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ORIGINAL ARTICLE

Phenotypic and Molecular Resistance Pattern of *E. coli* Isolated from School Children with Asymptomatic Bacteriuria

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ABSTRACT

Background: *Asymptomatic bacteriuria can develop systematic UTI, and considered as an omen sign for the presence of urinary tract abnormalities. Community transmission of ESβL and carbapenemases producing multidrug resistant E. coli has increased progressively and considered important public health problem.* ***Objectives:*** *This study was carried out during the period of January 2016 to January to2017 involving 1200 school children to determine the prevalence of ASB and antibiotic resistance pattern of urinary E. coli isolated from asymptomatic school children.* ***Methodology:*** *Urine analysis and culture with bacterial counting was done for detection of ASB. Antibiogram was done for E. coli that caused ASB by disk diffusion method with special regards to colistin and tigycycline. Phenotypic identification of ESβLs production was confirmed by the combined disk synergy test (CD-T) and analysed regarding to PCR results which detected SHV, TEM and CTX-M ESβL genes. Phenotypic identification of carbapenemase production with confirmatory (IPM/EDTA-CD) test was done.* ***Results:*** *Out of 1200 asymptomatic school chidren, 160(13.3%) had bacteriuria where 67(5.6%) had significant bacterial count. ASB was more prevalent in the age group of >12y(7.7%) and female children(8.8%). E. coli was the predominant bacterial pathogen causing ASB, 46/1200(3.8%). that represented 46/67(68.7%) of ASB children. and were sensitive to colistin and tigycycline by disc diffusion method. 43.5% of them by confirmatory (CD-T), and 47.8% by PCR were ESβL- producing. Percentage of ESβL genes among them(46) was as follow, 30.4%, 19.6% and 10.8% for SHV, TEM and CTX-M genes respectively. Also (16/46)34.8% were carbapenemase positive by screening disc diffusion test, (13/46)28.3% were (MβL) positive by confirmatory IPM/EDTA-CD test.* ***Conclusion:*** *ASB was significantly higher in age group >12 y and in female children. E. coli was the most prominent bacterial isolate. Multi-drug resistant E. coli producing ESβL or carbapenemases among ASB school children need adequate concern.*

INTRODUCTION

From all the infections that affect the human population, urinary tract infection comes in the 2nd place in frequency of occurrence in all ages either community or hospital acquired.[1] Over 8.3 million clinical visits occur because of UTI with females are far more affected than males.[2]

Globally, UTI occurs once at least in 8% of girls and 2% of boys at the age of 7 years, about 12-30% of them will experience recurrence within a year.[3]

Asymptomatic bacteriuria (ASB) is defined as a significant bacterial count (usually 10^5 organism /ml) of the same uropathogen present in clear-voided urine specimens in a person without urinary symptoms.[4]

About 10% of those having asymptomatic bacteriuria will develop systematic UTI, and it can be considered as an omen sign for the presence of urinary tract abnormalities.[4]

Some renal abnormalities and vesico-uretic reflux were found in about 10-35% of children having ASB. An association was observed between renal scarring in the childhood period and the risk of developing hypertension and chronic renal insufficiency at older age.[5]

Asymptomatic bacteriuria resolves spontaneously in about one third of the affected girls within weeks to months. However, about 24-80% of them, which is considered a high percentage, will suffer persistent infection or even reinfection with another stereotype or species.[6]

Many researchers reported that Gram negative bacteria especially *E. coli* are the commonest organisms to cause UTIs.[1] Unfortunately, some strains of *E. coli* that most frequently cause urinary tract infections are more resistant to different anti-bacterials rather than strains that seldom cause UTIs.[6]

In the developing countries, the uncontrolled and unregulated use of antibiotics is associated with the

emergence of multidrug resistance. This occurs as a result of the huge use and misuse of antibiotics either in the hospital, agricultural or the community settings.[7]

Bryce et al stated that routine antibiotic use is likely to be an important contributor to resistance, which may persist for up to 3 months post-antibiotic treatment.[8]

Worldwide, pediatric UTIs due to ESβL-producing bacteria are an important part of this problem because they limit therapeutic choices and increases morbidity of infection. [9, 3]

Infections with multidrug-resistant *E. coli* producing carbapenemases has increased progressively over the past few years and considered important public health problem.[10]

The aim of this study was to determine the prevalence of ASB among school children, to assess the phenotypic and molecular resistance pattern of the community transmitted urinary *E. coli* between school children with preventive and therapeutic guidelines.

