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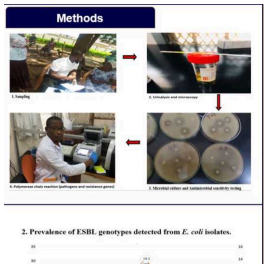
ANTIBIOGRAM AND PREVALENCE OF ESBL GENES IN COMMENSAL E. COLI ISOLATED FROM THE RESIDENTS OF GHANAIAN ELDERLY NURSING CARE HOMES V.1



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We use this protocol and it's working

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Abstract

We
isolated *E. coli* from urine samples of elderly patients and determined
the antibiogram of these pathogens. We further determined the Extended Spectrum
Beta-lactamase (ESBL) genes they harbored and their phylogenetic grouping. The
expected results were to reveal the prevalence and antibiogram of the isolated commensal
E. coli, their carriage rate of ESBL genes, and the
phylogenetic groupings.

Troubleshooting

Isolation of Escherichia coli.

1 The urine samples were cultured on Cystine-Lactose-Electrolyte-Deficient CLED agar for primary isolation. Presumptive *E. coli*, which appeared as yellow colonies, smooth, round, and moist after 24 hours of incubation were kept in nutrient broth to await secondary isolations. For secondary isolation, the suspected *E. coli* isolates were cultured on MacConkey and Blood Agar media (OXOID, Hampshire, England). The Blood agar base was supplemented with 10% horse blood. A loop full of each sample from the transport media was introduced on the media plate and was streaked appropriately with a sterile inoculating loop. The media plates were appropriately labeled and incubated at 37ºC for 24 hours. The following biochemical tests were performed to confirm further the suspected isolates: Indole test, Citrate test, oxidase test, and catalase test.

2 Biochemical Tests

Indole test

3 Peptone water suspensions were prepared in a bottle according to the manufacturer's protocol. Three to five pure isolates were then cultured in suspensions and grown overnight. Two to three drops of Kovac's reagent were added to the suspension and the bottle was shaken. The formation of a pink-colored ring that rose to the surface was observed, indicating a positive result.

Citrate test

4 The Simon's citrate agar was prepared according to the manufacturer's protocol. Pure isolates of the organisms on nutrient agar were inoculated into the citrate agar and incubated for 24 hrs. The citrate agar was green before inoculation. There was no color change because *E. coli* is citrate-negative.

Oxidase test

5 A drop of oxidase reagent, which contains tetramethyl-p-phenylenediamine was placed on a pure colony of the *E. coli*. No color change occurred indicating *E. coli* exhibits a negative oxidase reaction.

Catalase test

6 A few drops of hydrogen peroxide were added to pure colonies of *E. coli*. There was an immediate release of oxygen bubbles, indicating a positive reaction.

7 Antibiogram of *E.coli* isolates; Kirby-Bauer disc diffusion test

The Kirby-Bauer antimicrobial sensitivity test method was used to determine the antibiogram of the *E. coli* isolates (Bauer et al., 1966). Ten antimicrobial drugs were used. These were imipenem (IPM, 10 µg), ertapenem (ERT, 10 µg), aztreonam (AZM, 15 µg), cefepime (FEP, 30 µg), nitrofurantoin (F, 50 µg), cefuroxime (CXM, 10 µg), gentamycin (CN, 10 µg), amikacin (AK, 30 µg), ciprofloxacin (CIP, 5 µg) and levofloxacin (LEV, 10 µg).

Mueller-Hinton agar was prepared according to the manufacturer's protocol. The organisms were cultured on nutrient agar overnight. Between 4 and 5 isolated colonies of the organisms were suspended in about 2 ml of sterile saline by use of inoculating loop. The saline tube was vortexed to create a smooth suspension. The turbidity of the suspension was adjusted to a 0.5 McFarland standard. 200 ml of the suspension was introduced onto the Mueller-Hinton plate. A sterile glass spreader was used to spread the organisms on the plate. The surface of the plate was allowed to dry for 5 minutes before the antibiotic discs were placed on them. Sterile forceps were used to remove the antibiotic discs from the dispensers. After placing the discs on the agar, each disc was gently touched with the inoculating loop to ensure their contact with the agar surface. The plates were then incubated upside down for 24 hours at 37ºC.

