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Antimicrobial Resistance Profile of Extra-intestinal *Escherichia coli* Infections in a South Western Nigerian City

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Abstract There is a general increase in the attention being paid to extraintestinal *Escherichia coli* infections; this is because recent reports have identified various reserviour sources of multi-resistant clones of *E. coli* carrying mobile genetic elements capable of epidemic spread. The public health implication of an outbreak of *E. coli* is better imagined than experienced. We have therefore analyzed all extraintestinal *E. coli* isolates and their resistance profile in Abeokuta, Ogun State, Southwestern Nigeria between March 2010 and November 2010. About 339 isolates of *E. coli* were isolated at the Microbiology Unit of Federal Medical center, Abeokuta using standard Bacteriological techniques and tested against various antibiotics including 3rd generation Cephalosporins. Gender distribution was 105(31.0%) males and 234(39.0%) females, the highest recovery rate was recorded by age group 16-45 with 198(58.4%), followed by above 45 years 76(22.4%) and lastly 0-15 years 65(19.2%). Urine had the highest occurrence rate with 180(53.0%), followed by genital samples with 61(18.0%), and the least frequently isolated site was Blood, 27(8.0%). Other samples tested were grouped together and recorded 35(10.4%). Gentamycin was the most active antibiotic with 41.3% susceptibility against all isolates tested, followed closely by Amoxi/clav with 36.3% against all isolates tested. Very high resistance was recorded by the β-lactamases. There was poor sensitivity to the 3rd generation cephalosporins, 32.0% of isolates were susceptible to Ceftazidime and 34.4% to Cefuroxime in age group 16-45, and 46.4% susceptibility to ceftazidime were recorded against all isolates tested. In conclusion we report a high antimicrobial resistant rate in extraintestinal *E. coli* infection in Abeokuta with possible co-acquisition of different β-lactamase subtypes including ESBL and emerging Carbapenemases, the results of this study highlights the importance of regular surveillance of common pathogens such as *E. coli* in our environment.

Keywords Extraintestinal Escherichia Coli, Antimicrobial Resistance, β-lactamase, Abeokuta

1. Introduction

Enteriobacteriaceae is a broad group of heterogeneous medically important gram negative bacilli, consisting of 30 genera and more than 120 species[1]. This group of bacteria accommodates *Escherichia coli* is one of the most medically important bacteria known to man and animals alike, there have been associated with various diseases of man, but serve as a commensal in the intestinal tract of man and animals[2]. Pathogenic strains of *E. coli* causes both Intestinal and extraintestinal diseases such as Urinary tract infections (UTI), gastroenteritis, meningitis and sepsis, they are able to do this by means of possession of several virulence factors such as Adhesins and exotoxins[1]. Pathogenic intestinal *E. coli* strains have been classified according to their disease manifestation and type of toxins produced, 5 main groups have been identified enterotoxigenic E. coli (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorragic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), and Enteroaggregative E. coli (EAEC)[1]. The most commonly encountered strains are the EHEC and EPEC strains capable of causing life threatening diseases.

Several reports have demonstrated the severity of extraintestinal *E. coli* infections[3-4]. In the United States alone it is estimated that 6-8 million cases of uncomplicated UTI occur annually and about 130-175 million cases occur globally, with *E. coli* being responsible for over 80.0% of this figure[3]. Majority of *E. coli* sepsis have been traced to

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UTI and a few to gastroenteritis, the major reserviour of pathogenic strains of *E. coli* causing extraintestinal infection remains the alimentary tract, although evidence has shown that other sources such as contaminated food and food-animals like chicken can also cause extraintestinal *E. coli* infections[5-6]. It is an established fact that *E. coli* serves as a reserviour for the spread and dissemination of resistance phenotypes such as the dreaded ESBL and Carbapenemase resistance[7] this factors has also been recorded in extraintestinal *E. coli* infections, making treatment complicated and very expensive. Epidemics of extraintestinal *E. coli* infections have been recorded although this is not common[3]. The increasing global incidence of the shiga-toxin producing *E. coli* 0157:H7 and its disease implications particularly in heamolytic ureamic syndrome (HUS) associated UTI infections has lead to an increase in the attention paid to extraintestinal *E. coli* infections, there is however a need to constantly review the antimicrobial resistance pattern of extraintestinal *E. coli* infections in developing countries such as Nigeria where surveillance of this condition is poor.

In Nigeria there are some reports of high level resistance in *E. coli* infections from extraintestinal sources[7], although more research data is still needed before any conclusions can be drawn on the exact multi-resistance profile of Nigerian *E. coli* isolates. In Abeokuta there are some interesting reports on ESBL producing isolates of *E. coli* from extraintestinal sites which has shown that multi-resistant extraintestinal *E. coli* infection are an established disease condition in Abeokuta[7, 8]. This prompted the objective of our study which is to review the antimicrobial resistance pattern of extraintestinal *E. coli* infection in a major tertiary hospital in Abeokuta, South West Nigeria.

