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Phenotypic and Molecular Detection of Resistance Genes from Multi-drug Resistant *Escherichia coli* Isolated from Patients Attending Selected Hospitals in Damaturu, Yobe State, Nigeria

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ABSTRACT

Multidrug resistance among *Escherichia coli* causing urinary tract infections (UTIs) and diarrhea are major public health problem worldwide which cause difficulty in treating the infections caused by *Escherichia coli* due to the high resistances. The study is aimed to determine the phenotypic and molecular detection of multidrug resistant *E. coli* isolated from clinical samples of patients attending selected Hospitals in Damaturu, Yobe State-Nigeria.

Methods: Two hundred (200) clinical samples were collected aseptically from patient diagnosed with diarrheic (100 stool samples) and UTI's (100 urine samples) using sterile universal container. The samples were processed using standard microbiological methods for identification of *E. coli*. Samples were cultured on MacConkey agar (stool) and Cystine lactose electrolyte deficient agar (urine). The resulting colonies of isolates were further subculture on Eosin methylene blue agar for confirmatory and followed by gram stain, biochemical identification at Microbiology laboratory unit of Yobe State Specialist and Yobe State Teaching Hospital respectively. The antimicrobial susceptibility patterns were determined using Kirby-Bauer disc diffusion techniques and the phenotypic expression of extended spectrum beta-lactamases (ESBLs) were determined using modified double disc synergy test (MDDST) and also the three (3) resistance genes (*blaTEM, accC1* and *qnrA*) were detected using polymerase chain reaction.

Results: One hundred and twenty-two (122) isolates were resistant to antibiotics. The highest level of resistance was against amoxicillin (90.2%) while the least resistance was against sparfloxacin (24.3%). Thirty-seven (37) *E. coli* isolates shows MDR; the highest MDR was (24.3%) while least MDR was (5.4%). The PCR amplification of resistant genes *(blaTEM, accC1* and *qnrA*) were detected on *E. coli* that shows positive ESBL and the bands were separated using agarose gel electrophoresis.

Conclusion: The findings of this study show augmentin, ciprofloxacin and sparfloxacin are the most effective antibiotics against *E. coli* isolated from patients attending the two hospitals in Damaturu; who are diagnose with UTI and diarrheic infection. The resistant genes include; *blaTEM, accC1* and *qnrA* coding for beta-lactam, aminoglycoside and quinolones were present in *E. coli* isolated from patients attending selected Hospitals in Yobe State, Nigeria.

Keywords: Multidrug resistant, *Escherichia coli*, extended spectrum beta lactamase, resistance-associated genes, urinary tract infections, diarrheic.

1. INTRODUCTION

During the last few decades, the incidence of microbial infections has increased dramatically. Continuous deployment of antimicrobial drugs in treating infections has led to the emergence of resistance among the various strains of

microorganisms (Lee *et al*., 2013). Multidrug resistance (MDR) is insensitivity or resistance of a microorganism to the administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it or process by which microorganism's resist to three or more antimicrobial classes (Singh, 2013; Popęda, 2014). According to WHO, these resistant microorganisms (like bacteria, fungi, viruses, and parasites) are able to combat attack by antimicrobial drugs, which leads to ineffective treatment resulting in persistence and spreading of infections. Although the development of MDR is a natural phenomenon, extensive rise in the number of immunocompromised conditions, like HIV-infection, diabetic patients, individuals who have undergone organ transplantation, and severe burn patients, makes the body an easy target for hospital acquired infectious diseases, thereby contributing to further spread of MDR (Nikaido, 2009; WHO,2014). Antimicrobial resistance infection is associated with high mortality rates and high medical costs and has a significant impact on the effectiveness of antimicrobial agents. MDR provokes obstruction in disease control by intensifying the possibility of spreading of resistant pathogens, thus, declining efficacy of treatment and, hence, resulting in prolonged time of infection in-patient. The cost of treatment is increase due to multiple classes of antibiotics, and some turn out to be extensively drug resistance (WHO, 2014).

Escherichia coli is the first microorganism which was described by Theodor Escherich in 1885, which is a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens (Kaper and Nataro, 2004). As a commensal, it lives in a mutually beneficial association with hosts, and rarely causes disease. It is, however, also one of the most common human and animal pathogens, as it is responsible for a broad spectrum of diseases. The peculiar characteristics of the *E. coli*, such as ease of handling, availability of the complete genome sequence, and its ability to grow under both aerobic and anaerobic condition, makes it an important host organism in biotechnology. *E. coli* is used in a wide variety of applications both in the industrial and medical area and it is the most used microorganism in the field of recombinant DNA technology (Yoo *et al*., 2009).

