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Detection of Extended-spectrum β-lactamases (ESBLS) Among *Enterobacteriaceae* Inside Departments of Sabha Medical Center Hospital, Especially Nursery Department and Outpatient of Sabha City , Fazzan Area in Southwestern of LIBYA

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Abstract

To estimate a rate of spread of ESBL-producing *Enterobacteriaceae* among out and inpatient Sabha Medical Center in stool, urine samples. ESBLS activities were tested by the double disk synergy. The isolates were *Escherichia coli* 83(47.7%), 42(24.13%), *Klebsiella spp.* 21(12.0%), 11(6.3%), other type of *Enterobacteriaceae* 13(7.4%) 4(2.29%) for in and outpatient respectively. Forty four (25.28%) ESBLs strains were detected in 54.5%, 11.36% of *E. coli*, 15.9%, 4.5% of *Klebsiella spp.*, 11.36%, 2.27%of other, for in and outpatient respectively. Prevalence rates 22.72%, 13.6%, 11.36 %, 9.09 % , 6.81% , 6.81 %,4.5% , 4.5% , 2.27% of ESBLs were isolated from patients on the Nursery, Medicine, surgery , Pediatric, Urology, Obstetric, Kidney Ward, ICU and ophthalmic department respectively. Fecal carriage of ESBLs is 34% 6.8% for in and outpatient. Our study has provided a first ESBLS screening for *Enterobacteriaceae* in Sabha Medical Center. Faecal ESBLS-producing *Enterobacteriaceae* for inpatient is referring to the weakly antimicrobial policy implemented in the hospital and the aware risk of the spread it in the community. ESBLS- E. infection is prominent in Urinary Tract Infection for Nursery department and seems demonstrates the outbreak state in the hospital.

Keywords: *Enterobacteriaceae* ; Sabha; Nursery Department; outpatient; LIBYA.

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1. Introduction

Extended-spectrum beta-lactamases (ESBLS) were term used to indicate enzymes that able to resistance penicillin, the first, second and third generation cephalosporins which extended to monobactam. These enzymes are broken the beta-lactam ring causes inactivating the antibiotic. The first ESBLS described were in Greece in the 1960s and named TEM [1]. TEM was present on plasmid of gram negative bacteria; also other enzymes discovered were related to it. The most common ESBLS are in *Enterobacteriaceae* especially *E.coli* in Europe and other regions [2]. Other types of ESBLS are associated with a variety of mobile genetic elements on chromosome [3].

The worldwide prevalence of ESBLS among *Enterobacteriaceae is* a major note in both hospital nosocomial infection and community acquired infection [4]. On the other hand, these enzymes are causes multi resistant bacteria to other antibiotic options remain available [5]. ESBLS are the big challenges for antibiotic treatment in the last and present years [6], which increase especially by using the empirical management of community-associated infection [2]. The risk of increased antimicrobial resistance in the future is happen by development of ESBLS types which causes treatment difficulty year by year [4, 5, 3, 7] . Furthermore, ESBL testing is must be used as diagnostic test in hospital to avoid using the β-lactam antibiotics treatment and also useful for epidemiological purposes [8].

2. Objective

The studies on the prevalence of ESBLS in the outpatient especially in the feces carrier *Enterobacteriaceae* are little known in the Libya. Therefore, the aim was to estimate the rate of spread of ESBL-producing *Enterobacteriaceae* in outpatient and inpatient Sabha Medical Center Hospital (Libya) in stool and urine samples.

3. Methodology

3.1. Samples of patients

A total of 174 urine and stool samples were collected from out and in patent attending Sabha medical center. The study was carried out between June 2013 to Feb 2014, Sabha city, Libya. All sample were collected under-standard condition in this hospital.

