Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(9) 179-186
ISSN 2322-2948
doi: 10.14196/sjm.v2i9.798

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Distribution and multi-drug resistance pattern of escherichia coli isolated from patients in federal medical center, Owerri

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ARTICLE INFO

Article history:
Received 06 July 2013
Accepted 18 August 2013
Available online 29 September 2013

Keywords:
Escherichia coli
MDR
EMB
Ciprofloxacin
Tetracyclin
Owerri

ABSTRACT

This study was carried out to investigate the distribution and multi-drug resistance (MDR) pattern of Escherichia coli isolated from patients in Federal Medical Centre (FMC), Owerri, Imo State. 255 clinical specimens were collected, including urine, stool, wound swabs, high vaginal swabs, urethral swabs, endocervical swabs, sputum and aspirates. Specimens were cultured on MacConkey agar (MA, Oxoid) and Eosin Methylene Blue Agar (EMB, Oxoid). 100 E. coli isolates were obtained. A significantly high rate of infection was found in females ($p<0.05$). Susceptibility testing was done on 50 selected isolates using Disc diffusion method with Mueller-Hinton agar. Antibiotics used were Amoxicillin/Clavulanic acid- AMC (30µg), Cefpirome- CPO (30µg), Cefpodoxime- CPD (10µg), Cefotaxime- CTX (30µg), Cefoxitin- FOX (30µg), Ciprofloxacin- CIP (5µg), Tetracyclin- TE (30µg), Nalidixic acid- NA (30µg), Chloramphenicol- C (30µg), Ceftriaxone- CRO (30µg) and Sulphanethoxazole/Trimethoprim- SXT (25µg). Ceftriaxone and Ciprofloxacin had the highest percentage sensitivity (92.0%), while Nalidixic acid and Tetracyclin were the least sensitive (8.0%). Four isolates (8.0%) were resistant to all antibiotics used. This study established that Ceftriaxone and Ciprofloxacin are the most effective drugs for the treatment of E. coli infections in Owerri. However, it is advised that these drugs be taken only on prescription to prevent development of drug resistance.

1. Introduction

Bacterial antibiotic resistance is a well-recognized threat to public health. Particularly serious aspects of this phenomenon are bacteria with multi-drug resistance (MDR) mechanisms, which enable them to become resistant to structurally, unrelated antimicrobial agents. In addition, the MDR strains possess the potentials of quickly compromising the effectiveness of new antimicrobial agents (Gold and Moellering, 2000). Resistance to antibiotics in Gram-negative bacteria is due to various mechanisms that can act additively or synergistically; whereas some of them account for the intrinsic bacterial resistance, the expression of others is regulated in response to environmental changes. These mechanisms can be specific, such as the enzymatic inactivation of the antibiotic or the alteration of the target antibiotic. They could also be moderate or non-specific mechanisms that involve the presence of permeation barriers and efflux systems that impede access to or pump out a wide variety of drugs (Gold and Moellering, 2000).

Escherichia coli, a member of the Enterobacteriaceae family of bacteria, is the leading cause of community acquired (CA-) and hospital acquired (HA-) urinary tract infections (UTIs) (CDDEP, 2013). Although E. coli is naturally found in the human gut, certain food-borne strains can cause serious gastrointestinal infections. Infections with multidrug resistant E. coli, also known as extended spectrum beta-lactamase (ESBL), have been assumed to be a hospital phenomenon. However, a recent analysis across the United States (ICAAC, 2012) indicated that multidrug resistant E. coli is now spreading in the everyday world, in an undetected and untracked fashion.

This study therefore, was carried out to investigate the distribution and multi-drug resistance (MDR) pattern of Escherichia coli isolated from patients in Federal Medical Centre (FMC), Owerri, Imo State.