METHODOLOGY

Study design and Subjects:

Cross sectional study was carried out on Shebin Elkom city and nearby village school children, Menofia governorate, Egypt from January 2016 to January 2017. A total of 1200 urine specimens were collected from apparently healthy school children in the age of 6-15 years, including 502 females (44.2%) and 698 males (58.2%) from both rural and urban areas.

A study questionnaire was used for taking the personal history including: age, sex, school grade, residence, socioeconomic level, previous exposure to antibiotics in the preceding three months and, history of kidney stones, urinary catheterization or surgeries in the genital system.

Any child who had any of the following symptoms (burning, frequent micturation, groin or flanks pain, palpable masses, fever, high blood pressure, pallor, lower limb oedema, puffiness of eye lids) or used antibiotics within the last 2 weeks prior to urine sampling were excluded from the study.

Ethical Points of the Research:

1. An agreement from the medical ethics committee at Menofia faculty of medicine was taken as a first step.
2. The participants simply were informed about the purpose of this study and the steps which will be done. Oral consent form was taken from every participant to share in the study.

Methods:

All students enrolled in the study were subjected to the following**:** Midstream urine samples were collected from students into sterile bottles for urine analysis after explaining the need to collect the urine with as little contamination as possible, i.e. a clean-catch specimen.[11]

Samples were transferred to microbiological lab at the microbiology department, faculty of medicine, Menofia University within half an hour to one hour.

Urine was subjected to microbiologic investigations.[11]Where urine specimens were cultured using a standard sterile calibrated wire loop that used to place 0.001ml of well mixed urine on cysteine lactose electrolyte deficient (CLED) and MacConkey's agars to determine the colony forming unit. The plates were incubated at 37°C aerobically for 24 h. specimens that gave bacterial count $\geq 10^5$ colonies per ml (after multiplying the plate count by 1000) were considered as significant bacteriuria while those that gave $<10^5$ colonies per ml were considered as insignificant bacteriuria.[12,13]

E. coli isolates were identified by colony morphology, culture characteristics on MacConkey's agars, microscopic examination, conventional biochemical reactions [11], and API20E.

Urine was tested with urine dipstick tests for protein and sugar and tested for leukocytes.

Antibiogram of *E. coli* isolates:

Antimicrobial susceptibility testing for *E. coli* isolates was done by disk diffusion method. The standard suspension of each isolate was prepared using its overnight colony culture, which was adjusted to the turbidity of 0.5 McFarland standard before being used to swab the surface of a dried Mueller-Hinton (MH) agar (Oxoid, UK) plate. The following antimicrobial discs were placed on the MH agar after 20 min. of inoculation: (Oxoid); ampicillin (10µg), amoxycillin/clavulinic acid (20/10µg), amikacin (30µg), gentamycin (10µg), tobramycin (10µg), streptomycin (10µg), ciprofloxacin (5µg), norfloxacin (10µg), azteronam (30 µg), imipenem (10 µg), meropenem (10 µg), cefamandole (30 µg), ceftriaxone (30µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30µg), cefoxitin (30µg), trimethoprim/ sulfamethoxazole (1.25 µg/23.75µg), chloramphenicol (30µg), nitrofurantoin (300µg), tetracycline (30µg), colistin (10µg), tigecycline (30µg) and interpreted according to the Clinical and Laboratory Standards Institute guidelines (2015).

Multidrug resistant (MDR) and Extensive drug resistant (XDR) *E. coli* isolates

MDR and XDR isolates were identified according to the combined guidelines of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC).[14] Bacterial isolate resistant to at least one antimicrobial from three different groups of first line drugs tested was regarded as multidrug resistant (MDR). Extensively drug resistant (XDR) isolates were identified when the isolates were resistant to at least one agent in all but two or fewer antimicrobial categories .[3]

ESβLs susceptibility testing:

Screening for ESβLs production was performed by disk diffusion test using ceftriaxone (30µg), ceftazidime (30µg), cefotaxime (30µg) and aztreonam (30µg). If the zone of inhibition (ZOI) was ≤25 mm for ceftriaxone, ≤22 mm for ceftazidime and/or ≤27 mm for cefotaxime and aztreonam, the isolate was considered a potential ESβL producer as recommended by CLSI and further tested by confirmatory clavulinate combined disk test.[15]

In confirmatory clavulinate combined disk, cefotaxime (30µg) and cefotaxime/clavulanic acid (30/10µg) were used. *E. coli* was considered as ESβL-producer if there was ≥5 mm increase in diameter of cefotaxime/clavulanic disk than that of cefotaxime disk alone. The same was done with ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg).[15] (Figure 1)

