Determination of the Antibiotic Susceptibility Patterns of Local Isolates of *E. coli* O157:H7 from Edo State, Nigeria.

Jonathan Osariemen Isibor*[1] and Afe Omolola Ekundayo [2]

[1,2] Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
***Corresponding author: -** Tel: (234) 803 5515 110; E-mail: joe_isibor@yahoo.com

Abstract: *Escherichia coli* O157:H7 is considered one of the most serious of known food borne pathogens with a disease spectrum ranging from non bloody diarrhea to bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and other complications . To determine the prevalence and pattern of antibiotic susceptibility of isolates, we collected one thousand (1000) stool specimens from subjects within Edo State between May 2006 and April 2011. 316 (32%) of these yielded *E.coli* on culture, while 27 isolates tested positive for O157:H7 serotype. Susceptibility tests were performed by Bauer-Kirby disc diffusion on Mueller Hinton Agar (CM337-Oxoid) using the following antibiotics: Nitrofurantoin (300µg), Amoxicillin (25µg), Erythromycin (5µg), Tetracycline (10µg), Cloxacillin (5µg), Gentamicin (10µg), Cotrimoxazole (25µg), Chloramphenicol (30µg) (ABTEK Biologicals Ltd), Ofloxacin (30µg), Ciprofloxacin (5µg) and Cefuroxime (30µg) (Hi Media laboratories Ltd), and results were interpreted according to criteria developed by NCCLS. Most of the isolates displayed a high degree of resistance pattern (50% and above) in the following increasing order; Nitrofurantoin (40.7%), Ciprofloxacin (48.1%), Ofloxacin (51.8%), Cefuroxime (70.3%), Gentamicin (74.0%), Erythromycin (77.7%), Cloxacillin and Cotrimoxazole (85.1%), Tetracycline and Chloramphenicol (88.2%) and Amoxycillin (100%). There was joint resistance of *E. coli* isolates to almost all antibiotics tested, with the Multiple Antibiotic Resistance (MAR) index ranging from 0.54 to 1.0. Because antimicrobial resistance patterns are continually evolving and multi-drug resistant (MDR) organisms undergo progressive antimicrobial resistance, there is need to continuously update data on antimicrobial susceptibility profiles not only for epidemiological reasons but to also update information for use by those medical personnel that prescribe and dispense and use these antibiotics.
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1. Introduction.

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world. It is an important cause of foodborne human disease. Complications related to infection include diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Nataro and Kaper, 1998).Since the first reported case of *Escherichia coli* O157:H7 in 1982 in USA, outbreaks and sporadic cases of disease due to this organism have also had a fairly wide geographic distribution in the Africa continent.

In Swaziland, for example, a large outbreak of bloody diarrhea caused by *E.coli* O157:H7 was reported by Effler et al. (2001). Different prevalence rates of infection with *E. coli* O157:H7 have been reported from several cities in Nigeria (Okeke et al., 2000; Ngbede et al., 2006; Agbogu et al., 2006; Ekundayo and Isibor, 2008; Smith et al., 2009). Its apparent low frequency of reporting from our hospitals' microbiology laboratories is not due to a total absence from our environment or as a result of lesser importance of this bacterium than other gastrointestinal pathogens in causing gastrointestinal disease. Rather, *E.coli* O157:H7 for a long time has eluded the skillful eyes of microbiologists because of its peculiar biochemical characteristics, its inability to ferment Sorbitol - a nutrient not incorporated in the growth medium routinely used for the isolation of *E. coli* from stool specimens. In addition, and especially in developing countries of the world where economic considerations undermine the provision of effective medi-care, most clinicians do not readily request for the laboratory culture for this organism, even in cases of bloody diarrhea.

Consumption of faecally contaminated water and food, such as raw milk, raw vegetables, fruits, cheese, curd , sprouts, lettuce and juice, and eating of undercooked contaminated ground meat is an important route of transmission of *E. coli* O157:H7, especially in many regions of the world where good potable water is almost lacking and safe management of human waste is inadequate (Swerdlow et al., 1992; Swinbanks,1996). Since the infection primarily occurs via the fecal-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits

and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visit.

