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Plasmid Curing in Multi-Drug Resistant Hospital and Community Uropathogenic *Escherichia coli*

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ABSTRACT: *Escherichia coli* is the most prevalent organism responsible for urinary tract infections (UTIs) in hospital and community sources. The present study was carried out to detect multi drug resistant (MDR) *E coli* from urine samples and the role of plasmids in drug resistance. One hundred urine samples were collected from the hospital and community within the University of Port Harcourt. Microscopic and chemical examination was carried out on the urine samples. *E coli* were isolated and antibiotic sensitivity test was carried out on the isolates, the resistant *E. coli* were cured by acridine orange and further subjected susceptibility testing. Result obtained from the study showed 35% *E. coli* recovered from community samples and 65% from hospital samples. Antibiotic sensitivity testing before plasmid curing showed high level of resistance to Augmentin (99%), Cefuroxime (92%), Ceftazidime (78%) and Cefixime (71%). The lowest level of resistance was reported in Gentamicin (15%) and Nitrofurantoin (19%). All the isolates were resistant to Augmentin but upon plasmid curing the resistant rate of isolates to eight antibiotics reduced. Our findings showed that Augmentin and Cefuroxime (62 and 31%) were still resistant after the plasmids of the isolates were cured. For hospital and community sources Nitrofurantoin (1; 0%), Ceftazidime (3; 8%), Ciprofloxacin (1%), Gentamicin (10%) and Ofloxacin (10%). Sixty-two (62) percent of the hospital isolates were resistant to three or more antibiotics while 60% of the community isolates were multidrug resistant. Our study thus concludes that plasmids alone are not responsible for the resistance to antibiotics exhibited by *E,coli* from urine samples. Antibiotics should be produced to target genes that are responsible for resistance to prevent the spread of drug resistant organisms.

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Urinary tract infections (UTIs) in humans are caused mostly by *Escherichia* coli, they are responsible for community and hospital- acquired UTIs. *E. coli* is the most prevalent facultative anaerobic species in the gastrointestinal tract of human and animals, usually a harmless microbe, but it is also a medically important bacteria that is responsible for most illnesses. *E. coli* among the Gram-negative organism from human origin are responsible for antimicrobial resistance (Ejikeugwu *et al*., 2017). Antimicrobial resistance has become a worldwide concern due to the emergence of drug resistant organisms. It is therefore necessary to constantly create an awareness that the right antibiotics must be prescribed as well as the mechanism of resistance and the role of plasmids in drug resistance (Irona *et* al., 2013; Lee Dr *et* al., 2018). Plasmids are small DNA molecules that are found within the cell, the cells are physically separated from chromosomal DNA but they have the ability to coexist with the host. Plasmids are extrachromosomal mobile elements that are genetic which are found in bacteria, they contribute to antibiotic resistance, virulence, gene gain between species through horizontal gene transfer by conjugate and non-conjugate mechanisms (Schief and Wensmk, 1981; 1999; Dasmeh *et al*., 2015). Plasmids found in different bacteria vary from each other because some of the plasmids are stable and thus can be maintained from one generation to another during cell division into daughter cell. During the cell division process the cell receives one or more plasmid copy (Tevors, 1986). Urinary Antibiotics résistance that is acquired in hospital and community are different from each other. Most urinary tract infections are acquired when the bacteria gets into the urinary system. It is most often acquired from public utilities that lack proper maintenance. People in hospitals and public residential homes are more vulnerable to urinary tract infections. They are resistant to a wide range of antibiotics especially fluoroquinolones, they are also extended beta lactamase producers (Mathers *et al.*, 2015). Recent studies have shown that *E. coli* S731 contain epidemic plasmids with bla CTX-M and blaKPC plasmids which makes them multidrug

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resistant and they are high risk (Mathers *et* al., 2015). The wild dissemination of antimicrobial resistance among bacterial populations is an increasing problem worldwide. The aim of this study is to compare the rate at which *E. coli* can cause urinary tract infection between community and hospitalized patients, and the role of plasmid in multidrug resistant *E. coli.*

MATERIALS AND METHODS

Collection of Urine Samples: Urine samples were collected from outpatients attending a public hospital and from a community (Alakahia community in Obio-Akpor local Government area of Rivers state). Sterile urine bottles were distributed randomly in Alakahia community. The persons involved, were properly instructed on how to produce early morning mid-stream urine and consent was obtained from them. Urine samples freshly collected were examined macroscopically to check for colour, odour, and turbidity.

