990) and determination of minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) as antimicrobial activity nano-ZnO according to Chwalibog et al. (2010).

RESULTS

Incidence and antibiogram of broilers chickens Salmonella

From the result of this study, the incidence of *Salmonella* in broilers chickens as shown in Table 1 were 21% with high recovery rate from liver followed by yolk sac. Serotyping of recovered *Salmonella* isolates in Table 2 revealed that the most common serovars were *Salmonella* Kentukey, *Salmonella* Infantis and *Salmonella* Enteritidis whose antibiogram as shown in Table 3 high resistance to ampicillin (90%), nalidexic acid (88%), sulphamethoxasole + trimethoprim (82%) and tetracyclines (82%).

Effect of *L. mesenteroides* zinc nanoparticles on broilers chickens *Salmonella*

The selected virulent and multidrug resistant *Salmonella* serovars in the present study were exposed to *L. mesenteroides* zinc nanoparticles (Figure 1) in different concentrations to detect its antibacterial effect as novel therapy for avian salmonellosis. The results (Table 4) revealed that zinc nanoparticles 100 nm in size and 10 µg/ml in concentration has potent inhibitory effect against broad range of *Salmonella* serovars but its salmonicidal effect occurred only at 2000 µg/ml as shown in Figure 2) with destruction of *Salmonella* cell wall after treatment with nanoparticles.

DISCUSSION

Salmonellosis in poultry is a worldwide problem both for poultry and as a vehicle for human disease (Sharp,

Table 1. Incidence of *Salmonella* serovars in broiler chickens organs.

Organ	N = samples	n= positive	%	All %
Liver	45	14	31(14/45)	5.8(14/240)
Yolk Sac	60	12	20(12/60)	5(12/240)
Lung	40	4	10(4/40)	1.6(4/240)
Caecum	50	12	24(12/50)	5(12/240)
Spleen	45	8	17.7(8/45)	3.3(8/240)
Total	240	50	--	20.7%(50/240)

Table 2. Recovery of *Salmonella* serovars isolated from broilers chickens organs.

Organs	Serotypes	Number of serovars	%
Liver	*S.* Kentucky	7/14	50
	S. Enteritidis	4/14	29
	S. Pullorum	1/14	7
	S. Infantis	1/14	7
	S. Newport	1/14	7
Yolk sac	*S.* Kentucky	5/12	42
	*S.*Enteritidis	2/12	17
	S. Heidelberg	2/12	17
	S. Infantis	2/12	17
	S. Virginia	1/12	8
Lung	*S.* Hiedelberg	2/4	50
	S. Labadi	1/4	25
	S. Infantis	1/4	25
Caecum	*S.* Kentucky	7/12	58
	S. Typhi	1/12	8
	S. Infantis	4/12	33
Spleen	*S.* Kentucky	6/8	75
	S. Agona	1/8	12.5
	S. Infantis	1/8	12.5

1991). Pathogenesis of *Salmonella* depends on its ability to survive and replicate inside host cells. This virulence trait is linked to the ability to cause systemic infections and a large number of genes are required to enable *Salmonella* to cope with nutritional limitations, to avoid clearance by the host immune system or survive damage by antimicrobial peptides and radicals (Hegazy and Hensel, 2012). The occurrence of *Salmonella* Enteritidis has significant increase in poultry carcasses from 2000 to 2005 in the US. Studies between 2000 and 2009 showed that the predominance of *Salmonella* serovar in poultry was *Salmonella* Enteritidis, which was resistant to multiple antibiotics, including marked resistance to third generation cephalosporins. In the past years in the US, increased resistance to Ceftiofur was observed in poultry strains. In 1997, resistance to this antibiotic was 1.6%, and in 2003, it was 7.4% (Medeiros, 2011; Voss-Rech et al., 2015). In the current investigation, incidence of *Salmonella* in broiler chickens shown in Table 1 were 21% with high recovery rate from liver followed by yolk sac. Serotyping of recovered *Salmonella* isolates in Table 2 revealed that the most common serovars were *S.* Kentukey, *S.* Infantis and *S.* Enteritidis with antibiogram shown in Table 3 with high resistance to ampicillin (90%), nalidexic acid (88%), sulphamethoxasole + trimethoprim (82%) and tetracyclines (82%). Antibiotic resistance mechanisms can be categorized as (i) modification or destruction of the antimicrobial agent, (ii) pumping the

Table 3. Resistance pattern of *Salmonella* serovars recovered from broiler chickens.

Antimicrobial agents	Resistance patterns					
	R*	%	I*	%	S*	%
Sulphamethaxole + Trimethoprim (SXT)	41/50	82	0/50	0	9/50	18
Amikacin 30 µg	0/50	0	2/50	4	48/50	96
Imepenem 10 µg	0/50	0	3/50	6	47/50	94
Tetracyclines 30 µg	41/50	82	1/50	2	8/50	16
Ampicillin 10 µg	45/50	90	1/50	2	4/50	8
Nalidixic acid 30 µg	44/50	88	1/50	2	5/50	10
Chloramphenicol 30 µg	21/50	42	2/50	4	27/50	54
Gentamicin 10 µg	1/50	2	3/50	6	46/50	92
Ciprofloxacin 5 µg	29/50	58	17/50	34	4/50	8
Azetreonam 30 µg	8/50	16	3/50	6	39/50	78
Ampicillin + Sulbactam 20 µg	12/50	24	4/50	8	34/50	68
Cefepem 30 µg	8/50	16	2/50	4	40/50	80
Ceftriaxone 30 µg	7/50	14	5/50	10	38/50	76
Cephalothin 30 µg	16/50	32	12/50	24	22/50	44
Cefotaxime 30 µg	4/50	8	0/50	0	46/50	92
Ceftazidem 30 µg	4/50	8	0/50	0	46/50	92

*R: Resistant, I: intermediate, S: sensitive.

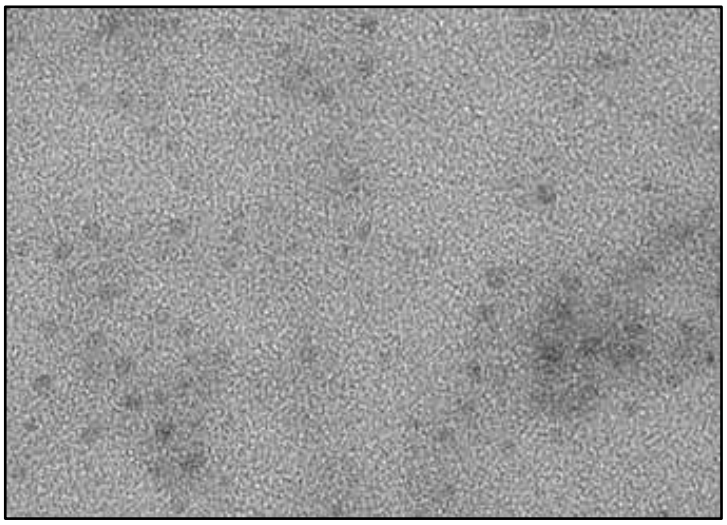

Figure 1. SEM images of *Leuconostic* Zn-Nps.

antimicrobial agent out from the cell by efflux pumps, (iii) modification or replacement of the antibiotic target and (iv) decrease in cell membrane permeability. Walsh (2003) also showed that resistance to antibiotics is due to temporary or permanent change of bacterial genetic information. Most resistance genes are found in plasmids. Acquired resistance is caused by the transfer of resistance genes from one cell to another (Tavares, 2001).