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections (Nawaz et al., 2009).In recent years antibiotic resistance of diarrheagenic pathogens has reached alarming proportions worldwide. The misuse of antibiotics has been found to be the most important selecting force in bacterial antibiotic resistance (Akinjogunla et al., 2008). *E. coli* is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics (Poppe et al., 2005).

Thus, the aim of this investigation was to determine the susceptibility patterns of *E. coli* O157:H7 prevalent in Edo State.

2. Subjects, Materials and Methods

Subjects and specimen types

One thousand (1000) fecal specimens were collected from consenting persons of both sexes and of all age groups. Those reporting with cases of diarrhoea and other gastrointestinal complaints as well as apparently healthy individuals (control population) were included for the study, while those who had been on any type of antibiotic treatment for the preceding two weeks were excluded.

Ethical clearance

The permission to use human subjects for this study was got from the Edo State Ministry of Health after the study proposal was considered and approved by the Research and Ethics Committee. Subjects enrolled for this study were those who gave their consent.

Collection of specimens

Each subject was requested to pass a small quantity of fresh stool specimen into a sterile plastic universal container (Sterilin, UK), and returned immediately to the laboratory. Specimens from distant cities were transported in Cary- Blair transport medium (Oxoid CM O519) and inoculated within 1- 2 hr
of receipt, in the Research and Diagnostic laboratory, College of Medicine, Ambrose Alli University, Ekpoma.

Bacteriological procedures

The specimens were then inoculated onto MacConkey Agar (Oxoid CM7) (for easy identification of lactose fermenting organisms), Eosin Methylene Blue Agar (Oxoid CM 0069) (for easy identification of the green metallic sheen appearance characteristic of *E. coli* colonies and Sorbitol MacConkey Agar (Oxoid, CM813) enriched with Cefixime-Tellurite supplement (Oxoid SR 172) (used to selectively differentiate the non-sorbitol-fermenting *E. coli* O157:H7 strains from other *E. coli* strains). Each stool specimen was streaked onto the media and incubated aerobically at 37^{o}C for 24 hr to isolate *E. coli*. Each colonial morphology and reactions on agar media, like colony size, consistency, shape and pigmentation, lactose and sorbitol fermentation, were noted while the colonies were gram stained, and their motility tested.

Serotyping of *E.coli* isolates.

All non-sorbitol-fermenting *E.coli* colonies (at least 5 colonies from each plate) were tested for agglutination with O157 latex reagents (Oxoid DR620) to determine if the isolates belonged to the O157 serogroup (Nataro and Kaper, 1998). To determine this, the test reagents were removed from the refrigerator and allowed to attain room temperature before use. A drop of the latex suspension was added to one side of a circle inscribed on the plastic reaction card, while a drop of sterile normal saline was added to the centre of the circle. A few colonies of the test organism grown on a non-selective agar medium was carefully emulsified in the saline drop using a sterile wire loop. The suspension and emulsified colonies were mixed together to cover the entire circle. The same was done with the control latex and control organism suspension. The card was then rocked gently for 1 minute and observed for agglutination. Agglutination indicated the presence of *E. coli* serogroup O157.

Biochemical diagnosis for *E. coli* O157: H7.

Biochemical identification tests were performed on presumptive *E. coli* colonies (Cheesbrough, 2006). For definitive biochemical diagnosis, strains of *E. coli* that agglutinated with O157 latex reagents were further tested for β -glucuronidase activity by inoculating the colonies on Sorbitol MacConkey agar with BCIG (Oxoid CM O981), and incubated at 37^{o}C for 24 hours. The suspect colonies were also tested for cellubiose, dulcitol and raffinose fermentation. *E. coli* strains that appeared pale or colourless and lacked the enzyme β-glucuronidase were presumed to be *E. coli* serotype O157:H7. Strains that agglutinated with latex reagents and were β- glucuronidase negative, and could not ferment cellubiose but fermented dulcitol and raffinose were confirmed as *E. coli* O157: H7 serotype (Thompson et al., 1990).