Chemical and Microscopic Examination of Urine Samples: Chemical examination was carried out on the urine samples by reagent strips (Combo 11). The reagent strips/sticks were dipped into the fresh urine to detect the presence of various analytes in the urine while comparing with the various colour codes on reagent container.

Microscopic examination was carried out by dispensing the urine samples into the test tubes (about 5ml each) and placed into a centrifuge (ensuring a balance in the centrifuge). The samples were centrifuged for 10 minutes and the supernatant were discarded while the pellets were resuspended. A small drop was placed on a neat slide and covered with a cover slip. It was then placed under a microscope and viewed at X40 magnification lens to identify cells and bacteria.

Isolation of E. coli from urine samples: A sterilized wire loop was used to pick a loopful of urine onto the Eosin Methylene Blue (EMB) agar plate and smeared. Thereafter, a zig-zag streak was made from the smeared point to the lower portion of the plate. The plates were incubated at 37˚C for 24 hours. A green metallic sheen on EMB is positive for *E. coli*. After 24 hours of incubation of organisms in growth media, organisms on green metallic sheen were streaked onto nutrient agar plates, inverted and incubated at 24 hours at 37˚C. Nutrient agar slants in bijou bottles were used for stocking sub-cultured organisms and stored in a refrigerator at 4˚C.

Identification of E. coli: E.coli was identified using standard microbiological techniques. The biochemical tests include; oxidase test, indole test, catalase tests, sugar fermentation test, motility test, citrate test, Methyl Red Voges Proskauer test (MRVP), and Triple Sugar Iron (TSI) test (Chesbrough, 2004).

Antibiotic sensitivity test by Kirby-Bauer Disc Diffusion: AST was carried out using the Kirby-Bauer disk diffusion technique as was described using single antibiotic disks comprising ceftazidime (CAZ, 30 µg), ofloxacin (OFX, 5 µg), ciprofloxacin (CPR 5 µg) augmentin(AUG 30 µg) Gentamicin (GEN 10 µg) cefixime(CXM 5 µg) and nitrofurantoin (NIF, 300 µg) (Oxoid, UK). The inhibition zone diameters (IZDs) were measured, interpreted and recorded as per the guidelines of the Clinical Laboratory Standard Institute (Bauer *et al*., 1966; CLSI, 2005).

Preparation of Acridine Orange: Plasmid curing of *E. coli* was carried out following the methods of Salisbury *et al*. (1972); Sambrook *et al*. (1989) Brown (2000) and Trevors (1986) with slight modification. Plasmid curing was carried out on the resistant *E.* coli. Organisms were sub-cultured from stock into nutrient agar plate for 24 hours. Organisms were inoculated into nutrient broth and incubated for 18 hours. The test tubes were compared with McFarland's standard. Serial dilution was carried out on acridine orange, it was inoculated with organism that matched the turbidity of 0.5 Mac Farland Standard. The tubes were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

A total of 100 samples collected from hospital and community. Results obtained from based on the prevalence of *E.coli* identified from the various samples showed the presence of 19 (35%) samples positive for *E.coli* in samples from the community and 35 (65%) from the hospital. High percentage of *E.coli* was observed in the clinical samples compared to those obtained from the community as shown in Figure 1. Routine test following urine routine test using combo stick, out of 50 urine samples from hospital (HO1-HO50), 46% had leukocyte and glucose in their urine. About 36% had both ketone and bilirubin while 64% had Ascorbic acid, 30% had protein in their urine, 22% samples indicated urobilinogen and 20% indicated nitrogen while only18 % indicated presence of blood. In the case of community urine samples, 36% had leukocyte, 24% had glucose 26% had ketone, 18% had bilirubin, 40% had Ascorbic acid, and 22% had protein. About 20% had urobilinogen and nitrogen in their urine while only 12% had blood present in their urine. These

statistics may be subject to error due to the high occurrence of ascorbic acid in urine samples which may interfere with other analytes and may lead to a false low or negative result.