Transmission of *Escherichia coli* occurs via the fecal-oral route after consumption of contaminated, undercooked liquids and foods. Recently *E. coli* becomes more resistant to antimicrobials especially to cephalosporin, aminoglycoside, beta-lactam, quinolones and others (Chill *et al.,* 2016; Karlawsky *et al.,* 2017). *E. coli* is one of the *Enterobacteriaceae* strains that produce extended-spectrum beta-lactamases enzymes (ESBLs) producing bacteria and becomes highly resistant against different beta-lactam antimicrobials lead to difficult to treat diseases and as well as other class of antibiotics, hence they are called multi-drug resistant (MDR) bacteria (Nathesiwan *et al.,* 2001). The emergence of *Escherichia coli* isolates with multiple antibiotic-resistant phenotypes, involving co-resistance to three or more unrelated families of antibiotics, has been previously reported and is considered a serious health concern (Pitout, 2012).

The extended spectrum β lactamases TEM, SHV, and CTX-M are the three main types of ESBLs. CTX-M, which has become more prevalent than SHV and TEM, includes a rapidly expanding family, which has spread among a wide range of clinically important bacteria and over wide geographic areas (Yusuf and Yahaya, 2013). Furthermore, strains that produce ESBL often demonstrate resistance to antibiotics belonging to other classes (i.e. aminoglycosides, quinolones, and sulfonamides), which makes strategies of treatment more complex (Liao *et al,* 2017).

The mechanism of resistance to β-lactam antimicrobial agents in *E. coli* is production of β-lactamase hydrolytic enzymes that disrupt the amide bond of the characteristic four-membered β-lactam ring, rendering the antimicrobial ineffective (Blair, 2015). Interestingly the β-lactamases are structurally relate to PBP's and they may have evolved from these β-lactam-binding enzymes of cell wall biosynthesis. These enzymes have been describe numerous times in both Gram-negative and Gram-positive organisms and in the Mycobacteria (Yusuf and Yahaya, 2013).

Aminoglycosides are important treatments against Gram-negative infections. They are particularly active against aerobic, Gram-negative bacteria and act synergistically against certain Gram-positive organisms (Jena and Deb, 2006). Aminoglycosides are a therapeutically essential class of antibiotics whose usefulness their toxic potential and residues in food animals often restrict. The aminoglycoside antimicrobial compounds are produce from strains of *Streptomyces* spp., *Micromonospora* spp., and *Bacillus* spp. Neomycin, streptomycin and kanamycin are examples of aminoglycosides (Shakil *et al*, 2008).

The primary mechanism for resistance to aminoglycosides of *E. coli* is enzymatic modification involving three families of enzymes: acetyltransferases (AAC), nucleotidyl (adenyl) transferase (ANT) and phosphotransferases (APH) (vaculenko, 2003; EFSA, 2008). Cross-resistance between aminoglycosides is complex and depends on the gene(s) present (EFSA, 2008).

Quinolones are one of the most commonly prescribed classes of antibacterial in the world and they are use in treating a variety of bacterial infections in humans. Because of the wide use (and overuse) of these drugs, the number of quinolone-resistant bacterial strains has been growing steadily since the 1990s. As is the case with other antibacterial agents, the rise in quinolone resistance threatens the clinical utility of this important drug class. Quinolones act by converting their targets, gyrase and topoisomerase IV into toxic enzymes that fragment the bacterial chromosome (Drlica *et al*., 2009). Various community and hospital-based studies from Nigeria and other African countries have reported a varying prevalence of antimicrobial resistant and phenotypic ESBL producing Enterobacteriaceae (Ugbo *et al*., 2016). However, there is no information on molecular detection of *E. coli* isolates causing UTIs and diarrheic in Yobe State, Nigeria. Therefore, this study was carry out to investigate the molecular detection of resistance genes in *E. coli* isolated from patients diagnosed with UTIs and diarrheic in Yobe State, Nigeria.

2. MATERIAL AND METHOD

2.1 Sampling site and sample collection

The research design were carried out in two (2) hospitals in Damaturu metropolis with geographical coordinate's 12°00′N 11°30′E / 12.000°N 11.500°E. The samplings were performed between the months of March 2020 to January 2021. Two hundred clinical samples were collected aseptically using a sterile universal container, 100 urine samples were collected from patients diagnose with urinary tract infections from the two hospitals and 100 stool samples were collected from patients diagnose with diarrheic from the two hospitals respectively. This study was approved by the Research Ethics Committee of the Yobe State Specialist Hospital (YSSH) and Yobe State Teaching Hospital (YSTH), Damaturu before the commencement of the study.