Antibiotic susceptibility test

The antibiotic susceptibility test was determined by the disc diffusion method as described by Bauer et al. (1966). The inoculum was standardised by

comparison with 0.5 MacFarland standard. A loopful of test organism suspension was transferred to the centre of a previously dried Mueller -Hinton agar (Oxoid CM 337), and with a sterile dry cotton wool swab the inoculum was evenly spread over the entire agar surface. The lid was left ajar for 3 to 5 min to allow for any excess surface moisture to be absorbed before applying the antibiotic discs. Using a sterile forceps, the antibiotic discs (previously brought to room temperature) were aseptically placed on the agar surface, and gently pressed down to make contact with the agar. The same inoculation technique was performed on the control organism (*E. coli* ATCC 25922).The following antibiotics were used for the sensitivity testing: Nitrofurantoin (300µg), Amoxicillin (25µg), Erythromycin (5µg), Tetracycline (10µg), Cloxacillin (5µg), Gentamicin (10µg), Cotrimoxazole (25µg), Chloramphenicol (30µg) (ABTEK Biologicals Ltd), Ofloxacin (30µg), Ciprofloxacin (5µg) and Cefuroxime (30µg) (Hi Media laboratories Ltd). The choice of the above antibiotics was because they are commonly prescribed and locally availability. The agar plates were then incubated aerobically at 37^{o} C overnight. The diameters of the zones of inhibition were measured to the nearest whole millimeter using a ruler and results were interpreted according to criteria developed by for Clinical Laboratory Standards (CSCL, 2010).

3. Results

A total of three hundred and sixteen (316) *E. coli* were isolated from 1000 stool specimens collected from people all over Edo state, giving a prevalence of 31.6 percent. Table 1 shows the distribution of *E. coli* strains isolated from stool specimens of persons studied. Apart from agglutinating O157 latex suspension, *E. coli* O157 strains tested negative in the β-glucuronidase test, due to lack of β-glucuronidase enzyme. Plate 1 shows the growth of *E.coli* O157:H7 colonies on Sorbitol MacConkey BCIG agar, which contains a chromogenic substrate that helps to demonstrate the inability of this strain to produce β-glucuronidase. Table 2 shows the antibiotic susceptibility pattern of stool isolates of *E.coli* O157:H7. The summary of antibiotic resistance profile of *E.coli* O157:H7 is reflected in Table 3. Table 4 shows the number of antimicrobial agents to which isolates are resistant and their respective multiple antibiotic resistant indices.

4. Discussions

Out of the three hundred and sixteen (316) *E.coli* isolated in this study, 27(8.5%) were found to be *E.coli* O157:H7 serotype, thus confirming the presence of this pathogen in this locality. The antibiotic resistance patterns of members of the *Enterobacteriaceae* and specifically *E.coli*, mostly reported over the years, have also been reflected `in this study. From the susceptibility pattern of *E.coli* O157: H7 isolates to a panel of antibiotics commonly used in this locality as shown in Table 2, it can be seen from the pattern that most of the isolates displayed a high degree of resistance (50% and above) in the following increasing order; Nitrofurantoin (40.7%), Ciprofloxacin (48.1%), Ofloxacin (51.8%), Cefuroxime (70.3%), Gentamicin (74.0%), Erythromycin (77.7%), Cloxacillin and Cotrimoxazole (85.1%), Tetracycline and Chloramphenicol (88.2%) and Amoxycillin (100%). A very high resistance rate (99.2%) of *E. coli* to Amoxycillin was also reported by Aibinu et al. (2004) and Ngwai et al. (2011). Also reflected in Table 2 is a high resistance rate (88.8%) for Tetracycline. Our findings agree with the study carried out in Ibadan, Nigeria, where the isolated strains of *E. coli* O157:H7 showed a high resistance rate of 91.4% to Tetracycline (2010). Olukoya et al. (1993) have earlier confirmed the presence and propagation of R plasmids to be a major cause of resistance to Tetracycline encountered in Nigeria.