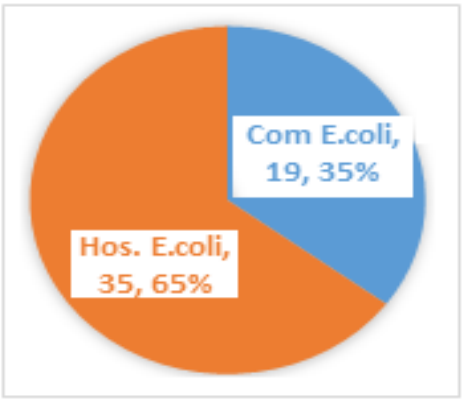

Fig 1: Prevalence of *E. coli* in hospital and community samples

Microscopy was carried out to identify the presence of cells and bacteria e.g. yeast cells, red cells, calcium oxalates, etc. Following microscopy results for 50 hospital samples 92% (45 samples) had bacterial cells, 32% (19 samples) had epithelial cell, Calcium oxalate was about 30% (18 samples), Red cells and Triple Phosphate was 11% while cast cell was seen in limited number (6%). However, 50 samples from the community showed slightly similar result as in hospital samples. From 43 samples 90% had bacteria, 29% (17 samples) had epithelial cells, and Calcium oxalate was same as community samples. Triple phosphate was 8%, and cast cells were 7%. This indicates that the presence of bacteria in urine is higher compared to other organisms found in urine.

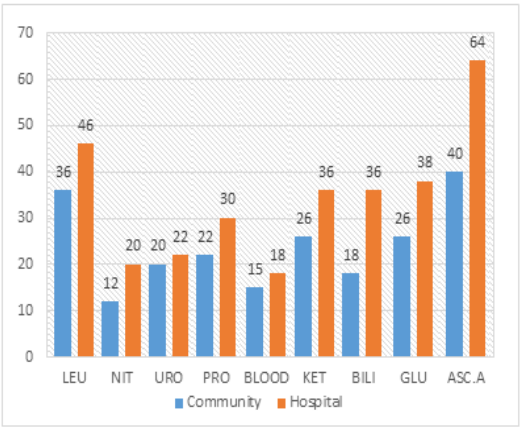

Fig 2: Percentage composition of analyte present in hospital and community urine samples

Antibiotic susceptibility test carried out on the isolates showed some level of resistance to some antibiotics. The highest resistance in the hospital isolates was found to be against Augmentin (99%). Other antibiotics to which high level of resistance was observed include; Cefuroxime (92%), Ceftazidime (78%), and Cefixime (71%). The lowest level of resistance was against Gentamicin (15%) as shown in Figure 3.

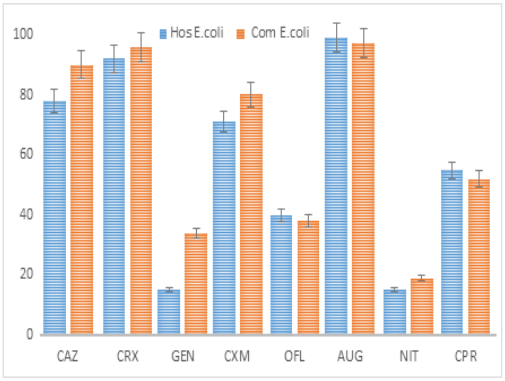

Fig 3: Percentage resistance of hospital and community *E. coli* to antibiotics. *Key*: CAZ Ceftazidime; CRX Cefuroxime; GEN Gentamicin; CXM Cefixime; OFL Ofloxacin; AUG Augmentin; NIT Nitrofurantoin; CPR Ciprofloxacin

This agrees with the findings of Raheed *et al*. (2014) and Kipkorir *et al*. (2016) reporting 4.6 % and 0% resistance to gentamicin. Similarly, the highest resistance in community isolates was found to be against Augmentin (97%) and Cefuroxime (96%). Other antibiotics that had high resistant levels were ceftazidime (90%), and Cefixime (80%). Ciprofloxacin had a moderate resistance level of (52%). Gentamicin remained the antibiotics with the lowest resistance. The results of this study are in conformity with the results obtained by Akingbade *et al*. (2014) where *E coli* isolated from urine samples were most susceptible to gentamicin, ceftazidime Levoxin and ciprofloxacin while resistance was observed in erythromycin, cefuroxime, cloxacillin, amoxicillin, ampicillin and cotrimoxazole. The resistance to these six antibiotics was observed in sixteen *E. coli* strains, they were resistant to more than two classes of antibiotics, this was attributed to the presence of resistant plasmid DNA which was detected in 6(37.5%) of the 16 E. *coli* strains. These strains had single plasmid with the same weight. High resistance of *E. coli* to augmentin in the present study was also confirmed by Umolu *et al*. (2006) reported high resistance levels of >75% for tetracycline, augmentin and amoxicillin while low resistance was noted nitrofurantoin (6%), Ofloxacin (19%). Sheik *et al*. (2013) reported 74% sensitivity to ciprofloxacin and 66% to augmentin while Shakyd *et al*. (2013) reported low resistance of 29% to augmentin and 8% to ciprofloxacin. Several studies have also reported low resistance to Ciprofloxacin