2. Materials and methods

2.1. Specimens collection

Isolates of stool, urine, high vaginal swabs(HVS), urethral swabs (US), wound swabs (WS), endocervical swabs (ECS), sputum and aspirates from different individuals were collected from patients in Federal Medical Centre (FMC), Owerri, Imo state in sterile bottles and appropriately labeled. The specimens were immediately transferred to the microbiology laboratory for bacteriological analysis and identification.

2.2. Isolation of *Escherichia coli*

Specimens were cultured on prepared, well-labeled MacConkey agar (MA) plates (CM0050, Oxoid) and incubated at 35-37°C for 20-24 hours, then sub-cultured on Eosin Methylene Blue (EMB) agar (CM0069, Oxoid). Presumptive isolates were identified by observing pink, lactose fermenting colonies on MA and dark colonies with green metallic sheen on EMB. Isolates were then transferred to Nutrient agar (NA) slants (CM003, Oxoid) and stored in the refrigerator for use during antibiotic susceptibility testing. Gram staining was done on isolates to observe Gram negative bacilli. Indole, Methyl Red, Voges-Proskauer and Citrate (IMViC) tests were used to identify E. coli. The isolates were confirmed using Microgen™ GNA-ID System for Enterobacteriaceae. The test was run according to manufacturer's instruction.

2.3. Antibiotic susceptibility testing

Antimicrobial sensitivities against Amoxicillin/Clavulanic acid- AMC (30µg), Cefpirome- CPO (30µg), Cefpodoxime- CPD (10µg), Cefotaxime- CTX (30µg), Cefoxitin- FOX (30µg), Ciprofloxacin- CIP (5µg), Tetracyclin- TE (30µg), Nalidixic acid- NA (30µg), Chloramphenicol- C (30µg), Ceftriaxone- CRO (30µg) and Sulphanethoxazole/Trimethoprim- SXT (25µg) (OXOID) were performed by Kirby Bauer Disc Diffusion technique. A sterile cotton wool swab stick was used to inoculate the entire surface of Mueller-Hinton agar (MHA) plate (CM0337, Oxoid) with the inoculum of E. coli, turbidity matching 0.5 MacFarland Nephalometer standard, before

antibiotic discs was laid on the surface. The plates were incubated overnight at 37ºC. The inhibition zone diameter (IZD) was evaluated according to the Clinical and Laboratory Standard Institute (CLSI, 2009) guidelines.

2.4. Serotyping of *E. coli* 0157:H7

The latex reagent (DR062OM, Oxoid) was brought to room temperature, making sure the latex suspension was mixed by vigorous shaking. A dropper was used to dispense one drop of the reagent on the circled region of the reaction card. A loopful of sterile saline was added to the circle in a way that the latex and the saline did not mix, and then a colony of the isolate was emulsified in the saline drop and finally mixed with the latex reagent. The card was rocked in a circular motion and observed for agglutination indicating the presence of E. coli 0157:H7.

3. Results

Table 1, shows the Microgen™ GNA-ID test result for some E. coli isolates.

Table 2, shows the age and sex distribution of E. coli in FMC, Owerri. More youths were infected with E. coli. More females (61) than males (39) also had E. coli infection ($p<0.05$, T=-1.02).

Table 3, shows the distribution of E. coli from various clinical specimens. Urine (14.9%) had the highest occurrence, followed by stool (9.8%), while E. coli was not isolated from aspirate.

Table 4, shows the percentage antibiotic susceptibility pattern of E. coli isolates in FMC, Owerri. Ceftrixone (92.0%) and Ciprofloxacin (92.0%) were the most potent. Tetracycline (8.0%) and Nalidixic acid (8.0%) were the least.

Table 1
Microgen™ GNA-ID test result for some E. coli isolates.