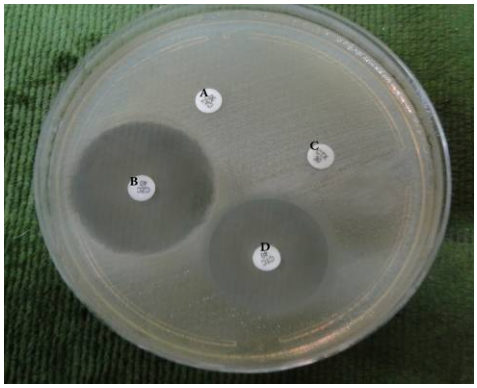

Fig 1: Detection of ESβL - producing *E.coli* using cephalosporin/clavulanate combined disk test. Letter A, C represent ceftazidime, cefotaxime disks alone, letter B, D represent ceftazidime, cefotaxime disks combined with clavulanic acid. There was an expansion of the bacterial growth inhibition zone around the combined disk by ≥ 5mm in diameter.

Carbapenems susceptibility testing:

Screening for carbapenemase production was performed by disk diffusion test using imipenem (IPM) and meropenem(MEM) disks (10µg for each) (Oxoid). The average diameters of zones of inhibition were measured and interpreted according to CLSI guidelines (2015), (For imipenem and meropenem; S≥23, R≤19**)**

Metalo β lactamases susceptibility testing:

Suspected Metalo β lactamases were confirmed by imipenem/EDTA combined disk (IPM/EDTA-CD) test, two (10µg) imipenem disks were placed on the plate at a distance of 15mm apart (center to center) and 5µl of sterile EDTA solution (930µg EDTA) was added to one of the imipenem disk and incubated aerobically at 37◦C for 18-24h. The presence of an expanded growth inhibition zone between two discs or increase of zone size more than 7mm in imipenem/EDTA disk than imipenem disk alone was considered as MβL positive.[16] (Figure 2a)

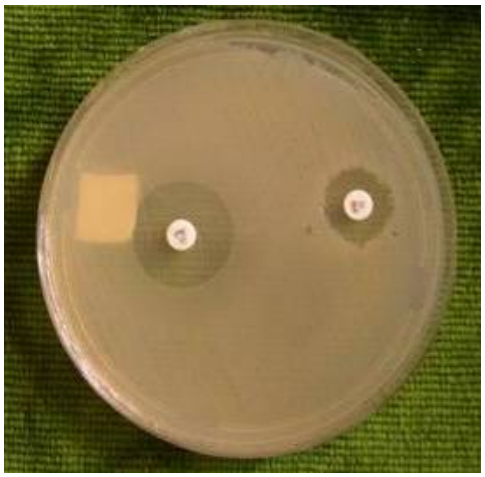

Fig 2.a: Detection of class B carbapenemase (MBL) producing *E.coli* using Imipenem/EDTA combined disk test. There was an expansion of the bacterial growth inhibition zone around the combined disks by ≥ 5 mm in diameter.

Molecular study for ESβL genes detection: done by using PCR.

DNA was extracted using illustra bacteria genomic Prep Mini Spin Kit (GE Healthcare, UK). The used primers were designed and synthesized by Eurofins MWG Operon (Germany).

PCR conditions for ESβL genes (*SHV*, *TEM* and *CTX-M*) study included: heating at 94°C for 3min, followed by 35 cycle of denaturation at 94°C for 45 s, annealing for 30s (at 54°C for *SHV*, 51ºC for *TEM* and 50°C for *CTX-M* regarding primer optimization) and extension at 72°C for 1 min. A final extension was at 72°C for 3 min.[17] (Table 1)

Table1: The used primers for detection of ESβLs genes

ESβLs gene primers	Primer sequence	Annealing temperature	Amplicon size (bp)	References
SHV-F *SHV*-R	CGC CTG TGT ATT ATC TCC CT CGA GTA GTC CAC CAG ATC CT	54°C	293 bp	**(*Bali EB, et al*., 2010)**.
TEM-F *TEM*-R	TTT CGT GTC GCC CTT ATT CC ATC GTT GTC AGA AGT AAG TTG G	51°C	403 bp	
CTX-M-F *CTX-M*-R	CGC TGT TGT TAG GAA GTG TG GGC TGG GTG AAG TAA GTG AC	50°C	569 bp	

Study was done using Mycycler™ Thermal cycler (BioRad, USA). Synthesized DNA fragments were detected on 1.5% agarose gels by ethidium bromide staining. A DNA ladder (100-3000 bp) was used to estimate allele sizes in base pairs (bp) for the gel.[1] (Figure 3a).