2. Materials and Methods

2.1. Study Design and Study Population

We conducted a retrospective study of all *E. coli* infections from extraintestinal sites and their antibiotic susceptibility and resistance pattern at the Federal Medical center Abeokuta, from March 2010 to November 2010. Laboratory data were extracted from the records of the Medical Microbiology Unit of FMCA on patients of all ages and gender during the study period. All patient data were treated with the highest degree of confidentiality in accordance to the Belmont report[9].

2.2. Bacteria Isolation and Identification

All laboratory investigations were done retrospectively according to standard bacteriological techniques[10]. Briefly, freshly collected samples were inoculated onto Mac-Conkay, Blood and Chocolate agar; inoculated plates were incubated at 37 ℃ aerobically while chocolate agar plates were incubated at 37 ℃ in a microaerophyllic condition for 48hrs. Isolates were identified as *E. coli* following standard biochemical procedures[10].

2.3. Antibiotic Susceptibility Testing

Susceptibility testing was done on nutrient agar plates inoculated with 0.5 Mac Farland standard inoculum, testing was done following the Kirby-Bauer method for disk diffusion and results interpreted according to NCCLS guidelines[11]. Antibiotics tested include multidisc, Amoxicillin (25µg), Erythromycin (25µg), Tetracycline (30µg), Cotrimoxazole (25µg), Streptomycin (10µg), Gentamycin (10µg) Amoxicillin/Clavunalate (30µg), Levoxacin (30µg), Ofloxacin (30µg), Nitrofurantion (300µg), supplied by (Abtek biological U.K.) and single disks Ceftazime (30µg), Cefuroxime (30µg), supplied by (Oxoid U.K.).

3. Results Analysis

During the period under review a total of 339 *Escherichia coli* isolates were recovered from extraintestinal sites, with a gender distribution of 105(31.0%) males and 234(39.0%) females. Table 1 shows the age and gender distribution, the highest recovery rate was recorded by age group 16-45 with 198(58.4%), followed by above 45 years 76(22.4%) and lastly 0-15 years 65(19.2%).

Table 1. Distribution of extraintestinal *Escherichia coli* isolates according to Age and Gender

Characteristics	No. (%)
Gender	
Male	105(39%)
Female	234(61%)
Age group	
0-15	65(19.2%)
>15-45	198(58.4%)
>45	76(22.4%)
Total	339(100.0)

Table 2 shows the distribution of isolates by sample site, from our results, Urine has the highest isolation rate for e. coli with 180(53.0%), followed by genital samples which includes high vaginal swab(HVS), Endocervical swab (ECS), and Semen, with a distribution of 61(18.0%), and the least frequently isolated site was Blood with 27(8.0%). Other samples tested which were grouped together; include sputum, Cerebrospinal fluid (CSF), Eye discharge and Ear swab, recorded 35(10.4%).

Table 2. Distribution of extraintestinal E. coli isolates recovered from different sample sites

Sample site	No. (%)
Urine	180 (53.0)
Blood	27 (8.0)
Genital	61 (18.0)
Wound	36 (10.6)
Others	35 (10.4)
Total	**339(100.0)**

Table 3. Antibiotic resistance pattern of Extraintestinal *E. coli* isolates against various Antibiotics

Age	Antibiotics{No tested (% resistant)}										
	Amox	Ery	Tet	Sterp	Gen	Am/cv	Lev	Oflox	Nit	Caz	Cef
0-15	65(100.0)	65(90.8)	65(97.0)	65(81.6)	65(51.0)	65(58.5)	0(0.0)	0(0.0)	65(95.4)	50(82.0)	50(64.0)
16-45	198(99.5)	198(96.5)	198(97.0)	198(89.0)	198(53.0)	198(61.0)	198(61.6)	198(74.0)	198(80.0)	120(71.0)	120(89.0)
>45	76(98.7)	76(100.0)	76(98.7)	76(89.2)	76(77.0)	76(71.0)	76(69.0)	76(90.8)	76(85.5)	60(85.0)	60(85.0)

Antibiotic sensitivity and resistance profiles of extraintestinal *E. coli* isolates according to age group revealed that Gentamycin was the most active antibiotic with 47.0% susceptibility in age group 16-45 and 41.3% susceptibility against all isolates tested, followed closely by Amoxi/clav with 38.9% sensitivity in age group 16-45 and 36.3% against all isolates tested. Very high resistance was recorded by the β-lactamases, Erythromycin and tetracycline, with Amoxicillin recording 99.4% resistance in all isolates tested and Erythromycin 96.2% resistance against all isolates tested. The Quinolones also performed below average with Levoxacin recording 38.4% sensitivity in age group 16-45 and 35.0% sensitivity against all isolates tested. There was poor even to the 3rd generation cephalosporins, for instance 32.0% of isolates were susceptible to Ceftazidime and 34.4% susceptibility was recorded for Cefuroxime in age group 16-45, and only 46.4% susceptibility to ceftazidime was recorded against all isolates tested, details of the antibiotic susceptibility are shown inn table 3 below.