Isolate Code	Lys	Orn	H2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal code	Identification
U32	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
HVS03	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
US47	+	-	-	+	+	-	+	+	-	+	-	-	4664	*E. coli*
ECS04	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
WS50	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
S02	+	-	-	+	+	-	+	+	-	-	-	-	4660	*E. coli*
U202	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
U07	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
U043		-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
SP111	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
S84	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
S67	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
HVS14	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*
U99	+	+	-	+	+	-	+	+	-	-	-	-	6660	*E. coli*
U80	-	-	-	+	+	+	+	+	-	-	-	-	0760	*E. coli*

Key:Lys-Lysine; Orn-Ornithine; H2S-Hydrogen sulphide; Glu-Glucose; Man-Mannitol; Xyl-Xylose; ONPG-Ortho-nitrophenol-galactosidase; Ind-Indole; Ure-Urease; VP-VogesProskauer; Cit-Citrate; TDA-Tryptophan deaminase acid; U=Urine, HVS= High Vaginal Swab; US= Urethral Swab; ECS= Endocervical Swab; WS= Wound Swab; SP= Sputum; S= Stool.

Table 5, shows the antibiotic susceptibility pattern of E. coli isolates from various clinical specimens. HVS was the most sensitive with no resistance recorded in 6 antibiotics.

Table 6, shows the antibiotic resistance profile (antibiogram) of E. coli isolated from various clinical specimens. MDR was observed in all antibiotics while 4 isolates were resistant to all antibiotics used.

Table 7, shows the summary of antibiotic resistance profile (antibiogram) of E. coli isolated from various specimens. The number of isolates resistant to more than two antibiotics were 44 (88.0%) out of the 50 tested. The highest rate of multidrug resistance was seen among ten antibiotics (16.0%) while the lowest rate was found among four antibiotics (2.0%).

Figure 1, shows Serotyping of E. coli 0157:H7 strains from various specimens. 5 (20.0%) isolates, out of the 25 stool specimens showed agglutination when serotyped.

Table 2

Age and sex distribution of E. coli in Federal Medical Centre, Owerri.

Age group of patients	Number of patients infected with *E. coli*		
	Male	Female	Total
0-10	16	3	18
11-20	1	6	7
21-30	16	30	46
31-40	3	10	13
41-50	0	3	3
51-60	2	3	5
61-70	0	2	2
71-80	0	2	2
81-90	1	1	2
91-100	0	1	1
Total	39	61	100

Table 3

Distribution of *E. coli* from various clinical specimens.

Specimens	Number screened	Number of positive specimens	Percentage (%)
Urine	87	38	14.90
HVS	29	12	4.71
US	17	5	1.96
ECS	17	6	2.36
WS	10	5	1.96
Sputum	35	9	3.53
Aspirate	4	0	0
Stool	56	25	9.80
Total	255	100	39.22

Key: HVS= High Vaginal Swab; US= Urethral Swab; ECS= Endocervical Swab; WS= Wound Swab

Table 4

Percentage antibiotic susceptibility pattern of *E. coli* isolates in federal medical centre, Owerri. (n=50)

Antibiotics tested	Number of susceptible isolates (%)
Amoxicillin/Clavulanic acid- AMC (30µg)	20 (40.0)
Cefpirome-CPO (30µg)	38 (76.0)
Cefpodoxime-CPD (10µg)	34 (68.0)
Cefotaxime-CTX (30µg)	16 (32.0)
Cefoxitin-FOX (30µg)	34 (68.0)
Ciprofloxacin-CIP (5µg)	46(92.0)
Tetracycline- TE (30µg)	4 (8.0)
Nalidixic acid-NA (30µg)	4 (8.0)
Chloramphenicol-C (30µg)	14 (28.0)
Ceftriaxone-CRO (30µg)	46 (92.0)
Sulphanethoxazole/Trimethoprim-SXT (25µg)	8 (16.0)

Key: n- number of isolates tested.

Table 5
Antibiotic susceptibility pattern of *E. coli* isolates from various human clinical specimens.