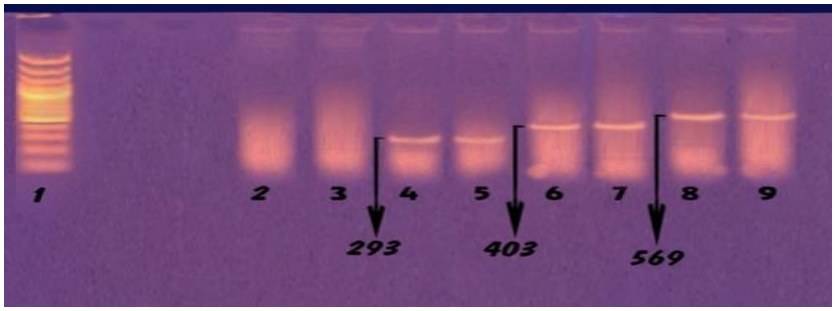

Fig. 3a: Gel electrophoresis of PCR amplification product of *SHV, TEM* and *CTX-M* ESβL genes. Lane 1; DNA ladder (100-3000bp), lanes 2 and 3; - ve for allgenes, lanes 4 and 5; + ve *SHV* gene (293bp), lanes 6 and 7; +ve *TEM* gene (403 bp) and lanes 8 and 9; +ve *CTX-M* gene (569 bp)

Statistical analysis of the collected data:

Results were collected, tabulated and statistically analyzed by an IBM compatible personal computer with SPSS statistical package version 20 (SPSS Inc. Realesed 2011. IBM SPSS statistics for windows, version 20.0, Armnok, NY: IBM Corp.).

Two types of statistical analysis were done:

a. Descriptive statistics e.g. was expressed in: Number (No), percentage (%) mean (**x**) and standard deviation (SD).
b. Analytic statistics e.g. - Chi-square test (χ^2) was used to study association between qualitative variables. Whenever any of the expected cells were less than five, Fischer's Exact test with Yates correction was used.

RESULTS

In this study, 1200 school children were screened for asymptomatic bacteriuria. Out of them, 1040 (86.7%) had no urinary growth. While, 160 (13.3%) children had bacteriuria, from them, *E. coli* represented 86/160(53.8%), Gram negative bacteria other than *E. coli* represented 44/160 (27.5%) and Gram positive bacteria represented 30/160(18.8%). Three chidren had Pyuria.

Regarding bacteriuria, *E. coli*, Gram negative bacteria other than *E. coli* and Gram positive bacteria represented 7.2%, 3.7% and 2.5% of the whole screened school children respectively.

The prevalence of ASB in the studied school children was about 67/1200(5.6%). Among them, *E. coli* was isolated from 46/67(68.7%). (Table 2)

Table 2: Comparison between *E. coli* and other organisms caused bacteriuria regarding the presence of ASB:

Screened urine specimens		bacteriuria (160/1200)	*E. coli* (86 /160) (53.8%) *(71.7)	Others (74/160)		bacteriuria (n=160)	Total Screened urine specimens (n= 1200)	χ^2	P value
				Gm -ve other than *E. coli* (n= 44)	Gm positive (n= 30)				
			No. (%)	No. (%)	No. (%)	No. (%)	No. (%)		
1200 screened urine specimens	bacteriuria 160 /1200 *(13.3)	(ASB)	46(68.7) *(3.8)	13 (19.4) * (1.1)	8 (11.9) *(6.7)	67 41.9 *(5.6)	160(13.3)	10.36	0.005
		Non-significantbact-eriuria	40(43.1) *(3.3)	31 (33.3) *(2.6)	22 (23.7) *(1.8)	93 58.1 *(7.8)			
	No growth 1040/1200 (86.7)*		-	-	-		1040(86.7)	-	

ASB= Asymptomatic bacteriuria, CFU= Colony forming units

ASB was significantly lower (p<0.01) in young children in the age group of 6 to 12 years 26/670(3.9%) than children in age group of >12 y 41/530 (7.7%).

Regarding gender, ASB was significantly higher (p <0.01) in females children 44/502 (8.8%) than male ones 23/698 (3.3%).