Currently nanotechnology has the potential to impact many aspects as: food security, disease treatment delivery system, new tools in cellular and molecular biology, new materials for pathogens detection (Weiss et al., 2006). Recent studies showed that nanoparticles particularly, zinc oxide had selective toxicity to micro-organisms (Reddy, 2007). The study is on evaluation of prepared *L. mesentroides* zinc nanoparticles as potent agent against broilers chickens Salmonellae; thus, selected *Salmonella* serovars which is virulent and multidrug resistant were exposed to different concentration of this molecules to detect its antibacterial effect against avian salmonellosis; the result in Table 4

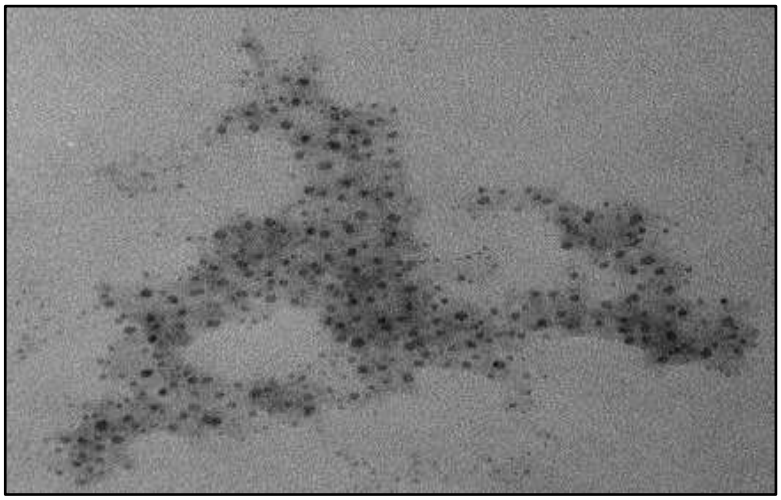

Figure 2. SEM images of *Salmonella* after exposure to *Leuconostic* Zn-Nps (complete damage of *Salmonella*).

Table 4. Anti-*Salmonella* effect of *L. mesentroids* Zn-NPs.

Zn-Nps concentration (µg/mL)	Agar diffusion methods (zone of inhibition of *Salmonella*) (mm)	Minimum inhibitory concentration, MIC (µg/ml)	Minimum bactericidal concentration, MBC (µg/ml)
10	8.5±1.24	< 10	1000
20	12.3±1.54	< 10	1000
40	14.7±1.34	< 10	1000
60	15.5±1.65	< 10	2000
80	16.2±1.22	< 5	2000
100	17.5±1.55	< 5	2000

showed that zinc nanoparticles 100 nm in size and 10 µg/ml in concentration has potent inhibitory effect against broad range of *Salmonella* serovars but its salmonicidal effect occurred only at 2000 µg/ml. Advances in the field of nanosciences and nanotechnology have brought to form nanosized inorganic and organic particles in medicine and therapeutics (Gajjar et al., 2009). Antimicrobial effect of zinc nanoparticles (Zn-NPs) occurs by different ways such as: formation of H_2O_2 which retard microbial growth, another way is by releasing of Zn^{+2} which damage microbial cell membrane and interact with intracellular contents (Moraru et al., 2003), while Violeta et al. (2011) attributed the antimicrobial activities of Zn-NPs to photocatalytic production of reactive oxygen species that damage cell components and interrupt energy transduction. Recently, new safe antimicrobial agents were needed to prevent and overcome bacterial infections. The large increase in the number and occurrence of antibiotic resistant bacterial strains has prompted a renewed interest in the use of metals as antibacterial agent (Odds et al., 2003).

Conclusion

This study suggests that broilers play a potential role as a reservoir of multi drug resistant and virulent *Salmonella* serovars with special reference to novel control methods by lactic acid bacteria (LAB) as *L. mesenteroides* zinc nanoparticles; the molecule proved as *in vitro* inhibitory agent for *Salmonella* in broiler chickens.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aman A, Siddiqui NN, Qader SA (2012).Characterization and potential applications of high molecular weight dextran produced by Leuconostoc mesenteroides AA1, Carbohydrate Polymer 87:910-915.

Case CL, Carlson MS (2002). Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in