2.2 Culture/Microbiological analysis

The clinical samples collected were streak separately onto cysteine lactose and electrolyte deficient (CLED) Agar for (urine) and MacConkey agar for (stool) plates under aseptic techniques and incubated at 37°C for 24hours. The resulting colonies were further sub-culture for confirmatory onto Eosin Methylene Blue

agar and incubated at 37°C for 24hours. The colonies were further identified using gram staining and biochemical techniques (citrate test, methyl red test, triple sugar iron test and indole test) (CLSI, 2016).

2.3 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of confirmed *Escherichia coli* isolates were performed on Mueller Hinton agar plates using a modified Kirby-Bauer disc diffusion technique. Ten antibiotics were used include; septrin (30µg), chloramphenicol (30µg), sparfloxacin (10µg), ciprofloxacin (30µg), amoxicillin (30µg), augmentin (10µg), gentamicin (30µg), pefloxacin (30µg), tarivid (10µg), streptomycin (30µg). The antibiotic disk was place on the surface of Mueller Hinton agar being streak with the confirmed colony of *Escherichia coli*, sufficiently separated from each other to avoid overlapping of inhibition zones. After 30 seconds of pre-diffusion, the plates were incubated at 37^{O}C for 24 hours after which the diameter of inhibition zones was measure with a meter rule (CLSI, 2016).

2.4 Phenotypic detection of extended spectrum β-lactamase (ESBLs)

The screening for ESBLs production in all positive *Escherichia coli* isolates were perform using combination of modified double disc synergy test (MDDST) method. The phenotypic detection was carried out using three types of antimicrobial agents (3rd generation cephalosporin's); ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg) and ceftriaxone (CRO 30µg). The single discs of ceftazidime, cefotaxime and ceftriaxone were place on each of the isolate inoculated on Mueller Hinton agar plates and incubated at 37°C for 24 hours for the detection of ESBL enzymes. The zone diameter around each of the discs was measure and the diameter around ceftazidime, cefotaxime and ceftriaxone were 5 mm and more, the bacterial isolates produced ESBL enzymes (CLSI, 2016).

2.5 Confirmatory test for detection of extended spectrum β-lactamase

The confirmatory test was performed with Augmentin (AMC 30µg) disc, which was place at the center of Mueller Hinton agar plate containing the streaked colonies of the positive isolate. Three discs ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg) and ceftriaxone (CRO 30µg) were place at side of Augmentin (AMC 30µg) disc with distance of 15mm from center; the plates were incubated overnight at 37^{o}C for 24 hours. The zone of inhibitions towards the Augmentin (AMC 30µg) were measure for positive production of ESBL (CLSI, 2016).

2.6 DNA extraction for PCR amplification

The DNA templates of each of the confirmed pure *Escherichia coli* isolates were generate by dispensing most of the pure colonies of overnight growth of the isolates onto 100-µL1X Tris- EDTA buffer, vortex mixed and boiled at 100°C for 10 minutes. Then it was transferred immediately to the freezer (-20°C) for 10 minutes, maintained at room temperature, vortex mixed again and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant containing DNA templates of the isolates were separate, stored at 4°C, and used as DNA template for PCR amplification (Yang *et al.*, 2008).

2.6.1 Molecular detection of ESBLs associated resistance genes

The polymerase chain reaction (PCR) was perform for beta-lactamase (*blaTEM*), aminoglycoside (*accC1*) and quinolones (*qnrA*) to detect the presence of extended spectrum beta-lactamase encoding genes and PCR analysis was carried out at Nigerian Institute for *Trypanosomiasis* Research Kaduna State, Nigeria. The PCR was carried out for eight (8) randomly selected ESBLs positives using a primer in (Table 1) and the PCR amplification was carried in a MyGene^{TM} Series Peltier Thermal Cycler MG96 (LongGene

Scientific Instruments Co. Ltd. China). The amplification mixture was all the same for three resistant genes (*blaTEM, accC1* and *qnrA*) respectively. The amplification reaction was carried out using (1µl) DNA templet, (1µl) each forward and reverse primers (from Inqaba Biotec, West Africa Ltd.) and (17µl) of PCR premix contain all PCR products (HotStart from Bioneer Company, South Korea) which make complete (20µl) reaction mixture (Zhang *et al.*, 2019). The amplification conditions used for each gene were as follows:

2.6.2 Amplification of the *blaTEM* resistance genes

The *blaTEM* resistant gene was amplified using initial denaturation at 95°C for 5min; 35 cycles of 94°C for 30sec, 52°C for 30sec and 72°C for 45sec; and final elongation step at 72°C for 7min. The annealing temperature was 52°C for *blaTEM* gene (Ensor *et al*., 2009).