Among ASB positive specimens, *E. coli* (46/67) was significantly lower (p<0.05) in young children with age group from 6 to 12 years (53.8%), than the age group of >12 year (78%). Also *E. coli* was found significantly higher (p <0.01) in females (75.0%) than males (56.5%) (Table 3)

Table 3: Comparison between *E. coli* and other organisms in children with ASB (n=67) regarding their socio-demographic charcterstics:

Socio-demographic charcterstics of children with ASB	Causative org. of ASB 67/1200 (5.6%)					
	***E. coli* (46/67) (68.7%)**	**Others (21/67) (31.3%)**		**Total (n=67)**	χ^2	**P value**
		Gm negative other than *E. coli* (13/21) (61.9%)	**No. (%)Gm positive (8/21) (38.1%)**			
	No. (%)	**No. (%)**	**No. (%)**	**No. (%)**		
Age:						
6-12:	14(53.8) *(1.2)	6 (23.1) *(0.5)	6 (23.1) *(0.5)	26 (38.8) *(2.2)	6.07	0.04
>12:	32(78.0) *(2.7)	7 (17.1) *(0.6)	2 (4.9) *(0.2)	41 (61.2) *(3.4)		
Gender:					6.69	0.03
Male:	13(56.5) *(1.1)	4 (17.4) *(0.3)	6 (26.1) *(0.5)	23 (34.3) *(2.0)		
Female:	33 (75.0) *(2.8)	9 (20.5) *(0.8)	2 (4.5) *(0.2)	44 (65.7) *(3.7)		
Residence:					0.17	0.91
Rural:	26 (66.7) *(2.2)	8 (20.5) *(0.7)	5 (12.8) *(0.4)	39 (58.2) *(3.3)		
Urban:	20 (71.4) *(1.7)	5 (17.9) *(0.4)	3 (10.7) *(0.3)	28(41.8 *(2.3)		
History of exposure to antibiotics						
No:	12 (60.0) *(1.0)	3 (15.0) *(0.3)	5 (25.0) *(0.4)	20 (29.9) *(1.7)	4.67	0.09
Yes:	34 (72.3) *(2.8)	10 (21.3) *(0.8)	3 (6.4) *(0.3)	47 (70.1) *(3.9)		
Chronic diseases:						
No:	44 (68.9) *(3.7)	12 (18.8) *(1.0)	8 (12.5) *(0.7)	64 (95.5) *(5.3)	0.67	0.70
Yes:	2 (66.7) *(0.2)	1 (33.3) *(0.08)	0 (0.0) *(0.0)	3 (4.5) *(0.3)		
Use of public W.C:						
No:	13 (76.5) *(1.1)	2 (11.8) *(0.2)	2 (11.8) *(0.2)	17(25.4) *(1.4)	0.89	0.46
Yes:	33 (66.0) *(2.8)	11 (22.0) (0.9)	6 (12.0) *(0.5)	50 (74.6) *(4.2)		

*2 diabetic cases in *E. coli* positive samples and one case of sickle cell anemia in Gm negative other than *E. coli*.

Among 46 *E. coli* isolates that caused ASB, eight isolates were MRD (17.4%) that were resistant to ampicillin, amoxycillin/clavulinic acid, trimethoprim/ sulfamethoxazole and tetracycline. Thirty three isolates were XDR (71.7%). Where, four (8.6%) *E. coli* isolates were resistant to 20 antibiotics tested in this study including 3rd generation cephalosporins, azteronam, quinolones and imipenem. Seven (15.2%) *E. coli* isolates were resistant to 17 antibiotics tested in this study including 3rd generation cephalosporins, azteronam and imipenem. All *E. coli* isolates were sensitive to colistin and tigycycline. No PDR isolates against antibiotics used in this study. (Table 4)

Table 4: Resistance phenotypes of *E. coli* isolated from children with ASB (n= 46).