nursery pigs. Journal of Animal Science 80:1917-1924.
Chwalibog A, Sawosz E, Hotowy A, Szeliga J, Mitura S, Mitura K, Grodzik M, Orlowski P, Sokolowska A (2010). Visualization of interaction between inorganic nanoparticles and bacteria or fungi. International Journal of Nanomedicine 5:1085-1094.
Clinical and Laboratory Standards Institute (CLSI) (2017). Clinical and Laboratory Standards Institute (Formerly NCCLS), Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. Approved Standard M100-S24, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.
Gajjar P, Pettee B, Britt DW, Huang W, Johnson WP, Anderson AJ (2009). Antimicrobial activities of commercial nanoparticles against environmental soil microbe *Pseudomonas putida* KT2440. Journal of Biological Engineering 13:1-13.
Gast RK (2003). Paratyphoid infections. In: Diseases of Poultry Saif YM, Barnes HJ, Fadly AM, Glisson JR, McDougald LR, Swayne DE (eds.), Iowa State University Press: Ames IA. pp. 583-613.
Goyal A, Katiyar SS (1996). Regulation of dextransucrase productivity of Leuconostoc mesenteroides NRRL B-512F by the maintenance media. The Journal of General and Applied Microbiology 42(1):81-85.
Hegazy WAH, Hensel M (2012). *Salmonella enterica* as a vaccine carrier. Future Microbiology 7:111-127.
International Organization for Standardization (ISO) (2002). Microbiology for Food and Animal Feeding Stuffs: Horizontal Method for the Detection of Salmonella Spp. Available at: https://www.salmonella360.com/cms3/assets/fullsize/955
Kauffmann F, Das-Kauffmann W (2001). Antigenic formulas of the Salmonella serovars. WHO co-operating center for reference and research on Salmonella.8th Ed, cited by pop off, M.Y., Paris, France.
Mashrai A, Khanam H, Aljawfi RN (2017). Biological synthesis of ZnO nanoparticles using C. albicans and studying their catalytic performance in the synthesis of steroidal pyrazolines. Arabian Journal of Chemistry 10:S1530-S1536.
Medeiros MAN (2011). Prevalência de *Salmonella* spp. e resistência antimicrobiana dos isolados em carcaças de frango congelado no varejo. Brasil, 2004 a 2006. In: Seminário Internacional de Salmoneloses Aviárias, Rio de Janeiro, RJ, Anais..., CD Room. 2011.
Moraru CI, Panchapakesan CP, Huang Q, Takhistove P, Liu S, Kokini JL (2003). Nanotechnology: a new frontier in food science. Food Technology 57(12):24-29.
Odds FC, Brown AJ, Gow NA (2003). Antifungal agents: mechanisms of action. Trends in Microbiology 11(6):272-279.
Otari SV, Patil RM, Nadaf NH, Ghosh SJ, Pawar SH (2012). Green biosynthesis of silver nanoparticles from an actinobacteria Rhodococcus sp. Materials Letters 72:92-94.
Perez C, Pauli M, Bazevque P (1990). An Antibiotic assay by the agar well diffusion methods. Acta Biologiae ET Medicine Experimentals 15:113-115.
Perez RH, Zendo T, Sonomoto K (2014). Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microbial Cell Factories 13(1):S3.
Reddy KM (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Applied Physics Letters 90(21):213902.
Servin AL, Coconnier MH (2003). Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Practice and Research Clinical Gastroenterology 17(5):741-754.
Sharp JCM (1991). Food borne infection in poultry. Journal of the Royal Society of Health 111:335-337.
Shida K, Nanno M (2008). Probiotics and immunology: separating the wheat from the chaff. Trends in Immunology 29(11):565-574.
Tavares W (2001). Manual de antibióticos e quimioterápicos antiinfecciosos." 3 ed. São Paulo: Editora Atheneu.
Vasiljevic T, Shah NP (2008). Probiotics from Metchnikoff to bioactives. International Dairy Journal 18:714-728.
Violeta V, Catalin P, Constantin F, Monica A, and Marius B (2011). Nanoparticles applications for improving the food safety and food processing. 7th International Conference on M a trials Science and Engineering, Bramat, Brasov, 24-26 February 2011, 77 p.
Voss-Rech D, Clarissa SL, Alves L, Coldebella A, Joice A, Leão JA, Rodrigues DP, Back A (2015). A temporal study of Salmonella enterica serotypes from broiler farms in Brazil. Poultry Science 94(3):433-441.
Walsh C (2003). Antibiotics: actions, origins, resistance. American Society for Microbiology. 335p. Available at: https://www.cabdirect.org/cabdirect/abstract/20043133125
Weiss J, Takhistov P, McClements DJ (2006). Functional materials in food nanotechnology. Journal of Food Science 71(9):107-116.
White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J (2001). The isolation of antibiotic-resistant Salmonella from retail ground meats. New England Journal of Medicine 345(16):1147-1154.

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Antibiotic resistance genes in diarrheagenic *Escherichia coli* (DEC) isolated from livestock organic wastes in Ouagadougou, Burkina Faso

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Diarrheagenic *Escherichia coli* (DEC) are often disseminated through the fecal matter of livestock and waste products including slurry and manure. The study aimed to characterize archived DEC recovered from cattle fecal matter, manure and slurry for quinolone resistance and extended spectrum beta-lactamases (ESBLs) with focus on trends in antimicrobial susceptibility patterns. The susceptibility of the bacteria was tested using standard laboratory procedures. Polymerase chain reaction (PCR) was carried out to detect the presence of *qnrA, qnrB, qnrS* genes and β-lactamase producing genes (bla_{ESBL}) such us bla_{TEM} and bla_{SHV}. About 91% of DEC strains were multidrug resistant (MDR) with non-susceptibility to ≥1 agent in ≥3 antimicrobial classes. The most common resistance was to amoxicillin-clavulanic acid (96.36%), followed by tretacycline (89.09%), ceftazidime (76.36%), and cefotaxime (70.780%). *qnrS* (18.2%) was the most prevalent quinolone resistant genes, followed by *qnrB* (7.2%) and *qnrA* (2%). bla_{TEM} (5.45%) was most prevalent than bla_{SHV} genes (3.6%). bla_{TEM} and bla_{SHV} genes were identified in double or multiple-carrying with *qnrS* and *qnrB*, no Beta-lactamase (ESBLs) producing strains were observed. This result highlights the importance of livestock fecal matter, manure, and slurries as a significant public health concern and a repository of antibiotic resistant gene.

Key words: Diarrheagenic Escherichia coli (DEC), Livestock's fecal matter, manure, slurry, antibiotics resistance, quinolone resistance genes, bla_{TEM}, bla_{SHV}, Burkina Faso.

INTRODUCTION

Diarrheagenic Escherichia coli (DEC) constitute one of the most important causes of gastrointestinal in developping countries (Okeke, 2009; Bonkoungou et al., 2012; Dembélé et al., 2015; Konaté et al., 2017)). Some

common pathotypes of DEC include the Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), (ETEC), and Enteroinvasive *E. coli* (EIEC) (Okeke, 2009; Sidhu et al., 2013). DEC may account for life-threatening infections and harbor virulence properties such as haemolysins, toxins, effacement factors, and cytotoxic necrotic factors (Kaper et al., 2004).

DEC are present in livestock's fecal matter,waste as slurry, manure and are largely contracted through environment (Manyi Loh et al., 2016; Bako et al., 2017). Furthermore, the environment is increasingly being recognized for the role it might play in the global spread of clinically relevant antibiotic resistance (Singer et al., 2016). The nature of this crisis and its health and economic burdens prompt us to identify new alternatives as well as to implement new policies to combat resistance. The emergence of antimicrobial resistance mechanisms, especially those associated with mobile genetic elements, may enhances the possibility that virulence factors genes and antibiotic resistance genes are spread simultaneously, inducing the emergence of new pathogens (Chen et al., 2011; Koczura et al., 2012).

The last report of World Health Organization on antibiotics resistance showed that *E. coli* is commonly resistant to third-generation cephalosporins, including resistance conferred by extended spectrum beta-lactamases (ESBLs), and to quinolones (WHO, 2014).

In *E. coli*, the resistance is primarily associated with the association of mutations in the quinolone-resistance determining regions (QRDRs) of *gyrA* and *parC*, which encode topoisomerase II (DNA gyrase) and topoisomerase IV respectively (Hopkins et al., 2005).

DEC that harbor bla_{ESBL} genes such as the bla_{TEM} and bla_{SHV} ESBL genes (Hoseini et al., 2014; Strau et al., 2015) render ineffective many widely used beta-lactam antibiotics including the third-generation cephalosporin such as cefepime through a secretion of beta-lactamase thereby, limiting available therapeutic options for the treatment of infections caused by these bacteria (Straus et al., 2015).