2.6.3 Amplification of the *accC1* resistance genes

The *accC1* resistant gene was amplified using initial denaturation at 95°C for 5min; 35 cycles of 94°C for 1min, 55°C for 1min and 72°C for 2min; and final elongation step at 72°C for 5min. The annealing temperature was 55°C for *accC1* gene (Hujer *et al*., 2006).

2.6.4 Amplification of the *qnrA* resistance genes

The *qnrA* resistant gene was amplified using initial denaturation at 95°C for 5min; 35 cycles of 94°C for 1min, 55°C for 1min and 72°C for 2min; and final elongation step at 72°C for 5min. The annealing temperature was 55°C for accC1 gene (Robicsek *et al*., 2006).

3. RESULTS

Table 1: The three (3) resistance genes used in this study are (*blaTEM, accC1* and *qnrA*) and the detection of the genes were perform using a primer by previous studies.

Table 2: One hundred and twenty two (122) *Escherichia coli* positive isolates were obtain from two hundred (200) samples; one hundred (100) samples from each hospital. The table shows the prevalence of *Escherichia coli* in stool and urine samples. Only 23 of the 61 stools were positive for *Escherichia coli*, which corresponded to an isolation rate of 37.7 %. Only 99 of the 139 urine samples gave positive *Escherichia coli* and hence prevalence rate of 71.2 %.

Table 3: Reveals the multi-drug resistance (MDR) pattern of 37 *Escherichia coli* isolates. The MDR patterns in the table were comprised of Yobe State Specialist and Yobe State Teaching Hospitals in Damaturu respectively. Under the Y.S.S.H (21) isolates were found to be resistance to three and above antibiotics; the highest level of MDR is 5 (23.8 %) and the least is 1 (4.8 %). While (16) isolates were found to have three and above antibiotics at Y.S.T.H; the highest level MDR is 5 (31.3%) and the least is 1 (6.3 %). Then, the combined MDR of the both hospitals were found to be 37 isolates and the highest level of MDR was found to be 9 (5.4 %) while the least is 2 (5.3 %) respectively.

Table 4: Shows the total number of positive samples for extended beta-lactamase and non- extended beta lactamase producing *Escherichia coli* among the MDR isolates. Among the (122) positive isolates were obtained in stool and urine from clinical samples of patients in the Y.S.S.H and Y.S.T.H Damaturu. Only (34) isolates were producing extended spectrum beta lactamase: 12 (32.3 %) stool and 22 (64.7 %) urine from the clinical samples of the both hospitals. Meanwhile, the remaining (88) isolates were found non-extended spectrum beta lactamase producing organisms; 11 (12.5 %) stool and 77 (87.5 %) respectively.

Table 1: Primers used in PCR for (3) antimicrobial resistance genes of *Escherichia coli*

Groups of antibiotics	Resistance genes	Product size (bp)	Primers	References
Beta-lactam	*blaTEM*	680	F:5'-CAGCGGTAAGATCCTTGAGA-3' R:5'-ACTCCCCGTCGTGTAGATAA-3'	Ensor *et al*., 2009
Aminoglycoside	*accC1*	890	F:5'-ATGGGCATCATTCGCACATGTAGG-3' R: 5'-TTAGGTGGCGGTACTTGGGTC-3'	Hujer *et al*., 2009
Quinolones	*qnrA*	810	F: 5'-ATTTCTCACGCCAGGATTTG-3' R: 5'-GATCGGCAAAGGTTAGGTCA-3'	Robicsek *et al*.,2009

Table 2: Prevalence of *Escherichia coli* isolated from clinical samples of patients attending Y.S.S.H and Y.S.T.H Damaturu.

Sample	Source	Number (%) of collected samples	Number (%) positive for *E. coli*
Stool	Y.S.S.H	31(50.8)	15 (48.4)
	Y.S.T.H	30 (4.2)	8 (26.7)
	Total	**61 (100)**	**23 (37.7)**
Urine	Y.S.S.H	69 (49.6)	45 (65.2)
	Y.S.T.H	70 (50.4)	54 (77.1)
	Total	**139 (100)**	**99 (71.2)**

KEY: Y.S.S.H = Yobe State Specialist Hospital, Damaturu.
Y.S.T.H = Yobe State Teaching Hospital, Damaturu.

Table 3: Multi-drug resistant pattern of *Escherichia coli* isolated from clinical samples of patient attending Y.S.S.H and Y.S.T.H Damaturu.