The reason may not be far-fetched. Tetracycline is the most commonly available antibiotic for use as growth promoter and routine chemoprophylaxis among livestock in Nigeria. Most individuals in Edo State consume chicken and meat products in their various homes. The public health significance of these findings is that antimicrobial resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from meat production facilities to the neighborhood. Indiscriminate and misuse of antimicrobials among livestock producers and marketers in our locality could also be responsible for the resistance pattern obtained in this study. There is no significant difference ($P > 0.05$) with regards to the susceptibility pattern of the isolates to the quinolones in this study. Ciprofloxacin, for instance, is a potent broad spectrum antibacterial agent. Prior to its use, resistance was rare (Barry et al., 1990). Egri-Okwaji et al. (1996) also reported 100% susceptibility of *E.coli* isolates to Ofloxacin while Kesah et al. (1999) recorded a 2% resistance of *E.coli* to fluoroquinolone. A previous assessment of some six antibiotics against clinical isolates of *E.coli* showed Ciprofloxacin to be the most efficacious (Isibor et al., 2003). However some workers have recently reported resistance to the quinolones in Nigeria (Daini et al., 2005; Umolu et al.2006) as well as in other countries (Oteo et al., 2005).

Table 1. Distribution of *E. coli* strains isolated from stool specimens of persons studied. (n=316).

E. coli strains	No. (%) of isolates
Sorbitol fermenting *E. coli* strains	236 (74. 7)
Non-O157 *E. coli* strains (No agglutination with O157 antisera)	50 (15.8)
O157 *E.coli* strains (agglutination with O157 antisera;*BCIG negative)	27 (8.5)
O157 *E.coli* strains (agglutination with O157 antisera;*BCIG positive)	3 (1.0)
Total	316 (100)

* BCIG = 5-Bromo-4-Chloro-3-Indolyl-β-D-glucuronic acid.

Table 2. Antibiotic susceptibility pattern of stool isolates of *E.coli* O157:H7. (n=27)

Antimicrobial agents	Disc content (µg)	Code	No (%) sensitive	No (%) resistant
Nitrofurantoin	300	NIT	16(59.2)	11(40.7)
Amoxycillin	25	AMX	0(0.0)	27(100)
Erythromycin	5	ERY	6(22.2)	21(77.7)
Tetracycline	10	TET	3(11.1)	24(88.8)
Cloxacillin	5	CLX	4(14.8)	23(85.1)
Gentamicin	10	GEN	7(25.9)	20(74.0)
Cotrimoxazole	25	COT	4(14.8)	23(85.1)
Chloramphenicol	30	CHL	3(11.1)	24(88.8)
Ofloxacin	5	OFX	13(48.1)	14(51.8)
Ciprofloxacin	5	CIP	14(51.8)	13(48.1)
Cefuroxime	30	CRM	8(29.6)	19(70.3)

Table 3. Summary of antibiotic resistance profile of *E.coli* O157:H7. (n= 27)

Number of antibiotics to which there was resistance.	Number (%) of strains showing resistance pattern.
Six antibiotics	6(22.2)
Seven antibiotics	4(14.8)
Eight antibiotics	5(18.5)
Nine antibiotics	7(25.9)
Ten antibiotics	4(14.8)
Resistance to all antibiotics tested	1(3.70)

Table 4. Frequency of multiple antibiotic resistance (MAR*) and multiple antibiotic resistance indices of *E.coli* O157:H7 isolates.