This study aimed to investiguate the quinolone resistant mutations and ESBL genes among DEC isolated in cattle fecal matter, slurries and manure in Ouagadougou, Burkina Faso and and how these mutations correlates with antibiotic susceptibility profiles.

MATERIALS AND METHODS

Diarrheagenic *Escherichia coli* (DEC) strain

The study involved a total of 55 DEC strains identified from previews study (Bako et al., 2017) (Table 1). *E.coli* strains have been isolated from cattle feces and organic waste (manure and slurry) from four livestock markets in the city of Ouagadougou, Burkina Faso between May 2015 and May 2016. A 16-plex Polymerase Chain Reaction (PCR), was used to screen simultaneously the virulence genes specific for Shiga-toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) and Enteroaggregative *E. coli* (EAEC) (Müller et al., 2007; Antikainen et al., 2009, Kagambèga et al., 2012). The 16-plex PCR is based on the detection of 15 different pathogroup-specific virulence genes (Table 2). In addition, one *E. coli* specific gene, *uidA*, was included. Strains comprised 52 strains of Enterotoxinogene *E. coli* (ETEC), two strains of Shiga Toxin *E. coli* (STEC) and one strain of Enteroaggregative *E. coli* (EAEC).

The antibiotic susceptibility tests

Antibiotic susceptibility test was done onto Mueller-Hinton media (Liofilchem, Italy) plate media following the standardized disk diffusion method as described (Bauer et al., 1966) using 16 antibiotic disks. The 16 antibiotics was: amoxicillin clavulanic-acid (AUG, 30 µg), chloramphenicol (C, 30 µg), norfloxacin (NOR, 10 µg), tetracycline (TET, 30 µg), nalidixic-acid (NA, 30 µg), imipenem (IPM, 10 µg), aztreonam (ATM, 30 µg), ceftriaxon (CRO, 30 µg), trimethoprim -sulfate (SXT, 25 µg), ceftazidime (CaZ, 30 µg), nutrofurantoin (F, 300 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 5 µg), cephalotin (KF, 30 µg), gentamicin (CN, 10 µg), cefoxitin (FOX, 30 µg).

Inhibition diameters of the antibiotics were interpreted according to the European Committee on Antimicrobial Susceptibility Instructions (EUCAST 2015, 2017). The Double Disk Synergy Test (DDST) was used to detect extended-spectrum β-lactamase (ESBL) producing strain according to the european committee on antimicrobial susceptibility testing description. This test is based on the detection of synergy between an amoxicillin clavulanic-acid disc and two discs of third generation cephalosporin's (ceftriaxone and cefotaxime) separated by 2 to 3 cm. The synergy between the discs, gave the appearance of "champagne cork" shape.

Detection of quinolone resistance genes and some ß-lactamase genes

DNA extraction

DNA was extracted by the thermal shock method. A loopful of bacteria previously cultured on MacConkey sorbitol agar and reisolated on Mueller-Hinton media was transferred to an Eppendorf tube with 250 µL water (nuclease free). The mixture was boiled for 10 min and centrifuged for 1 min at 13000 *g*. The supernatant was used for in the PCR reactions.

Primers and PCR assay

Quinolone resistance genes as *qnrA, qnrB* and *qnrS*, ß-lactamase gene as bla_{TEM} and bla_{SHV}, were detected by conventional PCR using primers as described by Cattoir et al. (2007). The following primers were used: bla_{TEM} (blaTEM-R: CCAATGCTTATTCAGTGAGG; blaTEM-F: ATGAGTATTCAACATTTCCG), bla_{SHV} (blaSHV-R:

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Table 1. DEC strains and their origins.

S/N	Strain code	Source of strain	Pathotype
1	OMBKilFB2.3		ETEC
2	OMBSNFB5.1		ETEC
3	OMBSNFB13.2		ETEC
4	OMBSNFB7.3		ETEC
5	OMBSNFB4.4		ETEC
6	OMBSNFB13.4		ETEC
7	OMBKFB3.3		ETEC
8	OMBSNFB4.1		ETEC
9	OMBSNFB7.2	Cows fecal matter	ETEC
10	OMBKFB12.2		ETEC
11	OMBSNFB13.1		ETEC
12	OMBSNFB8.4		ETEC
13	OMBSNFB1.2		ETEC
14	OMBSNFB1.4		ETEC
15	OMBSNFB3.3		ETEC
16	OMBSNFB6.1		ETEC
17	OMBSNFB9.3		ETEC
18	OMBSNFB12.3		ETEC
19	OMBOIFM10.1		ETEC
20	OMBSNFM9.1		ETEC
21	OMBSNFM10.1		ETEC
22	OMBSNFM1.4		ETEC
23	OMBSNFM2.1		ETEC
24	OMBSNFM6.1	Sheep fecal matter	ETEC
25	OMBSNFM13.4		ETEC
26	OMBSNFM5.2		ETEC
27	OMBSNFM3.2		ETEC
28	OMBSNFM3.1		ETEC
29	OMBSNFM4.3		STEC
30	OMBKFM4.1		ETEC
31	OMBSNFM2.2	Sheep fecal matter	ETEC
32	OMBSNFM1.2		ETEC
33	OMBOIFC2.3		ETEC
34	OMBSNFC7.3		ETEC
35	OMBKilFC1.3		ETEC
36	OMBOIFC9.4		ETEC
37	OMBSNFC4.1	Goat fecal matter	ETEC
38	OMBSNFC7.3		ETEC
39	OMBSNFC3.3		ETEC
40	OMBOIFC4.1		ETEC
41	OMBKilFC2.4		ETEC
42	OMBSNLB6.3		ETEC
43	OMBOILB11.1		ETEC
44	OMBOILB9.1		ETEC
45	OMBOILB12.1		ETEC
46	OMBOILB10.1	Slurry	ETEC
47	OMBSNLB3.3		ETEC
48	OMBSNLB6.2		ETEC
49	OMBSNLB7.2		ETEC
50	OMBSNLB2'.1		ETEC

Table 1. Contd

51	OMBSNF6.4		EAEC
52	OMBOIF2.2		ETEC
53	OMBOIF2.1	Manure	ETEC
54	OMBOIF4.2		STEC
55	OMBOIF6		ETEC

EAEC, Enteroaggregative *E. coli* ; ETEC, Enterotoxinogenic *E. coli*, STEC, Shiga-toxin producing *E. coli.*

Table 2. The virulence genes in the 16-plex PCR.