S/N	Number of Combination of Antibiotics Antibiotics	Y.S.S.H Isolates (%) (n = 21)	Number of Combination of Antibiotics Antibiotics	Y.S.T.H Isolates (%) (n = 16)	Multi Drug Resistant Isolates Combined (%) (n = 37)
1	3 AM, CPX, AU	3 (14.3)	3 CPX, AU, SP	2 (12.5)	5 (13.5)
2	3 AM, SXT, CPX	5 (23.8)	3 AM, CPX, AU	1 (6.3)	6 (16.2)
3	4 AM, SXT, SP, CPX	1 (4.8)	4 AM, CPX, AU, OFX	2 (12.5)	3 (8.1)
4	5 AM, SXT, CH, SP, CPX	3 (14.3)	5 AM, CPX, AU, OFX, S	3 (18.8)	6 (16.2)
5	6 AM, SXT, CH, SP, CPX, OFX	4 (19.0)	6 AM, CPX, AU, CN, OFX, S	5 (31.3)	9 (24.3)
6	7 AM, SXT, CH, SP, CPX, CN, OFX	2 (9.5)	7 AM, SXT, CPX, AU, CN, OFX, S	0 (0.0)	2 (5.4)
7	8 AM, SXT, CH, SP, CPX, CN, OFX, S	0 (0.0)	8 AM, SXT, CPX, AU, CN, PEF, OFX, S	3 (18.8)	3 (8.1)
8	9 AM, SXT, CH, SP, CPX, CN, PEF, OFX, S	3 (14.3)	9 AM, SXT, CH, CPX, AU, CN, PEF, OFX, S	0 (0.0)	3 (8.1)

KEY: AM = Amoxicillin, SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AU= Augmentin, CN= Gentamycin, PEF= Pefloxacin, OFX= Nalidic Acid, S= Streptomycin.
Y.S.S.H = Yobe State Specialist Hospital, Damaturu, Y.S.T.H = Yobe State Teaching Hospital, Damaturu.

Table 4: Total numbers and percentage of ESBL - producing and non- ESBL *Escherichia coli* isolated from clinical samples of patients attending Y.S.S.H and Y.S.T.H Hospital Damaturu.

Sources of Isolates	Y.S.S.H n = 60	Y.S.T.H n = 62	ESBL producing *E. coli* (%) n=34	Non-ESBL producing *E. coli* (%) n =88
Stool	15	8	12 (35.3)	11 (12.5)
Urine	45	54	22 (64.7)	77 (87.5)
Total	**60**	**62**	**34 (100)**	**88 (100)**

KEY: ESBL= Extended spectrum beta lactamase,
Y.S.S.H = Yobe State Specialist Hospital, Damaturu, Y.S.T.H = Yobe State Teaching Hospital Damaturu.

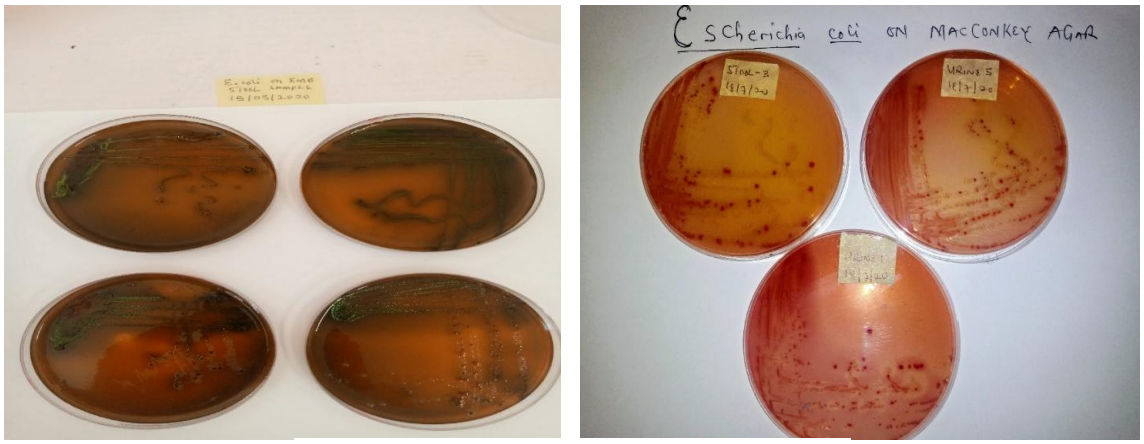

Figure 1: Shows *Escherichia coli* on EMB and MacConkey agar

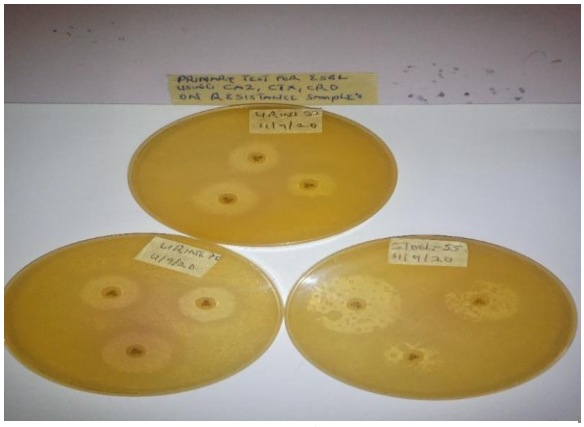

Figure 2: Shows primary test for extended spectrum beta-lactamase (ESBLs)