Antibiotic Tested	Number of susceptible isolates (%)							
	Urine n=20	HVS n=4	US n=5	ECS n=4	WS n=5	Sputum n=6	Stool n=6	Total n=50
AMC (30µg)	8(40.0)	1(25.0)	1(20.0)	1(25.0)	1(20.0)	2(40.0)	6(100.0)	20
CPO (30µg)	16(80.0)	4(100.0)	5(100.0)	2 (50.0)	5(100.0)	4(66.7)	2(40.0)	38
CPD (10µg)	8(40.0)	4(100.0)	5(100.0)	4(100.0)	3(60.0)	6(100.0)	4(66.7)	34
CTX (30µg)	0(0)	2(50.0)	4(80.0)	4(100.0)	2(40.0)	4(66.7)	0(0)	16
FOX (30µg)	12(60.0)	4(100.0)	5(100.0)	4(100.0)	2(40.0)	3(50.0)	4(66.7)	34
CIP (5µg)	20(100.0)	4(100.0)	5(100.0)	2(50.0)	5(100.0)	4(66.7)	6(100.0)	46
TE (30µg)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	4(66.7)	4
NA (30µg)	4 (20.0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	4
C (30µg)	4 (20.0)	4(100.0)	0(0)	0(0)	0(0)	2(40.0)	4(66.7)	14
CRO (30µg)	20(100.0)	4(100.0)	3(60.0)	4(100.00	3(60.0)	6(100.0)	6(100.0)	46
SXT (25µg)	2(10.0)	0(0)	0(0)	0(0)	2(40.0)	2(40.0)	2(40.0)	8

Key: AMC- Amoxicillin/Clavulanic acid; CPO- Cefpirome; CPD- Cefpodoxime; CTX- Cefotaxime; FOX- Cefoxitin; CIP- Ciprofloxacin; TE- Tetrecyclin; NA- Nalidixic acid; C- Chloramphenicol; CRO- Ceftriaxone; SXT- Suphanethoxazole/Trimethoprim; HVS- High Vaginal Swab; US- Urethral Swab; ECS- Endocervical Swab; WS- Wound Swab; n- number of isolates tested.

Table 6
Antibiotic resistance profile (antibiogram) of *E. coli* isolated from various human clinical specimens.

Antibiotic resistance profile	Number of resistant isolates
TE NA SXT C CTX AMC FOX CPD CPO CIP CRO	4
TE NA SXT C CTX AMC FOX CPD CPO CIP	4
TE NA SXT C CTX AMC FOX CPD CPO CRO	4
TE NA SXT C CTX AMC FOX CPD CPO	3
TE NA SXT C CTX AMC FOX CPD CIP	2
TE NA SXT C CTX AMC FOX CPD CRO	2
TE NA SXT C CTX AMC FOX CPD	1
TE NA SXT C CTX AMC FOX CPO	1
TE NA SXT C CTX AMC FOX CIP	2
TE NA SXT C CTX AMC FOX	3
TE NA SXT C CTX AMC CPD	1
TE NA SXT C CTX AMC CPO	1
TE NA SXT C CTX AMC	3
TE NA SXT C CTX FOX	2
TE NA SXT C CTX CPD	1
TE NA SXT C CTX CPO	1
TE NA SXT C CTX	1
TE NA SXT C FOX	2
TE NA SXT C CPD	1
TE NA SXT C CPO	1
TE NA SXT C	1
TE NA SXT	3
TE NA	2
TE	4

Key: AMC- Amoxicillin/Clavulanic acid; CPO- Cefpirome; CPD- Cefpodoxime; CTX- Cefotaxime; FOX- Cefoxitin; CIP- Ciprofloxacin; TE- Tetrecyclin; NA- Nalidixic acid; C- Chloramphenicol; CRO- Ceftriaxone; SXT- Suphanethoxazole/Trimethoprim.

Table 7
Summary of antibiotic resistance profile (antibiogram) *of E. coli* isolated from various specimens.