Pathogroup	Gene	Locus	Action	Reference
STEC	*stx1*	Phage	Shiga toxin 1	(Paton and Paton, 1998)
STEC	*stx2*	Phage	Shiga toxin 2	(Paton and Paton, 1998)
STEC, some EPEC	EHEC-*hlyA*	Virulence plasmid pO157	Enterohemolysin	(Paton and Paton, 1998)
STEC, EPEC	*eae*	LEE pathogenicity island in the chromosome	Intimin, a protein causing attaching/effacing lesions	(Nataro and Kaper, 1998)
STEC, EPEC	*escV*	LEE pathogenicity island in the chromosome	A conserved area in LEE pathogenicity island, type III secretion system structure protein	(Muller et al., 2007)
STEC, EPEC	*ent*	OI-122 pathogenicity island in the chromosome	Enterotoxin or enterohemolysin, a homolog to ShET2 enterotoxin of *Shigella flexnerii*	(Muller et al., 2007, Afset et al., 2008)
tEPEC	*bfpB*	EPEC adherence factor (EAF) plasmid	Subunit of Bundle forming pilus (BFP)	(Nataro and Kaper, 1998, Muller et al., 2007)
ETEC	*elt*	Plasmid	Heat-labile enterotoxin LT-I	(Nataro and Kaper, 1998, Kaper et al., 2004)
ETEC	*estIa*	Plasmid or transposon	Heat-stable enterotoxin STIa (STIp, porcine)	(Nataro and Kaper, 1998, Kaper et al., 2004)
ETEC	*estIb*	Plasmid or transposon	Heat-stable enterotoxin STIb (STIh, human)	(Nataro and Kaper, 1998, Kaper et al., 2004)
EIEC	*invE*	Virulence plasmid pINV	Transcription regulator, regulates the *ipa* genes	(Hale, 1991, Muller et al., 2007)
EIEC	*ipaH*	Virulence plasmid pINV and the chromosome	Invasion plasmid antigen	(Hale, 1991)
EAEC	*aggR*	Chromosomal island, plasmid pAA	AggR regulon, transcription activator, regulates the genes of fimbrial biogenesis	(Kaper et al., 2004, Harrington et al., 2006)
EAEC	*pic*	Chromosome	Serine protease	(Henderson et al., 1999, Muller et al., 2007)
STEC, EPEC, ETEC, EIEC, EAEC, E. coli	*astA*	Plasmid	EAEC heat-stable enterotoxin (EAST-1)	(Nataro and Kaper, 1998)
STEC, EPEC, ETEC, EIEC, EAEC, E. coli	*uidA*	Chromosome	β-glucuronidase	(Muller et al., 2007)

EAEC, Enteroaggregative *E. coli* ; ETEC, Enterotoxinogenic *E. coli*, STEC, Shiga-toxin producing *E. coli.*

GATTTGCTGATTTCGCTCGG; blaSHV-F: TTATCTCCCGTTAAGCCACC), qnrB (QnrB-F: GATCGTGAAAGCCAGAAAGG; QnrB-R: ACGATGCCTGGTAGTTGTCC), qnrS (QnrS-F: ACGACATTCGTCAACTGCAA; QnrS-R: TAAATTGGCACCCTGTAGGC), qnrA (QnrA-F: TCAGCACAAGAGGATTTCTC; QnrA-R: GGCAGCACTATTACTCCCA). The reaction mixture (20 µl) contained 4 µl of 5x FIREPol® Master Mix Ready to Load with 7.5 mM MgCl2 (Solis biodyne, Estonia), 1 µl of each primer, and 1 µl of DNA template 14 µl of water (nuclease free). The samples were gently vortexed and the PCR were performed using the thermal cycling condition including the annealing temperatures for each gene. Thermocycling conditions were 94°C for 5 min, following to35 cycles at 94°C for 30s and annealing temperatures were respectively 52°C, 54°C, 57°C, 55°C, 54°C, for bla_{TEM}, bla_{SHV}, *qnrB,*

Table 3. The prevalence of diarrheagenic *Escherichia coli* (DEC) resistance to antibiotics.

Antibiotics	Cattle fecal matter									Strain isolated from slurry (n=9)			Strain isolated from manure (n= 5)			Total
	Strain isolated from cow fecal matter (n= 18)			Strain isolated from sheep fecal matter (n= 14)			Strain isolated from goat fecal matter (n= 9)									
	STEC	ETEC	EAEC	STEC	ETEC	EAEC	STEC	ETEC	EAEC	STEC	ETEC	EAEC	STEC	ETEC	EAEC	
Aztreonam (ATM)	0	11 (61.11%)	0	1 (7.14%)	10 (71.42%)	0	0	5 (55.55%)	0	0	4 (44.44%)	0	0	3 (60%)	1 (20%)	63.63%
Amoxicillin Acid Clavulanic (AUG)	0	17 (94.44%)	0	1 (7.14%)	12 (85.71%)	0	0	9 (100%)	0	0	9 (100%)	0	1 (20%)	3 (60%)	1 (20%)	96.36%
Cefalotin (KF)	0	7 (38.88%)	0	1 (7.14%)	9 (64.28%)	0	0	5 (55.55%)	0	0	3 (33.33%)	0	0	3 (60%)	1 (20%)	52.72%
Cefoxitin (FOX)	0	0	0	0	2 (14.28%)	0	0	3 (33.33%)	0	0	0	0	0	3 (60%)	1 (20%)	16.36%
Cefotaxime (CTX)	0	13 (72.22%)	0	1 (7.14%)	9 (64.28%)	0	0	7 (77.77%)	0	0	6 (66.66%)	0	0	3 (60%)	0	70.90%
Ceftriaxon (CRO)	0	8 (44.44%)	0	1 (7.14%)	7 (50%)	0	0	3 (33.33%)	0	0	1 (11.11%)	0	0	3 (60%)	1 (20%)	43.63%
Ceftazidime (CaZ)	0	16 (88.88%)	0	1 (7.14%)	11 (78.57%)	0	0	5 (55.55%)	0	0	6 (66.66%)	0	0	2 (40%)	1 (20%)	76.36%
Imipenem (IPM)	0	1 (5.55%)	0	0	1 (7.14%)	0	0	1 (11.11%)	0	0	0	0	0	1 (20%)	0	7.27%
Chloramphenicol	0	8 (44.44%)	0	0	6 (42.85%)	0	0	3 (33.33%)	0	0	0	0	0	1 (20%)	0	32.72%
Gentamicin (CN)	0	5 (27.77%)	0	1 (7.14%)	3 (21.42%)	0	0	2 (22.22%)	0	0	1 (11.11%)	0	0	1 (20%)	1 (20%)	25.45%
Acide Nalidixique (NA)	0	4 (22.22%)	0	1 (7.14%)	3 (21.42%)	0	0	0	0	0	0	0	0	1 (20%)	0	16.36%
Norfloxacin (NOR)	0	0	0	0	2 (14.28%)	0	0	0	0	0	0	0	0	0	1 (20%)	5.45%
Ciprofloxacin (CIP)	0	2 (11.11%)	0	0	2 (14.28%)	0	0	1 (11.11%)	0	0	0	0	0	0	1 (20%)	10.90%
Tretacycline (TE)	0	16 (88.88%)	0	0	12 (85.71)	0	0	7 (77.77%)	0	0	9 (100%)	0	1 (20%)	3 (60%)	1 (20%)	89.09%
Trimethropim/Sulfamethoxazol (SXT)	0	8 (44.44%)	0	0	7 (50%)	0	0	3 (33.33%)	0	0	2 (22.2%)	0	1 (20%)	2 (40%)	1 (20%)	43.63%
Nitrofurantoin (F)	0	5 (27.77%)	0	1(7.14%)	9 (64.28%)	0	0	2 (22.22%)	0	0	2 (22.22%)	0	0	1 (20%)	1 (20%)	38.18%