3.2. Isolation and Identification

By using the standard loops, the urine samples were cultured on MacConky and blood agar plate. The stool samples were cultured on MacConky agar and Sallmonella-Shigell agar. All cultures were incubated at 37C$^{\circ}$ for 18-24 hours. The positive urine cultures were more than 10^5CFU/ml. Isolated colonies from significant plate were identified by using APIE20 system (Bio Me'rieux sa, 69280 Marcy l`Etoile-France) and the test performed according to the manufacture. Reference strain of *E.coli* ATCC 25922 was used as control in this study.

3.3. Sensitivity test

3.3.1. Preparation of bacterial inoculum

The inoculum was prepared by selecting a number of colonies from 18 hours cultured plate on a nutrient agar. The colonies were then emulsified into sterile normal saline. The turbidity of inoculum was adjusted to that of 0.5 McFarland [9].

3.3.2 The disk diffusion test

The antibiotic susceptibility testing was performed by the disk diffusion method according to the NCCLs recommendation M100-S25 [9]. Briefly, within 15 minute the adjusting suspension was streaked by the swab over the Muller-Hinton agar surface, the antibiotic disk dispensed onto the surface of the inoculated agar with no closer than 24mm from center to center. *E.coli* ATCC 25922 was used for each test run. All the plates were incubated at 35C° for 18 hours.

3.3.3. Double Disk synergy (ESBLS Detection)

This test was performed according to the technique reported by Monald and Thompson [10]. A single separated colony from an overnight blood agar culture was emulsified in 0.9% normal saline solution tube. The turbidity of suspension was adjusted to give 0.5% McFarland standard. The suspension was spread on Muller-Hinton agar plate by the sterile cotton swab. The disks containing the standard 30mg ceftazidium, ceftriaxone, cefotaxime and aztreonam were placed at 25mm a center to center from amoxicillin/clavulanic acid 20/10mg. The plates were incubated at 35C° to 18 hours.

3.4. Statistical analysis.

The statistical significance of difference between categorical variables was evaluated by T-test and Fisher Test. Significance was accepted when $P = \leq 0.05$. The statistical analysis of the data carried out by using Minitab program 16.

4. Result

4.1. The types of bacterial isolates

In this study 174 strains were isolated from urine and stool samples which contained different type of bacteria, *E.coli*, *Klebsiella spp.*, other type of *Enterobacteriaceae* from inpatient and outpatient isolates as showed in the Table (1). Distribution of *E. coli*, *Klebsiella spp.*, and bacteria isolated from in patients stool and urine samples according to type of bacteria; sample and hospital department is shown in Figure (1).

4.2. Sensitivity test

The *Enterobacteriaceae* collections were tested for antimicrobial susceptibility by Kirby-Bauer disk diffusion

method and Double Disk synergy. The antibiotic tested against *E.coli* for inpatient and outpatient were resistant by 20.8%,3.2% to cefotaxime , 16.8% , 5.6% to ceftriaxone, 8.8%, 10.4% to ceftazidium , 20.8% , 12.8% to aztreonam , 14.4%, 8.8% to amoxillin/clavulanic acid , 40.8% , 16% to cefoxitin, 28%, 16.8% to cephalothin, and 56.8%, 26.4% to ampicillin(Figure 2).

Table (1) The types of bacterial isolates in this study.

Bacteria isolates	**Inpatient No. (%)**	**Outpatient No. (%)**
E.coli	83(47.7)	42(24.13)
Klebsiella spp.	21(12.0)	11(6.3)
Other	13(7.4)	4(2.29)
Total	**117**	**57**