Degree of resistance	Resistance phenotypes	Total (46) No. (%)
5 (10.9%)	**Resistance to 2 antibiotics: (5)** - A, TE	5 (10.9%)
MDR 8 (17.4%)	**Resistance to 3 antibiotics** - A, AMC,TE (3) - A, TMP/SMX, TE (5)	 (6.5%) (10.9%)
XDR 33 (71.7%)	**Resistance to 5 antibiotics: (2)** - A, TMP/SMX, TE, TOB, NOR. **Resistance to 8 antibiotics: (3)** - A, AC, CN, TOB, TMP/SMX, C, N, TE. **Resistance to 11 antibiotics:(3)** - A, AMC, CN, TOB, TE, CIP, MA, TMP/ SMX, C, N, FOX. **Resistance to 14 antibiotics: (3)** - A, AMC, CN, TOB, ST, TMP/ SMX, C, N, TE, CIP, NOR, MA, CRO, FOX. **Resistance to 15 antibiotics: (6)** - A, AMC, CN, TOB, ST, TMP/ SMX, C, N, TE, CIP, NOR, MA, CRO, CTX, CAZ, **Resistance to 17 antibiotics: (7)** - A, AMC, AK, CN, TOB, ST, TMP/ SMX, C, N, TE, MA, CRO, CTX, CAZ, IMP, ATM, FOX. **Resistance to 18 antibiotics: (5)** - A, AMC, CN, TOB, ST, TMP/SMX, N, C, TE, FOX, CIP, NOR, MA, CTX, CRO, CAZ, IMP, ATM. **Resistance to 20 antibiotics:(4)** - A, AMC, AK, CN, TOB, ST, TMP/SMX, N, C, TE, FOX, CIP,NOR, MA, CTX, CRO, CAZ, IMP, MEM, ATM.	 (4.5%) (6.5%) (6.5%) (6.5%) (13%) (15.2%) (10.9%) (8.6%)
PDR	**Resistance to 23 antibiotics: (0)**	0 (0.0)

Among *E. coli* isolates, that assumed in ASB, 43.5% and 47.8% were ESβL-producing by confirmatory cephalosporin/clavulanate combination disks test and by PCR respectively. Regarding PCR results, the sensitivity of cephalosporin/ clavulanate combination disks test was 86% and its specificity was 96 %, 95% PPV, 88 % NPV with 91% overall accuracy for detection of ESβL-producing isolates (Table 5). Also, (34.8%) were carbapenemase positive by screening disc diffusion test and (28.3%) were class B carbapenemase (MβL) positive by IPM/EDTA-CD test. (Figure 2b)

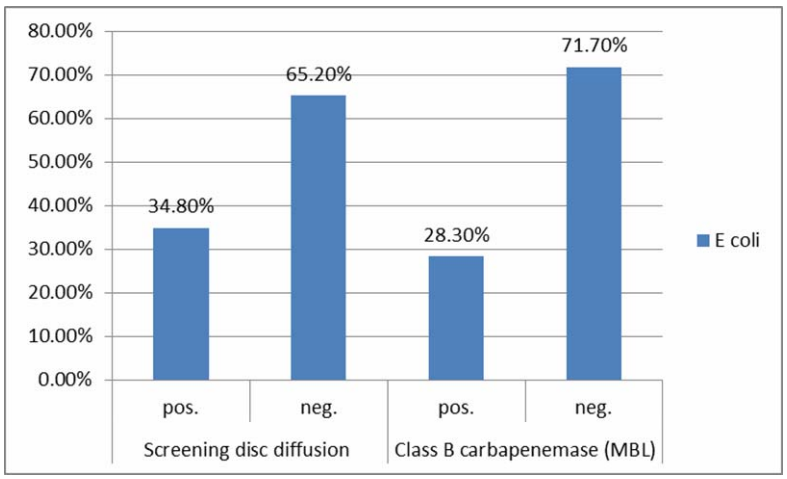

Fig 2b: Screening and confirmatory tests for carbapenemase detection among *E. coli* isolates (n=46) in children with ASB .

Distribution of ESβL genes among these *E. coli* isolates was as follow, 30.4%, 19.6% and 10.8% for *SHV*, *TEM* and *CTX-M* genes, respectively. (Figure 3b)