Number of antimicrobial agents to which isolates are resistant.	Number of isolates with MAR	MAR indices
6	6	0.54
7	4	0.63
8	5	0.72
9	7	0.81
10	4	0.90
11	1	1.0

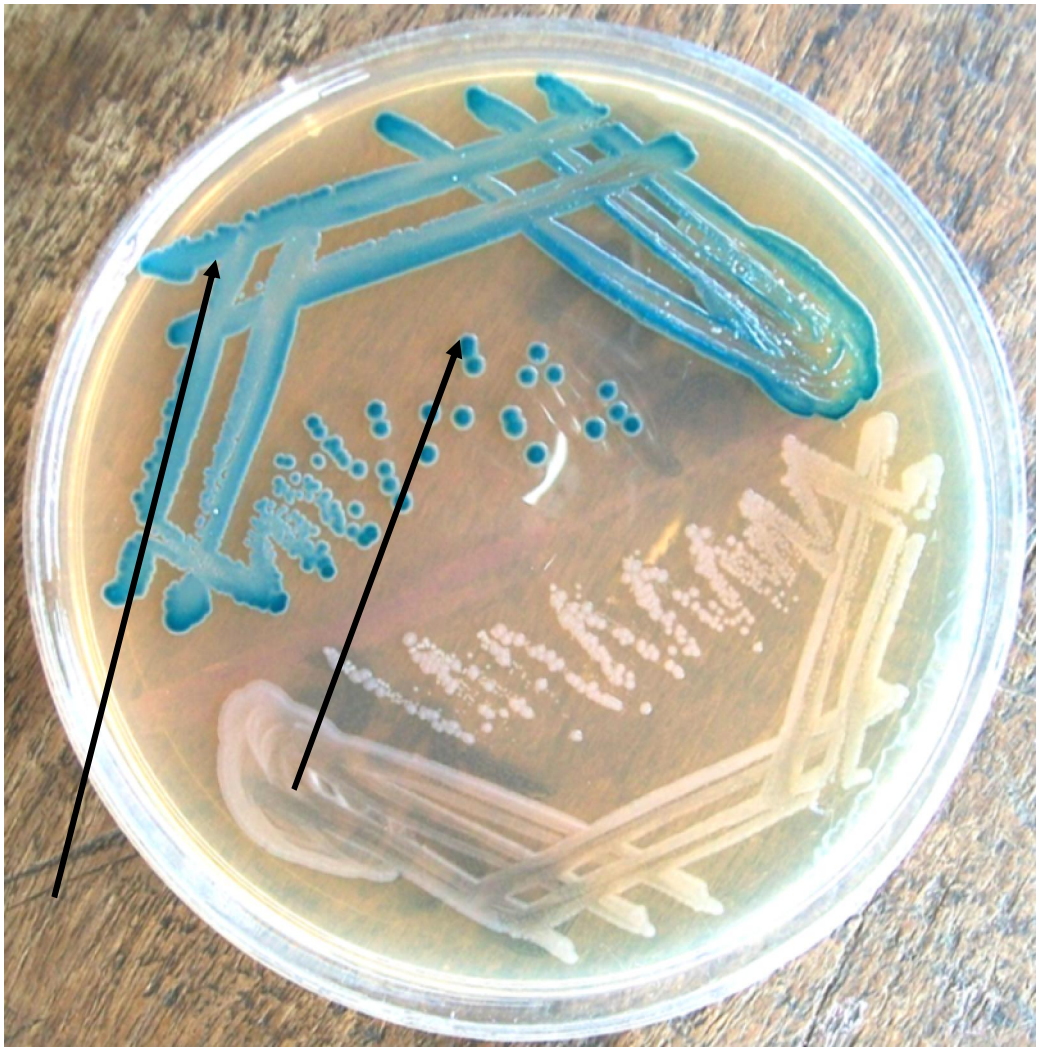

Plate 1. BCIG positive and BCIG negative *E.coli* colonies growing on Sorbitol MacConkey BCIG agar after 24hrs incubation at 37^{o}C.

A summary of the multidrug resistance pattern of *E.coli* O157:H7 to the antimicrobial agents used (Table 3) shows that resistance was expressed in all the isolates, with most (7 of them) being jointly resistant to nine (9) antibiotics, while one isolate was resistant to all the antibiotics tested. The Multiple antibiotic resistance (MAR) indices ranged from 0.54 to 1.0 (Table 4). The frequency of Multiple antibiotic resistance MAR has been defined as joint resistance of *E. coli* isolates to more than two antibiotics (Ngwai et al., 2011).MAR was determined using the formula MAR=x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for susceptibility (Krumperman, 1983; Akinjogunla and Enabulele, 2010).