Other= other type of *Enterobacteriaceae*

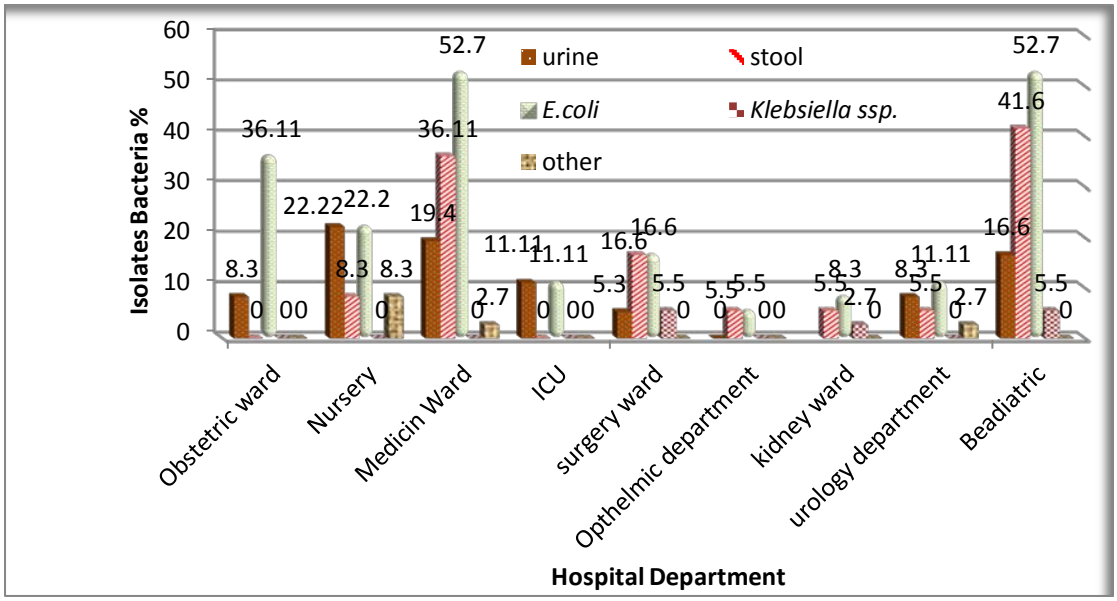

Figure 1: The sources and Bacterial isolates % form Hospital department types.

The *Klebsiella spp.* for inpatient and outpatient were resistant by 12.9%, 9.6% to cefotaxime , 9.6% , 3.2% to ceftriaxone, 12.9%, 6.4% to ceftazidium ,19.3%,6.4% to aztreonam 6.4%, 0 % to amoxillin/clavulanic acid , 45.16% , 12.9% to cefoxitin, 19.3%, 9.6 % to cephalothin, and 54.8%, 19.3% to ampicillin (Figure 3).

Other type of *Enterobacteriaceae* for inpatient and outpatient were resistant by 25%, 0% to cefotaxime and ceftriaxone, 31.25%, 6.25% to ceftazidium ,37.5%,6.25% to aztreonam 18.5%, 6.25 % to amoxillin/clavulanic acid , 62.5% , 18.7% to cefoxitin, 62.5%, 12.5 % to cephalothin, and 87.5%, 18.7% to ampicillin (Figure 4).

Statistically significant different was between inpatient and outpatient isolates P<0.05, P=0.05 for both *E.coli*, other type and *Klebsiella spp.* respectively.

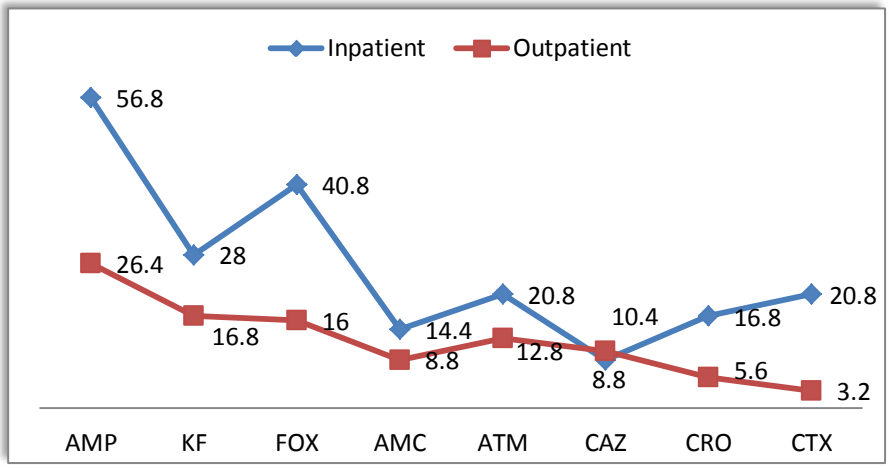

Figure 2: Antibiotic susceptibility test (resistant %) for *E.coli* P<0.05.