4. Discussion

The importance of *E. coli* as a pathogen of man over the years cannot be overemphasized, with its increasing ability to acquire and disseminate multiple resistant traits genetically to similar and often unrelated pathogenic bacteria[7, 12] has made its medical importance all so evident. In recent past attention was paid to *E. coli* as an enteric pathogen with its various forms of disease presentations. However with emerging evidence of severe infections often life threatening and more recently laboratory based evidence of outbreaks of extraintestinal *E. coli* infections, an example is the widely reported UTI outbreak caused by multiple serotypes of *E. coli* in California which lasted for about 4 months[5]. Epidemiologic reports have thus identified various reserviour sources of extraintestinal pathogenic *E. coli* infections, which include food animals such as chicken, pets, and feacal carriage by asymptomatic carriers[3].

In Abeokuta, there have several reports indicating the presence of multi-resistant strains of *E. coli* possessing EESBL and Amp C properties isolated from extraintestinal sites[7, 13-14]. This prompted the objective of our study. From our report we reviewed 339 *E. coli* isolates causing extraintestinal infections from various anatomical sites in Abeokuta. Gender distribution showed that females had more extraintestinal *E. coli* infections than males ($p>0.05$) with a frequency of 69.0% this is in agreement to a similar report from the same study site as our where enterohemorrhagic *E. coli* causing UTI was investigated[8]. This observation can be attributed to the female anatomy and the proximity of the female urethra and genital tract the anal region this allows for cross contamination in unhygienic situations and increased probability of UTI in direct comparison to males. Age range distribution showed that the peadiatric age group had the lowest *E. coli* recovery rate with 19.2% while age range 16-45 years had the highest, similar rates have been reported[8], this can also be attributed to the fact that more adult presented with various infectious diseases as compared to children during the study period, and the fact that adult women particularly pregnant women in their late trimester are more prone to UTI and bacteriuria[15-16].

Distribution according to sample site naturally recorded the highest rate in Urine samples with 53.0% followed by Genitals with 27.0%, 8.0% of recovered isolates came from Blood, this is consistent with previous reports that UTI are the principal extraintestinal disease of man[3, 8]. The rate of 8.0% recovered from blood also calls for attention as a previous report from our center placed *E. coli* as the second most incriminated bacteria in peadiatric sepsis in Abeokuta[14]. This report has further highlighted the importance of regular surveillance and strict infection control, because majority of *E. coli* sepsis have been reported to originate from untreated or poorly treated UTI, while significant number of paediatric *E. coli* sepsis originate from nosocomial infection during birth or shortly after child birth[14, 17]. We also recovered a few isolates from sites such as CSF, which is indicate of the level of invasiveness achievable by *E. coli* if not quickly attended to.

Antibiotic susceptibility to the various antibiotics tested was generally poor with resistance rates as high as 99.4% against amoxicillin in all isolates tested, only the Aminoglcosides and the Quinolones performed fairly when compared to others, with Gentamycin recording 41.3% susceptibility to all tested and 47.0% in age group 16-45. Amoxicillin clavunalic acid, which used to be very active against β-lactamase producers, was not very active against our isolates with a resistant rate of 61.0% in age group 16-45 and 71.0% in age group 46 and above the fact that even the 3rd generation cephalosporins were not very active

amongst our isolates with Ceftazidime showing 71.2% resistance in age group 15-45 and 76.7% resistance to all isolates tested confirms previous reports of ESBL and Carbapenemase producing *E. coli* dissemination in our study setting[7, 18].

Our current high level resistance to β-lacteamase antibiotics including expanded spectrum β-lactamases such as cephalosporins shows current high level ESBL circulation and possible co acquisition of possible Cabapenases resistance, this call for immediate response from local bacteriologist to draw out modalities for a robust study on the current molecular epidemiological profile of multiple drug resistant profiles, as this will give insight into possible novel multi-resistant genes such as New Deli β-lac, or $bla_{oxa\text{-}48}$ acquired by our gram negative isolates an give insight into their origin and possible control and eradication.

5. Conclusions

We have reported a very high antimicrobial resistant rate in extraintestinal *E. coli* infection in Abeokuta, Ogun State, Southwestern Nigeria with possible co-acquisition of different β-lactamase subtypes including ESBL and emerging Carbapenemases, the results of this study highlights the importance of regular surveillance of common pathogens such as *E. coli* in our environment and call for an urgent investigation into the molecular epidemiology of gram negative multi-resistant bacteria such as *E. coli*. This will likely lead to the discovery of new rapidly transmissible resistant genes sun as NDM β-lactamase and can give insight into the origin of such resistant properties and their possible eradication measures.

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