Antibiotics tested	Number of resistant isolates (%)
One antibiotic	4(8.0)
Two antibiotics	2(4.0)
Three antibiotics	3(6.0)
Four antibiotics	1(2.0)
Five antibiotics	5(10.0)
Six antibiotics	7(14.0)
Seven antibiotics	5(10.0)
Eight antibiotics	4(8.0)
Nine antibiotics	7(14.0)
Ten antibiotics	8(16.0)
Eleven antibiotics	4(8.0)

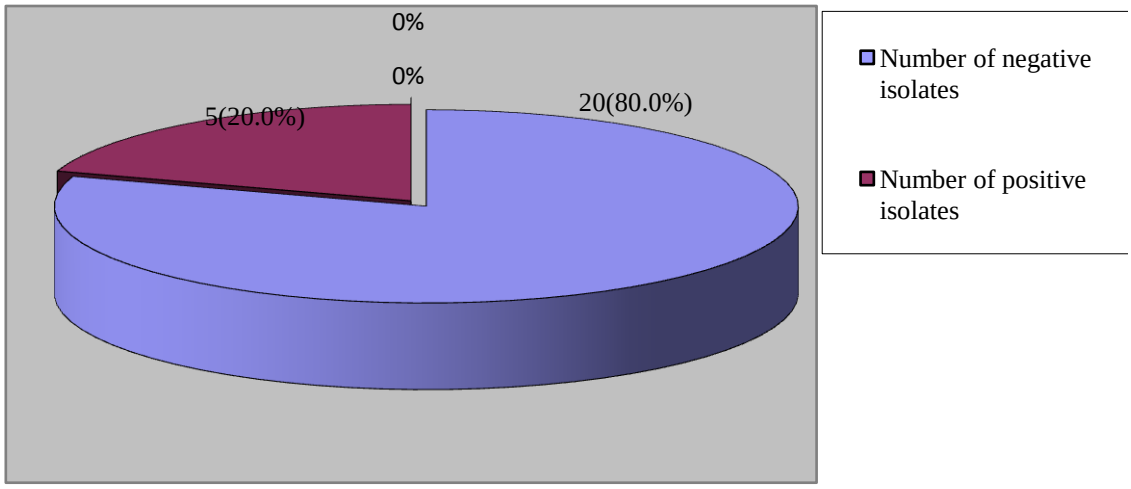

Fig. 1. Serotyping of E. coli 0157:H7 strains from various specimens.

4. Discussion

Since the pathogenecity and spread of E. coli is mainly dependent on exposure to risk factors, the rate of occurrence and its distribution in different localities may not be the same. A prevalence of 39.22% was recorded in this study. This is higher than the 25.0% reported by Emerenini and Okolie (2012) in Benin City, Nigeria. The presence of E. coli was higher in female patients (61.0%) than in male patients (39.0%). The significant difference in occurrence ($p<0.05$) indicates that females are at higher risk of getting infected. The highest percentage of occurrence in urine (14.90%) recorded in this study suggests the presence of urinary tract infection in Owerri. Acute urinary tract infections have been reported to be common (McCarty et al., 1999), occurring in 10-20% of otherwise healthy women during their lifetime. This is presumably due to the possession of short urethra by women which could pose a possible threat in a poorly managed and unhygienic environment.

The highest rate of E. coli infection amongst individuals within the age range of 21-30 may be as a result of poor hygiene. Other contributing risk factors include sex, nosocomial and community acquired infections and urinary tract abnormalities. The presence of E. coli 0157:H7 in diarrhoegenic stool among individuals from 0-10 years could be attributed to eating and drinking of contaminated food and water respectively. This result agrees with earlier reports by WHO (2009) which stated that E. coli-associated diarrhea illness or food poisoning occurred

most amongst children within the age range of 0-10 years. This may be due to their under-developed immune system.