EAEC, Enteroaggregative *E. coli* ; ETEC, Enterotoxinogenic *E. coli*; STEC, Shiga-toxin producing *E. coli.*

qnrA, qnrS, and elongation at 72°C for 60 s. The ultimate extension was 72°C for 10 min. The amplicons were visualized by electrophoresis on 1% (weight / volume) gel agarose after migration in the TAE (Tris Acetic acid EDTA) buffer.

Statistical analysis

SPSS statistics 20 and Microsoft Excel were used for statistical analysis. Bivariate Spearman's rank correlation test was used to determine the association between variables of this study.

RESULTS AND DISCUSSION

The antibiotic susceptibility tests

The profile of antibiotics resistance revealed that DEC were resistant to all antibiotics used in this study. The most common resistance (Table 3) was for amoxicillin-clavulanic acid (96.36%) followed by tetracycline (89.09%), ceftazidime (76.36 %) and cefotaxime (70.90%). The resistance rates for ciprofloxacin and norfloxacin (anibiotics belonging to the family of quinolone) were 10.90% and 5.45% respectively. No ESBLs phenotype was reported in this study.

The prevalence of resistance to amoxicilin clavulanic acid (84.2%) is comparable to that obtained by Iweriebor et al. (2015) in a similar study conducted on DEC in Cape Town, South Africa.

This type of resistance is acquired and could be expressed by a decrease of the activity of the β-lactamase inhibitor which is clavulanic-acid, resulting from a penicillinase hyperproduction, or the inactivation of the inhibitor itself (Kamga et al., 2014). This fact is considered to be a consequence of selection pressure related to the abuse of these antibiotics (Kamga et al., 2014).

The resistance to tetracycline observed in this study is comparable to those obtained in South Africa (96.84%) and Nigeria (64.3%) in diarrheagenic Escherichia coli isolated from effluents from cattle (Ajayi et al., 2011; Iweriebor et al., 2015).

The resistance to tetracycline is widely disseminated in *E. coli*, where it is generally mediated by tetracycline efflux pumps, such as *tetA* (Stavropoulos and Strathdee, 2000; Møller et al., 2016). This high prevalence can be explained by the fact that in Burkina, oxytetracycline one of

antibiotic belonging to the tetracycline family is the most antibiotic used in animal health (Samandoulougou et al., 2016).

Ceftazidime is third generation cephalosporin antibiotics belonging to the family of β-lactam.

The resistance of strains to ceftazidime in this study is comparable to those obtained in South Africa (32%) and Nigeria (50.6%) in Escherichia coli isolated from cattle fecal matter and manure (Iweriebor et al., 2015; Ajayi et al., 2011).

The resistance to nalidixic acid, and ciprofloxacin can be explained in general by the fact that fluoroquinolones such as ciprofloxacin and nalidixic acid are less used in dairy cattle than in other species such as poultry (Lanz et al., 2003).

91% of DEC strains comprising 47 ETEC, 2 STEC and 1 EAEC isolated from cow fecal matter, sheep fecal matter, goat fecal matter, manure and slurry were multi Drug resistant with non-susceptibility to ≥1 agent in ≥3 antimicrobial classes. Among this multi-drug resistant strain, 2 (ETEC) strains isolated originated from cow fecal matter and sheep fecal matter were resistant to 14 antibiotics of 16 used in this study. No statistic significant correlation was found between the multi drug resistant character of the strains as well as the parameters such as the origin of the strain, the type of DEC. The multi-resistance could be explained by the combination of several resistance mechanisms which in most cases are encoded by molecular supports.

No statistic significant correlation was noted with the resistance to de different antibiotic family and the type of DEC pathotype.

Carriage of *qnrA, qnrB, qnrS*, bla_{TEM} and bla_{SHV} genes by DEC

This study is the first to focus on the sharing of genes coding for quinolone (qnrA, qnrB, qnrS) and Beta-lactam resistance (blaTEM, blaSHV) by DEC isolated from livestock's fecal matter, manure and slurries in Burkina Faso.

The PCR revealed the presence (Table 4) of *qnrA, qnrB, qnrS*, and bla_{ESBL} genes such us bla_{TEM} and bla_{SHV} among DEC. For quinolone gene, *qnrS* (18.2%) was most prevalent followed by *qnrB* (7.2%) and *qnrA* (2%). All *qnrS* positive strains comprised 14 ETEC from cattle fecal matter, six from slurry and one EAEC from manure. *qnrA* positive strains comprising three ETEC were isolated from sheep and cow fecal matter. *qnrB* positive strains comprising 11 ETEC were isolated from cattle fecal matter. Among all *qnr* gene positives strains, only 9 strains were resistant to antibiotic belonging to the quinolone family such us nalidixic-acid (NA), ciprofloxacin (CIP) and norfloxacin (NOR). Correlation has been found between the susceptibility of DEC to ciprofloxacin and the presence of the *qnrA* gene ($p = 0.003$). Correlation was also found between the resistance of DEC to cefoxitin, antibiotic form of cephalosporin class and the presence of *qnrS* ($p = 0.009$).

The prevalence of *qnrS* and *qnrB* in this study are comparable to those obtained for *qnrS* (5.60%) and *qnrB* (0.43%) from *E. coli* isolated from farm animal in China (Yue et al., 2008). There are no data concerning the carrying of qnrA gene by DEC isolated from livstock's fecal matter, manure and slurries.

In general, the presence of these acquired genes does not confer high level of fluoroquinolones resistance (Rodríguez-Martínez et al., 2011). This could explain the fact that a statistically significant correlation between the susceptibility to the antibiotics belonging to quinolone family was not obtained.

Double-carrying *qnrB* + *qnrS* (3.6%) and *qnrA* + *qnrS* (1.8%) has been also identified among DEC.

bla_{TEM} gene (5.45%) was the most prevalent bla_{ESBL} genes followed by bla_{SHV} gene (3.6%). bla_{TEM} or bla_{SHV} only positive strain (9.05%) were constituted by five ETEC, three from cattle fecal matter, one from manure and the second one from slurry. All positive bla_{TEM} gene and or the bla_{SHV} gene DEC resisted at least one antibiotic of the ß-lactam class.