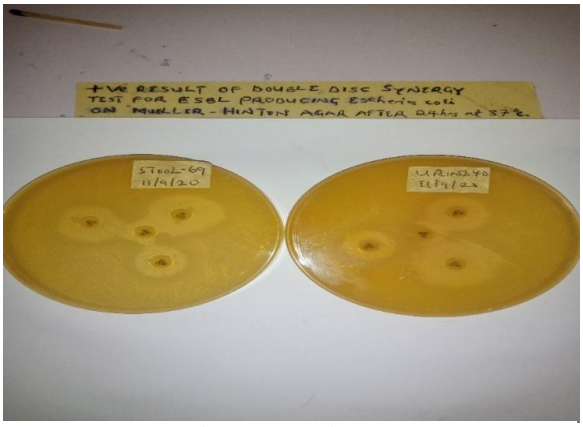

Figure 3: Shows confirmatory test for extended spectrum beta-lactamase (ESBLs)

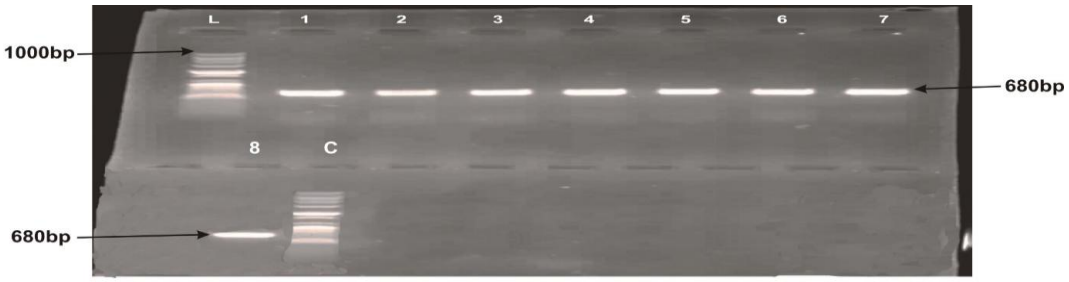

Fig 4: PCR amplified products from extracted DNA of 8 positive ESBL producing *Escherichia coli*, amplified with blaTEM gene shows positive results at 680bp.
L: DNA Molecular Ladder
C: Positive Control Sample

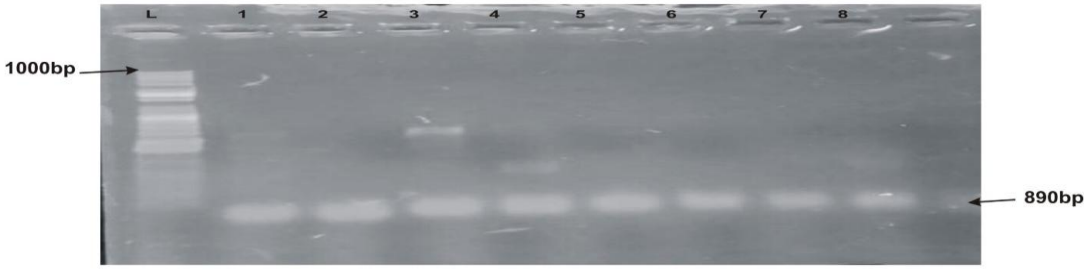

Fig 5: PCR amplified products from extracted DNA of 8 positive ESBL producing *Escherichia coli*, amplified with accC1 gene shows positive results at 890bp.
L: DNA Molecular Ladder