with values ranging from28.5% by Akhtar *et al*. (2016) and 49% by Iqbal *et al*. (2012). A study carried out by Ayub *et al*. (2016) reported *E.coli* as the most prevalent microorganism in gestational UTIs, results obtained in that study not in agreement with the present study where the percentage of resistance in most of the antibiotics before plasmid curing was higher. Their study reported resistance to norfloxacin (67%), ofloxacin Amox-Clav, cetriaxone and ceuroxime all had resistance of 43%, Meropenem was o% resistant (100% sensitive). Nitrofurantoin had low resistance of 14% which confirmed with the present study before plasmid curing. The present study revealed multidrug resistance which has been confirmed by previous studies of 98.7% resistance to at least 3 antibiotics (Dash *et al*., 2008; Ejikengwu *et al* 2017; Halifoiu *et al*., 2017). Dash *et al*. (2015) reported 100% of the *E. coli* in their study was multi drug resistant. Our study showed 79% of the community isolates were multidrug resistant and 91% of the hospital isolates were multidrug resistant. Percentage of multidrug resistance in the present study is however lower than the findings of some researchers (Halifoiu *et al*., 2017; Dash *et al*., 2015).

Table 1: Multidrug Resistance Pattern and Percentage of *E.coli* in Urin Samples from Hospital Sources

S/N	Antibiotics	Nob. of MDR E. *coli* from Hospital	Percentage of MDR *E.coli*
1	AUG-CAZ-CRX	5	15
2	AUG-CAZ-CRX-GEN	2	6
3	AUG-CPR-CRX-OFL	3	9
4	AUG-CAZ-NIT-OFL	1	3
5	AUG-CAZ-CRX-NIT-OFL	1	3
6`	AUG-CPR-CRX-CXM-OFL	1	3
7	AUG-CAZ-CPR-CRX-CXM	1	3
8	AUG-CPR-CRX-GEN-OFL	1	3
9	AUG-CAZ-CPR-CRX-OFL	1	3
10	AUG-CAZ-CRX-CPR-OFL	1	3
11	AUG-CAZ-CRX-CXM-OFL	1	3
12	AUG-CAZ-CRX-CXM-NIT-OFL	2	6
13	AUG-CAZ-CPR-CRX-CXM-OFL	1	3
14	AUG-CAZ-CPR-CRX-CXM-NIT	1	3
15	AUG-CAZ-CPR-CRX-GEN-OFL	3	9

Table 2: Multidrug Resistance Pattern and Percentage of *E.coli* in Urine Samples from Community Sources

S/N	Antibiotics4	Nob. of MDR *E. coli* from Community	Percentage of MDR *E.coli*
1	AUG-CAZ-CRX	3	20
2	AUG-CAZ-CRX-GEN	4	27
3	AUG-CAZ-CRX-CXM	2	13
4	AUG-CAZ-CRX-CXM-GEN	1	7
5	AUG-CAZ-GEN-OFL-NIT	1	7
6	CAZ-CRX-CPR-CXM-GEN-OFL	1	7
7	AUG-CAZ-CPR-CRX-CXM-OFL	1	7
8	AUG-CAZ-CRX-CXM-GEN-OFL-NIT	1	7
9	AUG-CAZ-CPR-CXM-GEN-OFL	1	7

Comparing the resistance in the hospital and community isolates, both sets of isolates were more resistant to four out of eight antibiotics tested. In the community isolates, the antibiotics that had more resistance were; ceftazidime (90%), cefuroxime (96%), cefixime (80%), and augmentin (97%) (Fig.3 & Table1). Community isolates also showed more resistance to the cephalosporins (ceftazidime 90%)), cefuroxime (96%), cefixime (80%), and penicillin (augmentin 97%). ciprofloxacin (59%), aminoglycoside (Gentamicin (34%)), and (nitrofurantoin (19%)) antibiotic groups, compared to community isolates (Fig. 4 & Table 2). Though the degree of resistance between the two sets of isolates varied, it only ranged only from 4% to 11% difference. The highest resistance difference (11%) was observed for Cefuroxime and the lowest (4%) was for Ofloxacin.