The prevalance of blaTEM gene is comparable to those obtained in South Africa (27%) and South Korea (17.5%) in DEC (STEC) isolated from dairy cattle farms (Iweriebor et al., 2015; Dong et al., 2017).

Multiple carrying (Table 4) of blaESBL genes and quinolo resitance gens were also noted (24.2%) in DEC. These strains were composed to nine ETEC isolated from cattle fecal matter, one ETEC from slurry and one ETEC from manure. These strains were resistant to at least one antibiotic belonging ß-lactam family.

No significant statistic correlation was found between susceptibility to Beta-lactamin antibiotics involved in this sudy and the carrying of the bla_{TEM} and bla_{SHV} genes. Double carrying between bla_{TEM}, bla_{SHV}, *qnrB* and or *qnrS* gene was observed in 24.2% of the strains in this study. Indeed, *qnr* genes have been frequently associated with bla_{ESBL} genes such as blaTEM and blaSHV genes (Boyd et al., 2004; Woodford and Carattoli, 2009).

Conclusion

The study showed that there are a lot of multi drug resistant diarrheagenic *E. coli* which can get to the environment through cattle fecal matter slurry and manure from livestock market located in Ouagadougou, Burkina Faso. This is in line with WHO's observations on the emergence of resistance to beta-lactams, third-generation cephalosporins and quinolones. In fact, these pathogens carry molecular support such us qnrA, qnrB, qnrS, blaTEM and blaSHV. These results show the risk incurred by the population to the exposure of livestock cattle fecal matter and organic waste products of animal origin such as manure and slurries.

Table 4. Quinolone and Beta-lactam resistance genes identified among diarrheagenic *Escherichia coli* (DEC).

Resistance genes	Cattle Fecal matter (n=41)		Slurry (n=9)		Manure (n=5)		Total (n=55) (%)
	Nbr (%)	Pathotype	Nbr (%)	Pathotype	Nbr (%)	Pathotype	
Quinolone resistance only							
qnrA	1(2.4)	ETEC	0		0		1.8
qnrB	4(9.75)	ETEC	0		0		7.27
qnrS	5(12.19)	ETEC	5(55.55)	ETEC	0		18.2
qnrB + *qnrS*	2(4.87)	ETEC	0		0		3.64
qnrA + *qnrS*	1(2.43)	ETEC	0		0		1.8
Beta-lactamin resistance							
bla_{TEM}	2(4.87)	ETEC	0		1(20)	ETEC	5.45
bla_{SHV}	1(2.43)	ETEC	1(11.11)	ETEC	0		3.64
Beta-lactam + quinolone resistance							
bla_{SHV} +*qnrS*	2(4.87)	ETEC	0		0		3.64
bla_{SHV} + *qnrB*	2(4.87)	ETEC	0		0		3.64
bla_{TEM} +*qnrS*	2(4.87)	ETEC	1(11.11)	ETEC	1(20)	EAEC	7.27
bla_{TEM} + bla_{SHV}+ *qnrB*	1(2.43)	ETEC	0		0		1.8
bla_{TEM} + bla_{SHV} + *qnrS*	1(2.43)	ETEC	0		0		1.8
bla_{TEM} + bla_{SHV} + *qnrB* +*qnrS*	1(2.43)	ETEC	0		0		1.8

EAEC, Enteroaggregative *E. coli* ; ETEC, Enterotoxinogenic *E. coli*, STEC, Shiga-toxin producing *E. coli.*

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Afset JE, Anderssen E, Bruant G, Harel J, Wieler L, Bergh K (2008). Phylogenetic backgrounds and virulence profiles of atypical enteropathogenic *Escherichia coli* strains from a case-control study using multilocus sequence typing and DNA microarray analysis. Journal of Clinical Microbiology 46:2280-2290.

Ajayi AO, Oluyege AO, Olowe OA, Famurewa O (2011). Antibiotic resistance among commensal *Escherichia coli* isolated from feces of cattle in Ado-Ekiti, Nigeria. Journal of Animal and Veterinary Advances 10(2):174-179.

Antikainen J, Tarkka E, Haukka K, Siitonen A, Vaara M, Kirveskari J (2009). New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. European Journal of Clinical Microbiology & Infectious Diseases 28(8):899-908.

Bako E, Kagambèga A, Traore KA, Bagre TS, Ibrahim HB, Bouda SC, Bonkoungou, IJO, Kaboré S, Zongo C, Traore AS, Barro N (2017). Characterization of diarrheagenic *Escherichia coli* isolated in organic waste products (cattle fecal matter, manure and, slurry) from cattle’s markets in Ouagadougou, Burkina Faso. International Journal of Environmental Research and Public Health 14:1100.

Bauer AW, Kirby WMM, Sherries JC, Turck M (1966). Antibiotic susceptibility testing. The American Journal of Pathology 45:493-496.

Bonkoungou IJO, Lienemann T, Martikainen O, Dembelé R, Sanou I, Traoré AS, Siitonen A, Barro N, Haukka K (2012). Diarrhoeagenic *Escherichia coli* detected by 16-plex PCR in children with and without diarrhea in Burkina Faso. Clinical Microbiology and Infection 18(9):901-906.

Boyd DA, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM, Bryce E, Gardam M, Nordmann P, Mulvey MR (2004). Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrobial Agents and Chemotherapy 48(10):3758-3764.

Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. Journal of Antimicrobial Chemotherapy 60(2):394-397.

Chen B, Zheng W, Yu Y, Huang W, Zheng S, Zhang Y, Guan X, Zhuang Y, Chen N, Topp E (2011). Class 1 integrons, selected virulence genes, and antibiotic resistance in *Escherichia coli* isolates from the Minjiang River, Fujian Province, China. Applied and Environmental Microbiology 77(1):148-155.

Dembélé R, Bonkoungou IJO, Konaté A, Bsadjo Tchamba G, Bawa HI, Bako E, Bagre TS, Kagambèga A, Zongo C, Traore AS, Barro N (2015). Serotyping and antimicrobial resistance of enteropathogenic Escherichia coli and enterohemorrhagic E. coli O157 isolated from children under five years of age with diarrhea in rural Burkina Faso. African Journal of Microbiology Research 9(14):1053-1059.

Dong HJ, Lee S, Kim W, An JU, Kim J, Kim D, Cho S (2017). Prevalence, virulence potential, and pulsed-field gel electrophoresis profiling of Shiga toxin-producing *Escherichia coli* strains from cattle. Gut Pathogens 9(1):22.

Hale TL (1991). Genetic basis of virulence in Shigella species. Microbiology Reviews 55:206-224.