The antibiotic susceptibility testing showed that Ceftriaxone and Ciprofloxacin had the highest sensitivity of 92.0% while Tetracyclin and Nalidixic acid had the highest rate of resistance (84.0%). This does not completely agree with a study (Winokur et al., 2001) which reported that ESBL strains show high level of co-resistance to ciprofloxacin, aminoglycosides, tetracycline, and Suphanethoxazole/Trimethoprim. It was also different from Farooqui et al (2000) who reported a relatively high resistance of 25% to quinolones and Khan and Ahmed (2001) who reported 46% resistance in Pakistan. Another study (Iqbal et al., 2002) reported a high resistance (34-39%) to third generation cephalosporins. McCarty et al (1999) however, recorded virtually no resistance to quinolones in their study. Emerenini and Okolie (2012) reported that E. coli was resistant to virtually all the antibiotics used, including Chloramphenicol, Tetracyclin and Nalidixic acid. Rezaee et al (2011) reported a resistance of 72.8%, 47.6% and 60.7% to Tetracyclin, Ciprofloxacin and Nalidixic acid respectively.

The cephalosporins used for this study were Cefpirome (fourth generation); Cefotaxime, Ceftraixone (third generation); Cefoxitin and Cefpodoxime (second generation). The second generation cephalosporins had a resistance of 32% while the third generation cephalosporin, Cefotaxime, had a higher resistance of 68.0%. This is surprising, as third generation cephalosporins, with few exceptions, are known to have better gram-negative activity. This could however, explain why Ceftriaxone had a higher sensitivity. Furthermore, Cefpirome recorded a resistance of 24.0%, suggesting cross-resistance with third generation cephalosporins; as fourth generation cephalosporins are known to be effective to both Gram- positive and –negative bacteria (Itokazu, 2013).

Cephalosporins were used instead of β-lactam drugs due to increased resistance to β-lactamase by most strains of E. coli. Three decades after the discovery of penicillin in 1929, it was documented to be the most effective β-lactam drug used for treatment of E. coli infection, but now, it is one of the least effective. Resistance to penicillin made scientists produce cephalosporins which show resistance to inactivation by β-lactamases (ESBL). Some organisms (especially Enterobacteriaceae) showed resistance to these Cephalosporins, making them to further produce extended spectrum β-lactamase drugs. Extended spectrum penicillins, like the other broad spectrum penicillins, are however destroyed by many β-lactamase producing organisms. This made penicillin + β-lactamase inhibitors the best choice for treatment of Enterobacteria. An example is Augumentin, a combination of Amoxicillin and Clavulanic acid which was used to test for ESBL resistant strains. The increased rate of resistance (60.0%) recorded in this study however, depicts that resistance to Amoxicillin/Clavulanic acid have long been developed. IF impson et al (1990) stated that due to emergence of E. coli with β-lactamase over-production, the usefulness of antibiotics like Amoxicillin/Clavulanic acid for UTI treatment is continuously reduced.

This study recorded a very high resistance (84.0%) in Sulphanethoxazole/Trimethoprim (SXT). This was very high compared to the 17% recorded by Talan et al (1998) but similar to the 60.0% and 77.0% reported by Farooqui et al (2000) and Iqbal et al (2002) respectively. This high rate of resistance in SXT depicts its unreliability in the therapy of E. coli infection, suggesting its discontinued use as first line therapy. The 72.0% resistance seen in Chloramphenicol was also very high compared to the 20.7% reported by Rezaee et al (2011).

The number of isolates resistant to more than two antibiotics were 44 (88.0%) out of the 50 tested. This is similar to the 84.2% recorded by Rezaee et al (2011) and higher than the 70.5% reported by Okeke et al (2000). Four isolates were resistant to all antibiotics tested. This is similar to Okeke et al (2000) who reported that 3 isolates were resistant to all drugs tested, two of which had low resistance to Nalidixic acid and Chloramphenicol. Virtually all the isolates were however, resistant to Tetracyclin, Nalidixic acid and Sulphanethoxazole/Trimethoprim, suggesting that these antibiotics are not effective in the treatment of E. coli infections in Owerri.

Conclusion

This study established a higher rate of infection in female than in males. Ceftriaxone and Ciprofloxacin are the most effective drugs for the treatment of E. coli infections in Owerri. However, it is advised that these drugs be taken only on prescription to prevent development of drug resistance.

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