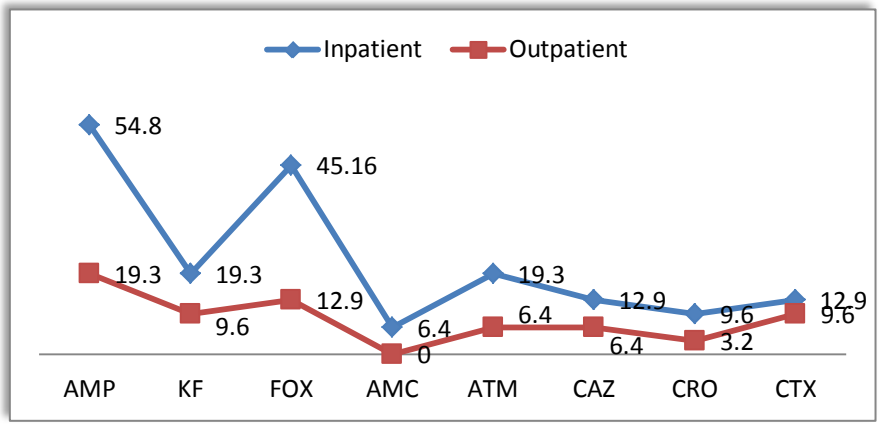

Figure 3: Antibiotic susceptibility test (resistant %) for *Klebsiella spp.* P=0.05.

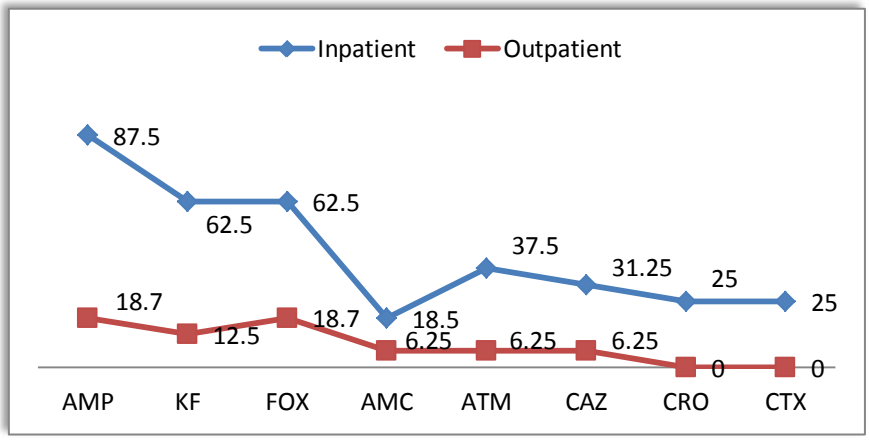

Figure 4: Antibiotic susceptibility test (resistant %) for other type of *Enterobacteriaceae* isolates P<0.05.

4.3. Double Disk synergy (ESBLS Detection)

ESBLs strains were detected in all isolates for inpatient and outpatient as shown in the table (2). Only 8 (4.59%) ESBLS strains were for outpatient. Of the 36 (20.68%) clinical isolates were for inpatient ESBLS *Enterobacteriaceae*. Of the Nursery department were 9 *E. coli*, 1 *Klebsiella spp*. (70% from urine), in Medicine Ward 5 *E. coli*, 1 other types of *Enterobacteriaceae* (50% form stool and urine), in surgery ward 3 *E. coli*, 2 *Klebsiella spp.* (60% from stool) and Pediatric Ward 3 *E. coli*, 1 *Klebsiella spp.* (75% from stool sample). Where were for Urology department 2 *E. coli*, 1 other types (66% from stool), and for Obstetric Ward 3 *E. coli* (100% from urine). On the other hand, in the Kidney Ward and ICU were 1 *E. coli*, 1 *Klebsiella spp*, and 2 *E. coli* (100% from stool) (100% from urine) respectively, then ophthalmic department (100% stool) (5).