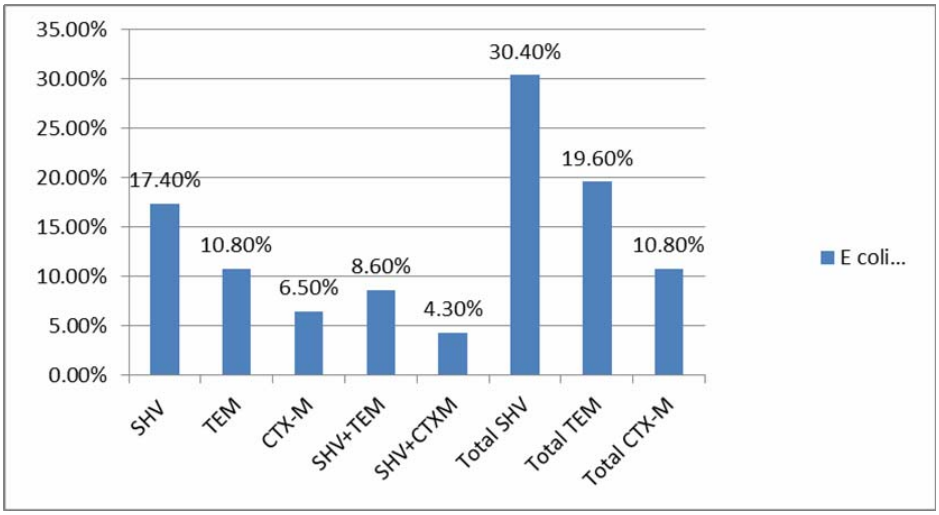

Fig 3(b): Distribution of ESβL genes among *E. coli* isolated from school children with ASB

Table 5: **Sensitivity and specificity of cephalosporin/clavulanate combination disks test in relation to PCR for detection of ESβL among *E. coli* isolated from school children with ASB (n=46):**

E. coli (n=46)		PCR for ESβLs genes		Sensitivity	Specificity	Accuracy	PPV	NPV
		+ve (n=22) No. (%)	-ve (n=24) No.(%)					
Phenotypic confirmatory combined test	+ve (n=20)	19 (86.4)	1 (4.2)	86%	96%	91%	95%	88%
	-ve (n=26)	3 (13.6)	23 (95.8)					

DISCUSSION

This study was carried out over school children of Shibin Elkom city and nearby villages to detect the prevalence of asymptomatic bacteriuria with multidrug resistant *E. coli*, particularly ESβL and carbapenemase producing strains.

In this study, the prevalence of ASB was (5.6%) among studied school children. That was lower than Kondapaneni et al [18] result, where (16.5%) of school children had significant bacteriuria but closer to Jha et al [4], where (1.39%) of studied school children had ASB

In this work, *E. coli* was the predominant bacterial pathogen causing ASB 46/67 (68.6%). This was matched to Jha et al [4] result, where *E. coli* was the predominant bacterial pathogen causing ASB in school children 4/7(57.14%). Both Motamedifar et al and Dada et al, had also put forward similar observation [19,20]. Also, Kondapaneni et al [18] had the same conclusion 9/33(27.27%). Onanuga and Selekere[1] reported something different. Where *K. pneumoniae* and *S. aureus* were the most frequently recovered organisms causing ASB in young adult students whilst *E. coli* was the least.

In the present study, (3.9%) of children in the age group of 6- 12 y had ASB. That was statistically significant lower (P<0.05) than that of age group >12 y where (7.7%) of them had ASB. This was close to Jha et al [4] who found that the prevalence of ASB in children of 5 to 10 years of age was about 1% to 1.5%.

In our study, regarding gender, ASB was significantly higher female children (8.8%) than male ones (3.3%). This was in agreement to Jha et al [4], where female (1%) to male (0.39%) ratio was 2.5:1. between age group 5 and 13 years Also, Dada et al [20] and Kondapaneni et al [18] mentioned that the prevalence of ASB was significantly higher in females than in males.

In the present study, residence, history of antibiotics exposure, presence of chronic diseases (2 children were diabetics and one child was sickle cell anemic) and using public W.Cs showed no statistically significant difference (P>0.05) on the overall prevalence of ASB. This is in contradiction to Salem et al [21], who documented a higher rate (30%) of ASB in Egyptian children with diabetes. Yauba et al [22] had a slightly higher prevalence of ASB in the children with sickle cell anemia (8.1%). This explained by low number of children with chronic diseases included in this study.

Very high ASB rate (48%) documented by Alo et al[23] among children in a rural area who have a poor health consciousness and low level of hygiene.

This work showed that, ASB caused by *E. coli* was more predominant in female (6.6%) than male (1.9%) children. This goes with Onanuga and Selekere [1] who observed that *E. coli* was the most frequently isolated microorganism in female than in male urine specimens.