Harrington SM, Dudley EG, Nataro JP (2006). Pathogenesis of

enteroaggregative Escherichia coli infection. FEMS Microbiology Letters 254:12-18.
Henderson IR, Czeczulin J, Eslava C, Noriega F, Nataro JP (1999). Characterization of pic, a secreted protease of Shigella flexneri and enteroaggregative Escherichia coli. Infection and Immunity 67:5587-5596.
Hopkins KL, Davies RH, Threlfall EJ (2005). Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. International Journal of Antimicrobial Agents 25(5):358-373.
Hoseini SS, Dastmalchi Saei H, Ownagh A (2014). Molecular detection of extended spectrum β-lactamase (ESBL) genes *blaCTX-M*, *blaTEM* and *blaSHV* in *Escherichia coli* isolated from water buffalo (*Bubalus bubalis*) feces in West Azerbaijan province. Iranian Journal of Veterinary Research 69(3):203-212.
Iweriebor BC, Iwu CJ, Obi LC, Nwodo UU, Okoh AI (2015). Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in feces of dairy cattle farms in Eastern Cape of South Africa. BMC Microbiology 15(1):213.
Kagambèga A, Martikainen O, Siitonen A, Traoré AS, Barro N, Haukka K (2012). Prevalence of diarrheagenic *Escherichia coli* virulence genes in the feces of slaughtered cattle, chickens, and pigs in Burkina Faso. Microbiologyopen 1(3):276-284.
Kamga HG, Nzengang R, Toukam M, Sando Z, Shiro SK (2014). Phénotypes de résistance des souches de *Escherichia coli* responsables des infections urinaires communautaires dans la ville de Yaoundé (Cameroun). African Journal of Cellular Pathology 3:1-4.
Kaper JB, Nataro JP, Mobley HL (2004). Pathogenic *Escherichia coli.* Nature Reviews Microbiology 2(2):123-140.
Koczura R, Mokracka J, Jablonska L, Gozdecka E, Kubek M, Kaznowski A (2012). Antimicrobial resistance of integron-harboring *Escherichia coli* isolates from clinical samples, wastewater treatment plant and river water. Science of the Total Environment 414:680-685.
Konaté A, Dembélé R, Zongo C, Kaboré WAD, Bonkoungou IJO, Traoré AS, Barro N (2017). Occurrence of Multiple Antibiotic Resistances of *Escherichia coli* Isolated from Diarrheal Children Less Than Five Years in Burkina. European Journal of Pharmaceutical and Medical Research 4(1):165-171.
Lanz R, Kuhnert P, Boerlin P (2003). Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Veterinary Microbiology 91(1):73-84.
Manyi-Loh CE, Mamphweli SN, Meyer EL, Makaka G, Simon M, Okoh AI (2016). An Overview of the Control of Bacterial Pathogens in Cattle Manure. International Journal of Environmental Research and Public Health 13(9):843.
Møller TSB, Overgaard M, Nielsen SS, Bortolaia V, Sommer MOA, Guardabassi L, Olsen JE (2016). Relation between *tetR* and *tetA* expression in tetracycline resistant *Escherichia coli*. BMC Microbiology 16(1):39.
Müller D, Greune L, Heusipp G, Karch H, Fruth A, Tschäpe H, Schmidt MA (2007). Identification of unconventional intestinal pathogenic *Escherichia coli* isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. Applied and Environmental Microbiology 73(10):3380-3390.
Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 11:142-201.
Nordmann P, Poirel L (2005). Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy 56(3):463-469.
Okeke IN (2009). Diarrheagenic *Escherichia coli* in sub-Saharan Africa: Status, uncertainties and necessities. Journal of Infection in Developing Countries 3(11):817-842.
World Health Organization (WHO) (2014). Antimicrobial resistance: global report on surveillance. Who. https://doi.org/1.4.2014
Pakzad I, Ghafourian S, Taherikalani M, Abtahi H, Rahbar M, Jamshidi N (2011). *qnr* Prevalence in Extended Spectrum Beta-lactamases (ESBLs) and None-ESBLs Producing *Escherichia coli* Isolated from
Urinary Tract Infections in Central of Iran ESBL screening methods. Iran. Iranian Journal of Basic Medical Sciences14(5):458-464.
Paton JC, Paton AW (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clinical Microbiology Reviews 11(3):450-479.
Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual Á (2011). Plasmid-mediated quinolone resistance: An update. Journal of Infection and Chemotherapy 17(2):149-182.
Samandoulougou S, Ilboudo AJ, Ouedraogo GS, Bagre T S, Tapsoba FW, Compaore H, Dao A, Zoungrana A, Savadogo A (2016). Physico-chemical and nutritional quality of eggs from local chicken and of improved race consumed in Ouagadougou, Burkina Faso. International Journal of Biological and Chemical Sciences 10:737–748.
Sidhu JPS, Ahmed W, Hodgers L, Toze S (2013). Occurrence of virulence genes associated with diarrheagenic pathotypes in *Escherichia coli* isolates from surface water. Applied and Environmental Microbiology 79(1):328-335.
Singer AC, Shaw H, Rhodes V, Hart A (2016) Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. Frontiers in Microbiology 7:1728.
Stavropoulos TA, Strathdee CA (2000). Expression of the *tetA*(C) tetracycline efflux pump in *Escherichia coli* confers osmotic sensitivity. FEMS Microbiology Letter 90(1):147-150.
Strau LM, Dahms C, Becker K, Kramer A, Kaase M, Mellmann A (2015). Development and evaluation of a novel universal β-lactamase gene subtyping assay for *blaSHV, blaTEM* and *blaCTX-M* using clinical and livestock-associated *Escherichia coli*. Journal of Antimicrobial Chemotherapy 70(3):710-715.
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2015). European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf.
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017). Breakpoint tables for interpretation of MICs and zone diameters, version 7.0, 2017. 0–77. http://www.eucast.org
Um MM, Barraud O, Kérourédan M, Gaschet M, Stalder T, Oswald E, Dagot C, Ploy MC, Brugère H, Bibbal D (2016). Comparison of the incidence of pathogenic and antibiotic-resistant *Escherichia coli* strains in adult cattle and veal calf slaughterhouse effluents highlighted different risks for public health. Water Research 88:30-38.
Woodford N, Carattoli AKE (2009). Complete nucleotide sequences of plasmids pEK204, pEK499 and pEK516, encoding CTX-M enzymes in three major *Escherichia coli* lineages from United Kingdom, all belonging to international 025:H4-ST131 clone. Antimicrobial Agents and Chemotherapy 53:4472-4482.
Yue L, Jiang, HX, Liao XP, Liu JH, Li SJ, Chen XY, Chen CX, Lü DH, Liu YH, Liu, YH (2008). Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine clinical isolates of *Escherichia coli*. Veterinary Microbiology 132(3-4):414-420.

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