Table (2) ESBL production percentage in bacterial isolates types**.**

Inpatient	**Outpatient**
No. (%)	**No. (%)**
E.coli 24(54.5)‡	*E.coli* 5(11.36)‡
Klebsiella spp. 7(15.9)‡	*Klebsiella spp.* 2(4.5)‡
Other 5(11.36)	Other 1(2.27)
Stool 15(34)*	Stool 3(6.8)*
Urine 21(47.7)*	Urine5(11.36)*
Total ESBL No. (%) = 44 (25.28)	
ESBLS stool samples = 18 (40. 9)	
ESBLS urine samples = 26 (59.09)	

‡=Fisher Test P > 0.05,*= Fisher Test P>0.05

5. Discussion

The *Enterobacteriaceae* collections were become resistant to our usual antibiotics by acquiring genes encoding enzymes that destroy important antibiotics, this demonstrated in the results which increased resistant to Penicillin and then the first generation of Cephalosporin (Figure 2. 3. 4), while recession resistant to the Monobactam then third generation of Cephalosporin to both inpatient and outpatient [11] with statistical different significantly (P<0.05). The Ampicillin resistant were higher than recorded in our results by both Abujnah A. A**.** and his colleagues [11] and Ghenghes K. S. *and his colleagues* [12]. The third generation Cephalosporin has been used in the recent years [11]**.**

Generally, our study was detected of ESBLS, for inpatient and outpatient in the Sabha Medical Center Hospital. ESBLS positive rate is higher than that reported in Zawiya Teaching Hospital [11]. When compared the results

with the other hospitals in Arabic area like in Algerian hospitals, ESBLs are detected around 16.4-31.4% of the clinical samples, In Egypt, ESBLs were found in 11-42.9% of samples in both hospitals and among the community [13]. In Tunisia, ESBLs, pAmpC, and carbapenemases were present and the spreading ranges from 11.7 to 77.8% in city hospitals and were 0.7 and 7.3% in two communities [14]. Normally, the results are different by increase the number of hospitals under study. The results showed that the ESBL-producing isolates were highly inpatient isolates than outpatient, that the reversed results which reported by Abujnah A. A. and his colleagues [11]. Non statistical significant difference were between ESBLS- producing by both E. c*oli* or *Klebsiella spp*. and types of patients category (P>0.05).