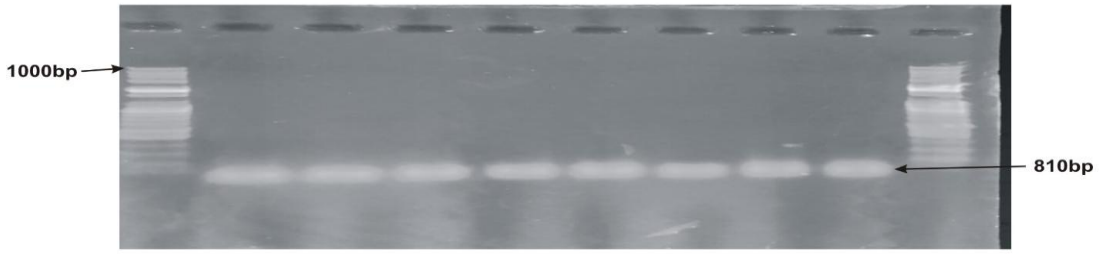

Fig 6: PCR amplified products from extracted DNA of 8 positive ESBL producing *Escherichia coli*, amplified with qnrA gene shows positive results at 810bp.
L: DNA Molecular Ladder
C: Positive Control Sample

Figure 4, 5 and 6: Shows PCR representative gel for the detection of resistance genes of *blaTEM*, *accC1* and *qnrA* respectively.

4. DISCUSSION

The main goal/aims of this study was to investigate the prevalence of multidrug resistance in *Escherichia coli*, ESBLs producing and molecular detection of antimicrobial resistance-associated genes (*blaTEM*, *accC1* and *qnrA*) in *Escherichia coli* isolated from patients diagnosed with urinary tract and diarrheic infections in Yobe State Specialist and Yobe State

teaching hospitals Damaturu, Nigeria. *E. coli* is one of the major causes of UTIs and diarrheic infection affecting humans of all ages. The emergence of multidrug resistance (MDR) *Escherichia coli* strains and the progressive rise in antimicrobial resistance threatens the effective treatment of UTIs and diarrheic infection leading to increased morbidity, prolonged hospital stays, increase in the cost of treatment and disease related mortality (Stefano *et al*., 2013). This early detection of antimicrobial resistance of the *E. coli* in a particular region will help to quick adapting strategies that can reduce the potential misuse of antimicrobial agents and prevent the emergence and subsequent spread of such multidrug resistance isolates (Salah *et al*., 2016).

The antimicrobial susceptibility pattern of one hundred and twenty two (122) *Escherichia coli* in this study shows that most isolates of *E. coli* were resistant to antimicrobial especially third generation cephalosporin's, but 37% isolates of *E. coli* were found to be resistance to three and above class of antibiotics and hence called multidrug resistant (MDR). The MDR rate in this study range from 24.3% while 5.4% lower MDR among (Table 3). In addition, in this study, UTIs isolates were the most common ESBLs producing strains with 64.7% followed by diarrheic isolates with 35.3% (Table 4).

In this study, the MDR and ESBL producing *E. coli* was significantly observe in isolate obtain in urine and this was similar to previous findings, which were significantly associated with the increased resistance of *E. coli* to beta-lactam, aminoglycoside and quinolones (Roshan *et al*., 2011). Most *E. coli* strains may be having natural resistance to beta-lactam antibiotics mostly ampicillin, amoxicillin and clavulanic acid and the resistance to ESBLs could happen in class A chromosome beta-lactamase TEM and SHV genes are expressed (Salah *et al*., 2016). These genes are capable of enabling bacteria to resist to different antimicrobial agents including third generation cephalosporin, aminoglycosides and others (Essack *et al*., 2004). In Iraq, the prevalence of MDR Enterobacteriaceae and ESBLs producing gram negative bacteria has been reported by some researcher (Al-mayahie, 2013). In addition, it was reported at previous findings that *E. coli* had the highest resistance to the all drugs tested (Adam and Turgut, 2019). Quinolone and gentamicin which were among effective agents of choice relies for treatment of most bacterial infections in the last one decade were now observed in this study to be largely ineffective on these *E. coli* isolates. The increasing level of resistance has reported in recent studies from other developing countries where there is no strict policy on the use of antibiotics in their communities (Muhammad and Swedan, 2015). The high effectiveness of sparfloxacin, augmentin and ciprofloxacin against the *E. coli* in this study on the treatment of UTI and diarrheic infection caused by *E. coli* support the previous findings (Muhammad *et al*., 2015, Abujnah *et al*., 2015 and Salah *et al*., 2016). Hence, the known effectiveness of colistin, nitrofurantoin and cefoperazone on UTI and diarrheic infection that caused by *E. coli* in combination with augmentin, ciprofloxacin and sparfloxacin can be used in treatment of such infections. Although, nitrofurantoin is an oldest UTI drug, which is not un-like to be attributed to, its unpleasant side effects that has largely discourage its frequent misuse and this support previous findings (Muhammad *et al*., 2015 and Abujnah *et al*., 2015). The combined multidrug resistance in this study was 37% (Table 3) which is around the previously reported findings of 39-85% in various parts of Nigeria and other African countries (Aboderin *et al*., 2009, Anago *et al*.,2015 Ugbo *et al*., 2016, Salah *et al*.,2016, Adebola *et al*., 2019 and Olaruntoba *et al*., 2020). The observed differences might be due to differences in the screening methods used in the selected hospitals in Yobe State during this study. However, the observed prevalence rate of MDR in this study indicates that the isolates obtained might have been expose to these

antimicrobial agents from either clinics or agricultural products since *E. coli* can easily be expose to the drugs used in animal husbandry and food industry during the processing to ingestion. These necessitate the controlling of using drugs in both clinical and agricultural usage to avoid or reduce the prevalence of MDR in patients.