In the present study, among 46 *E. coli* isolates that caused ASB, eight isolates were MRD (17.4%) that were resistant to ampicillin, amoxycillin/clavulinic acid, trimethoprim/ sulfamethoxazole and tetracycline. Thirty three isolates were XDR (71.7%) with no PDR isolates. This is nearly reverse to Parajuli [3], where out of 739 *E. coli* isolates, 64.9% were multidrug resistant (MDR) and only 5% were extensively drug resistant (XDR)

In our study, regarding *E. coli* isolates that caused ASB, four (8.6%) were resistant to twenty antibiotics including 3rd generation cephalosporins, azteronam, quinolones and imipenem, 5 (10.9%) were resistant to eighteen, 7(15.2%) were resistant to seventeen, 6 (13%) were resistant to fifteen, 3 (6.5%) were resistant to either 14, 11 or 8 antibiotics and two (4.5%) were resistant to five antibiotics. While Oladoja and Onifade[9] results on *E. coli* isolated from apparently healthy school children, Nigeria, were as follow, fourteen (6.79%) were resistant to all the eleven antibiotics tested, 39 (17.48%) to ten, 43 (20.87%) to nine, 56 (27.18%) to eight, 36 (14.56%) to seven, 12 (5.83%) to six, 4 (1.94%) to five and 2 (0.97%) to four antibiotics respectively.

Our results go with the work of Parajuli [3] that all *E. coli* isolates were sensitive to colistin and tigycycline. Where in Salem et al [21] study, Egypt, imipenem and levofloxacin were the most effective drugs against *E. coli* isolates.

In the present study, among *E. coli* isolates that assumed in ASB 43.5% by confirmatory cephalosporin/ clavulanate combination disks test and 47.8% by PCR were ESβL producing. This is near to Parajuli et al [3], where ESβL was detected in (38.9%) of the *E. coli* isolates and close to the findings reported by other studies in different parts of Asian region including (37.7%) by Shettigar et al [24] , India, (30.5%) by Rezai et al [25] , Iran and (27.4%) by Al Mously et al [26] from Saudi Arabia. Extremely higher rates of ESβL- producing *E. coli* (83%) had also been reported by Chinnasami et al [27], India. However, lower rates were also reported, particularly from developed countries including 9.3% from USA by Degnan et al [28] and 10.2% from Korea by Han et al [29].

These variations in the rate of ESβL producing *E. coli* might be attributable to the geographical difference, local antibiotic prescribing policy, the extensive use of broad spectrum antibiotics especially third generation cephalosporins and endemicity of drug resistance pathogens in the locality as explained by Parajuli et al [3]

In our study, regarding PCR results, the sensitivity of cephalosporin/clavulanate combination disks test was 86% and its specificity was 96 %, 95% PPV, 88 % NPV with 91% overall accuracy for detection of ESβL-producing isolates. This is in agreement with the results of Färber et al [30] and Bali et al [17] .

In the present study, among *E.coli* isolates that caused ASB, 16(34.8%) were carbapenemase positive by screening disc diffusion test, 13 isolates (28.3%) were class B carbapenemase (MβL) positive by IPM/EDTA-CD test. That is higher than detected by Bora et al [31], Nepal, 41/216(18.98%) *E.coli* isolates were carbapenemase positive by screening test, with fairly positive results in combined disc test for MβL. In Chakraborty et al [10] study, 29/300(9.5%) isolates were carbapenemase producers of which 15 (5%) were MβL producers.

The predominant ESβL gene of *E. coli* isolates tested was *SHV* gene. 14/46(30.4%). *TEM* and *CTX-M* genes were presented among 9/46(19.6%) and 5/46(10.8%) respectively. That was in agreement with Färber et al[30] study but were in disagreement with Akila et al [32], where 66.67% of their isolates had *TEM* type, one (5.56%) had *SHV* and one (5.56%) had both *TEM* and *SHV* genes**.** And Bali et al [17] stated that *TEM* gene was the most prevalent ESβL gene. In the study conducted by Cheng et al [33], 90% of the ESβL-producing *E. coli* harbored *CTX-M* genes, 59% and 32% possessed *TEM* and *SHV* genes respectively. Also Daoud [34] found that 83% of isolates tested positive for *CTX-M*, 29.6% for *SHV* and 68.2% for *TEM*.

Conclusions and Recommendations:

ASB was higher in children in age group of more than 12 y specially females, where *E. coli* was the most prominent cause and were sensitive to colistin and tigycycline. 43.5% by confirmatory (CD-T), 47.8% by PCR were ESβL-producing. *SHV* gene was the most prevalent ESβL gene among *E. coli* isolates. 34.8% of *E. coli* that caused ASB were carbapenemase positive by screening test and 28.3% were MβL producing by confirmatory test.

Routine and early laboratory checkup is very much recommended for school age children to prevent unnecessary morbidity and would help to reduce risk of chronic renal insufficiency. Antibiotic prescribing policy needs adequate concern to decrease the resistant pattern of bacteria.

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