According to Krumperman (1983), MAR indices above 0.2 indicate that such isolates originate from environment where antimicrobial agents are freely available and accessible with high potential for abuse. This is true of most of the cities in Edo State where people access antibiotics over-the-counter, almost without restrictions. This poses a serious public health concern.

The high MAR indices as recorded in this study also agree with that recorded by Ngwai et al. (2011) in their investigation of multidrug resistant *E.coli* from HIV patients in Keffi, Nigeria. Over the last two decades antimicrobial resistance has been reported for all classes of diarrhoegenic *E.coli* and specifically from African isolates (Vila et al., 1999). Similar to the high antibiotic resistance pattern observed in this study, Tobih et al. (2006) have also shown that pathogenic isolates of *E. coli* have relatively high potentials for developing resistance. Okeke et al. (2000) have expressed the opinion that normal intestinal flora is a reservoir for resistance genes and resistance of *E.coli* to almost all agents has increased over time.

Finally, in order to forestall the continuous perpetuation of the high antibiotic resistant profiles in our community as evidenced in this study, the existing antibiotic prescribing policies by relevant health professionals need be re-evaluated, while the purchase and use of antibiotics over- the- counter by the general public have to be strictly monitored.

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Corresponding Author:
Dr. Jonathan Osariemen Isibor
Department of Microbiology
Faculty of Natural Sciences
Ambrose Alli University
P.M.B.14, Ekpoma
Edo State- Nigeria.
E-mail: joe_isibor@yahoo.com
Telephone: +(234) 80 35 5151

References

1. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998; 11:142.
2. Effler P, Isaacson M, Arntzen L, Heenan R, Canter P, Barret TL, Mambo C,Levine W, Zaidi A, Griffin PM. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. Emerg Infect Dis 2001; 7:812-819.
3. Okeke IN, Fayinka ST, Lamikanra A. Antibiotic resistance in *E.coli* from Nigerian students, 1986-1998. Emerg Infect Dis 2000; 6:393- 396.
4. Ngbede JE, Jideani IA, Agbo EB. Prevalence of *Escherichia coli* O157:H7 from diarrheal patients in Jos hospitals, Nigeria. J Food Agric Environ 2006; 4:20-22.
5. Agbogu VN, Umoh VJ, Okuofu CA, Smith SI, Ameh JB. Study of the bacteriological and physiochemical indicators of pollution of surface waters in Zaria, Nigeria. Afr Jour of Biotech 2006;5 (9): 732-737.
6. Ekundayo AO, Isibor JO. Screening of human and non-human specimens for *Escherichia coli* O157:H7 and typhoid organisms in Benin City, Nigeria.E3 J Med Res. 2012; 1(4):052-056.
7. Smith SI, Bello OS, Goodluck H A, Omonigbehin EA, Agbogu VN, Odeigah P. Prevalence of EHEC O157 from human and environmental samples from Lagos and Zaria. Pak J Med Sci 2009; 25 (3):398-408.
8. Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell,HD, Geldreich E, Payne BJ, Meyer A, Wells JG. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhoea and death. Ann Intern Med 1992; 117:812-819.
9. Swinbanks D. Japan shuns radishes after 'possible link' to *E. coli*. Nature 1996; 382:567.
10. Nawaz SK, Riaz S, Hasnain S. Screening for anti-methicillin resistant *Staphylococcus aureus* (MRSA) bacteriocin producing bacteria. Afr J Biotech 2009; 8 (3): 365-368.
11. Akinjogunla OJ, Inyang CU, Ekoi OH, Ntinya AJ. Prevalence and antibiogram of *Salmonella typhi* and *Salmonella paratyphi* in stools of