8 **PCR Screening for ESBL Genes**

The DNAs of the 41 *E. coli* isolates were extracted using a zymogen extraction kit, based on the manufacturer's protocol. The isolates were then screened to determine the types of beta-lactamase (*bla*) genes they harbored. A total of forty-one *E. coli* isolates were screened for the presence of 10 *bla* genes. The

protocols employed by Kiiru and colleagues (Kiiru et al., 2012) were used with slight modifications. The reactions were carried out in a 10 µl reaction volume. This consists of 5 µl of 2X SYBR green master mix, 0.2 µl each of the primer sequence, 2.6 µl of the Nuclease free water, and 2 µl of the DNA template. The primer concentration was 0.2M. The PCR cycle conditions were as follows: 3 mins of initial denaturation at 94 ºC, (94 ºC of denaturation for 30 seconds, annealing for 30 seconds, elongation at 68 ºC for 30 seconds) x 30 cycles, and final elongation at 68 ºC for 10 minutes. The annealing temperatures were different for the different primers. The primer sequence and the annealing temperatures are listed in Table 1

Table 1: Primer sequences of the ESBL genes, amplicon sizes, and annealing temperatures

9 **Phylogenetic Grouping by PCR**

As described by Clermont *et al.*, 2000, the phylogenetic grouping of the isolated *E. coli* was determined. The positive *E. coli* strains were investigated for various genes that would determine their phylogenetic grouping by multiplex PCR (Clermont et al., 2000). The procedures were performed in a 10 µl reaction mixture. The reaction included 5 µl of 2X SYBR green master mix, 0.2 µl each oligonucleotide primer, 2.6 µl of nuclease-free water, and 2 µl of template DNA. Primer concentration was 0.2 M. Conditions of the reaction mixtures were 3 mins at 94 ºC initial denaturation, (94 ºC of denaturation for 30 seconds, annealing at 59.2 °C for 30 seconds, elongation at 68 ºC for 3 minutes) x 30 cycles, and final elongation at 68 ºC for 10 minutes. The marker-specific primer sequences and their amplicon sizes are listed in Table 2. Based on the presence or absence of specific genes (*chuA, yjaA, TspE4.C2,* and *arpA*), the isolates were clustered in group A, B1, B2, or D. Both group B1 and group A lacks the *chuA*gene. However, group B1 possesses the *TspE4.C2* gene, which is absent in group A. The *chuA* gene is present in both B2 and D isolates. The difference between the two is that group B2 has *yjA* genes while group D lacks it

Table 1: Primer sequences of the ESBL genes, amplicon sizes, and annealing temperatures
SKS

Target Gene	5'-3' sequence	Size (bp)	Ann. Temp(°C)
	ATGAGTATTCAACAT TTC CG		
*bla*TEM	CCAATGCTTAATCAG TGA GG	840	55.1
	TTCGCCTGTGTATTATCTCCCTG		
*bla*SHV	TTAGCGTTGCCAGTGYTCG	854	51.2
	ATGTGCAGYACCAGTAARGTKATGGC		
*bla*MA	TGGGTRAARTARGTSACCAGAAYCAGCGG	593	51.2
	CGC TTT GCC ATG TGC AGC ACC		
CTX-M 825	GCT CAG TAC GAT CGA GCC	307	57.7
	GCT CAG TAC GAT CGA GCC		
CTX-M 914	GTA AGC TGA CGC AAC GTC TG	474	56.3
	GTGGTGGATGCCAGCATCC		
*bla*CMY-1	GGTCGAGCCGGTCTTGTTGAA	915	56.1
	GCACTTAGCCACCTATACGGCAG		
*bla*CMY-2	GCTTTTCAAGAATGCGCCAGG	758	52.5
	ATGAAAAACACAATACATATCAACTTCGC		
*bla*OXA-1	GTGTGTTTAGAATGGTGATCGCATT	820	51.2
	ACGATAGTTGTGGCAGACGAAC		
*bla*OXA-2	ATYCTGTTTGGCGTATCRATATTC	602	56.1
CF	ATGATGAAAAAATCGTTATGC	1200	56.1
	TTGCAGCTTTTCAAGAATGCGC		

Table 2: The marker-specific primer sequences for phylogenetic grouping and their respective amplicon sizes

Target Gene	5'-3' sequence	Size (bp)
	GAGTAATGTCGGGGCATTCA	
TspE4.C2	CGCGCCAACAAAGTATTACG	840
	GACGAAC CAACGGTCAGGAT	
chuA	TGCCGCCAGTACCAAAGACA	854
	TGAAGTGTCAGGAGACGCTG	
yjaA	ATGGAGAATGCGTTCCTCAAC	593

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