The differences observed in the hospital and community *E.coli* from urine samples is due to the uncomplicated nature of the community UTIs, they show various virulence traits that favors colonization of UTI. This varies from hospital UTIs which are

caused by different virulent traits and different strains. The present study reported high resistance in hospital samples which might be due to the presence of opportunistic organisms with low virulence in the hospital. Thus, there is a transfer of drug resistant gene from one bacteria to another thereby increasing the level of resistance in the hospital isolates (Courvalin *et al*., 1980; Chopade *et al*., 1985; Montofour *et al*., 2008).

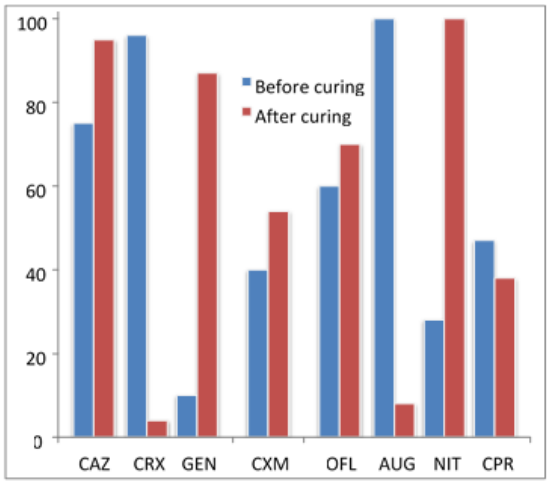

Fig 4: Resistant Rate of *E.coli* Isolates to Antibiotics before and after Plasmid curing

Plasmid profiling was done on the organism using acridine orange, the cured isolates were further subjected to antibiotic susceptibility test. After plasmid curing, it was observed that the resistant rate of isolates to the eight antibiotics reduced drastically. Both isolates showed less resistant to four out of eight antibiotics tested. In the hospital isolates, the antibiotics had less resistance to nitrofurantoin (1%), ceftazidime (3%), ciprofloxacin (1%), gentamicin (10%), Ofloxacin (10%). However, augmentin and cefuroxime maintained a high resistance of about 62% -31% respectively. Community isolates were also less resistant to antibiotics. There was an insignificant resistance to Nitrofurantoin (0), Ofloxacin (10%), and Ceftazidime (8%). Augmentin and cefuroxime still maintained high resistance to antibiotics. This suggests that plasmids are not totally responsible for the resistance of the isolates to the antibiotics. Results obtained from hospital isolates showed a high infection rate of about 50% with about 80-85% resistance rate to antibiotics, this may be attributed to the fact that most patients do not observe a good hygiene, inpatients indwelling with catheters, patients undergoing urological manipulations, long-stay elderly male patients and patients with debilitating diseases. According to Subbiah *et al.* (2017) and Raj (2002) stated that decreased resistance reported after plasmid curing can be attributed to some plasmid mediated beta lactamase strains that might have been selected. This confirms the relationship that exists between plasmids and multidrug resistance.

The organisms are usually from patient's intestinal flora, but occasionally from a moist site in the hospital environment. Nosocomial pathogens causing urinary tract infections (UTIs) tend to have a higher antibiotic resistance than community. Also, in the course of plasmid profiling, there was a general decrease in resistance to antibiotics after plasmid curing. Infection control policies are important in limiting the number of hospital-acquired UTIs. Results from the community isolates showed a limited number of infections at about 20-25% and resistance was about 60%. It also showed that Acridine orange at 20 micrograms per milliliter, there was observed decrease to Cefuroxime and Augmentin after plasmid curing. For some of the isolates curing of the plasmids had no significant effect on the level of resistance. This might be due to the possible selection of plasmid mediated beta-lactamases producing strains. It is essential to monitor plasmids mediated resistance and antibiotics susceptibility testing to reduce the level of drug resistance and a better cure for patients with UTI.

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