symptomatic typhoid patients. Int J Biotechnol Allied Sci 2008; 3 (1):377-382.
12. Poppe C, Martin LC, Gyles CL, Reid-Smith R, Boerlin P, McEwen SA, Prescott JF, Forward KR. Acquisition of resistance to extended spectrum cephalosporin by *Salmonella enterica* subsp. Enterica serovar Newport and *Escherichia coli* in Turkey poultry intestinal tract. Appl Environ Microbiol 2005; 71: 1184- 1192.
13. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press, Cambridge, 2006; p. 434.
14. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to Identify verocytotoxin-positive strains of *Escherichia coli* serotype O157..J Clin Microbiol 1990; 28:2165 – 2168
15. Bauer AW, Kirby WM, Sherris JC. Antibiotics susceptibility testing by a standardized single disk method. Am J Clin Patho 1996; 45:493-496.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing- nineteenth informational supplement. CLSI document M100-S20. Clinical and Laboratory Standard Institute, Wayne, Pennsylvania, USA. 2010.
17. Aibinu I, Adenipekun E, Odugbemi T. Emergence of quinolone resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos state hospitals, in Nigeria. Nig J Health Biomed Sci 2004; 3 (2): 73 -78.
18. Ngwai YB, Nwankwo HN, Adoga MP. Multi-drug resistant *Escherichia coli* from human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients in Keffi, Nigeria. Int Res Jour Microbiol 2011;2(4):122-125.
19. Olukoya DK, Daini OA, Niemogha MT. Preliminary epidemiological studies on tetracycline resistant plasmids isolated from enteric bacteria in Nigeria.Trop Geogr Med 1993; 45 (3):117-20.
20. Barry AL, Fuchs PC, Pfaller MA, Allen SD, Gerlach EH. Prevalence of fluoroquinolone resistant bacterial isolates in four medical centers during the first quarter of 1990. Euro J Clin Micro Infect Dis1990; 9: 906–8.
21. Egri-Okwaji MTC, Iroha EO, Kesah CN, Odugbemi T. Bacterial pathogens causing neonatal sepsis in an out-born neonate unit in Lagos, Nigeria Nig Qtr J Hosp Med 1996; 6: 149 – 152.
22. Kesah CN, Egri-Okwaji MTC, Iroha EO, Odugbemi T. Common antimicrobial resistance of nosocomial pathogens. Nig Postgrad Med J 1999; 6: 60 - 65.
23. Isibor JO, Erhunmwunse PI, Nwobu GO. Antimicrobial susceptibility of clinical isolates of *Esch. coli* to six antimicrobial agents. J Appl Bas Sci 2003; 1(1and2):37-40.
24. Daini OA, Ogbolu OD, Ogunledun A. Quinolones resistance and R- Plasmids of some Gram-negative enteric bacilli. Afr Jour Clin Exper Microbiol 2005 ; 6(1):14-20.
25. Umolu PI, Omigie O, Tatfeng Y, Omorogbe FI, Aisabokhale F, Ugbodagah, OP. Antimicrobial susceptibility and plasmid profiles of *Escherichia coli* isolates obtained from different human clinical specimens in Lagos – Nigeria. Jour Am Sci 2006; 2(4): 70-76.
26. Oteo J, Lazaro E, de Abjo FJ, Baquero F, Campos J, Spanish members of EARSS. Antimicrobial - resistant invasive *Escherichia coli*, Spain. Emerg Infect Dis 2005; 11 (4): 546-553.
27. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. Appl Environ Microbiol 1983; 46:165-170.
28. Akinjogunla OJ, Enabulele OI. Virulence factors, plasmid profiling and curing analysis of multi-drug resistant *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp isolated from patients with acute otitis media. Journal of American Science 2010; 6 (11): 1022-1033.
29. Vila J, Vargas M, Casals C, Urassa H, Mshinda H. Antimicrobial resistance of diarrheagenic *Escherichia coli* isolated from chicken under the age of 5 years from Ifakara, Tanzania. Antimicrob Agents Chemother 1999; 43: 3022-3024.
30. Tobih JE, Taiwo SS, Olowe O.A., Olaosun OA, Adejumo SO. Microbiological profiles of discharging ears in Osogbo, Nigeria. Trop Doc 2006; 36 (3): 165-1