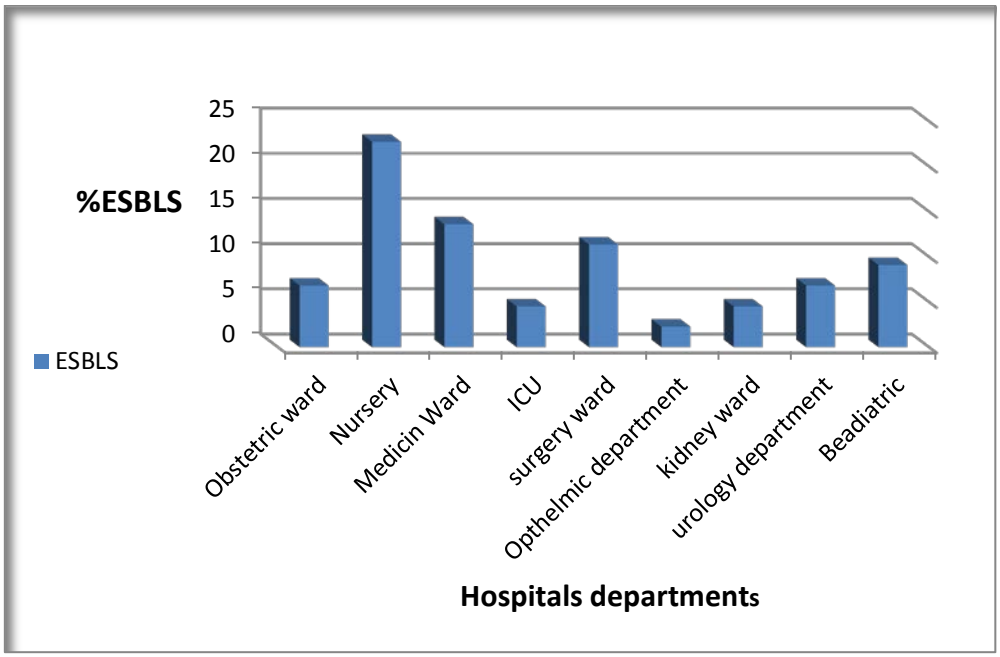

Figure 5: ESBL production percentage in Hospital departments types P<0.05.

The results showed that the ESBL-producing isolates were highly in the Nursery department than the other department, almost of them in urine samples. This is demonstrated the importance risk of outbreak around infant and risk die. The Nursery department is must be closed due to outbreak of ESBL-producing *Enterobacteriaceae* and dealing the contamination by wisely manner. The control measures were established with emphasis on hand hygiene, contact precautions, personalization of all medication and care products, and limiting access to the units. The doctor is must be try to avoid using the β-lactam antibiotics, while the treatment by β-lactam/β-lactamase inhibitor combinations, carbapenems and amikacin should first be considered. This is agreement with the other study was reported that 37 infants were affected by ESBLS outbreak on 28 February 2012, in a German neonatal intensive care unit (NICU) which closed until on February 2015[15]. However, we are communication with Sabha Hospital Center a demonstration about ESBLS cases results. Another hospital department showed highly ESBLS-producing was Medicine Ward 13.6% for both urine and stool samples then surgery ward, Pediatric Ward, Urology department from stool samples. Where Obstetric Ward and Kidney Ward were only from urine samples but ICU, Ophthalmic department from stool samples. Our ESBLS- producing

isolates distributed results in other hospital departments were lower than reported abroad [16**].** The statistical different significantly were between hospital department results (P<0.05).

Generally, the present proportion of ESBLS products in our samples is not surprising because the country has an excess of antibiotic used [11]. On the other side, the observed ESBLS-producing *Enterobacteriaceae* in hospital fecal samples is lower than that reported in the other study [17, 18, 19]. The faecal ESBLS-producing *Enterobacteriaceae* among inpatient was higher than outpatient. Non statistical significant difference were between samples ESBLS- producing types (stool or urine) and types of patients category (P>0.05). However the presence of ESBLS among faecal *E. coli* in Libya confirms its pervasiveness in the community and raises concern regarding this highly virulent and resistant clone. Finally, the results showed that the ESBL-producing isolates were highly for *E. coli* than *Klebsiella spp.* which in agreement with Hilty M. and his colleagues [20] and no with other reported [11].

6. Conclusion

Our study has provided a first ESBLS screening for *Enterobacteriaceae* in Sabha Medical Center**.** The findings of this study showed that *E. coli* and *Klebsiella spp.* were the predominant our strains and ESBL-producing isolates highly in *E. coli* strains. The present of faecal ESBLS-producing *Enterobacteriaceae* for inpatient is referring to the weakly antimicrobial policy implemented in the hospital and the aware risk of the spread it in the community. On the other hand, the laboratories should be aware of the implications of modified drug susceptibility testing reports on antibiotic prescription policies. Furthermore, ESBL *E. coli* infection is prominent in UTI for Nursery department and seems demonstrates the outbreak state in the hospital.

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