The phenotypic detection of ESBLs in this study were identified with 34% (Table 4) of the *E. coli* isolates using modified double disc synergy test (MDDST) that is ceftazidime, cefotaxime and ceftriaxone combination discs which has higher percentage with previous finding 24% (Adebola *et al.*, 2019). However, the percentage in this study was high because the use of the multiple discs including augmentin, ceftazidime, cefotaxime and ceftriaxone during the screening of ESBLs enhanced the highest rates of detection among the positive isolates obtained from the selected hospitals in Yobe State, Nigeria. Also, the finding in this study shows high percentage with previous studies in Amassoma, South-Southern with 9.6%, Oshogbo, South-Southern Nigeria with 26%, 6.7% in Libya, 25% in Cotonou, Benin Republic and 22.3% in Iran respectively (Onanuga and Selekere, 2016, Ogbolu *et al*., 2011, Abujnah *et al*., 2015, Anago *et al*., 2015 and Ahadiri *et al.*, 2014). The higher prevalence of ESBLs in this study were almost the same with previous studies reported at Benin South-Southern Nigeria with 44.4%, Jordan with 54% and Togo with 93.4% respectively (Ogefere *et al*., 2015, Muhammad and Swedan, 2015 and Salah *et al*., 2016). The prevalence of ESBLs producing UTI and diarrheic infection caused by *E. coli* is a worldwide problems which has different degree of raised according to each counties from region to regions and its significantly associated with the extensive use of broad spectrum antibiotics especially third generation cephalosporins which has been progressively increasing in many countries (Bora *et al.*, 2014). There are differences in the screening procedure of ESBLs producing *E. coli* across the various study centers, which might contribute to the observed different varying values above.

The molecular detection of ESBLs resistance genes in this study carried out with eight (8) positive isolates randomly selected from clinical samples of the both hospitals in Yobe State, Nigeria. The PCR amplification of resistance genes (*blaTEM, accC1* and *qnrA*) from this study reveals that all eight (8) positive isolates shows the resistant genes (Fig 4, 5 and 6). The resistant genes obtained from this study were similar to various previous studies (Oloruntoba *et al*., 2020, Muhammad and Swedan, 2015, Anago *et al*., 2015 and Machado *et al*., 2007). Likewise, the findings on resistant genes in this study correspond with many previous studies (Momtaz *et al*., 2012, Ensor *et al*., 2009, Robicsek *et al*., 2006, Hujer *et al*., 2006 and Adebola *et al*., 2019). However, code for beta-lactam, aminoglycoside and quinolones resistant (Adenipekun *et al*., 2016), knew those resistant genes detected in this study. The detection of those resistant genes in *E. coli* isolates in this study suggests that resistance to these antimicrobial agents are genetically mediated possibly because of long-term use or misuse of the antibiotics. The reports of antibiotic resistant genes found in *E. coli* which might indicates there is a possibility of presence of other classes of antimicrobial associated resistant genes that are not detected in this study. Therefore, there is a need for a strategies to control the rapid dissemination of these antimicrobial resistant genes through implementation of strict guidelines for counter misuse of antibiotics and also rational use of antibiotics in both human and agricultural activities and these could be the possible explanation for the emergence of multidrug resistance genes (Oloruntoba *et al*., 2020).

CONCLUSION

The findings of this study show augmentin, ciprofloxacin and sparfloxacin are the most effective antibiotics against *E.*

coli isolated from patients attending the two hospitals in Damaturu; who are diagnose with UTI and diarrheic infection. The resistant genes include; *blaTEM, accC1* and *qnrA* coding for beta-lactam, aminoglycoside and quinolones were present in *E. coli* isolated from patients attending selected Hospitals in Yobe State, Nigeria that are responsible for the multidrug resistance observed. However, there is an extremely needful to strengthen a strict compliance to the use of antibiotics and enforcement of infection control practices in all our hospitals, primary health cares and pharmacies as means of controlling the increasing spread of multidrug resistance bacteria in Yobe State, Nigeria.

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