Prevalence of Extended Spectrum-β-Lactamases (ESBLs) in Faecal *Escherichia coli* among Healthcare Workers in Fayoum University Hospital

Thesis
Submitted for partial fulfillment
of the MD Degree in Medical Microbiology and Immunology
Presented by

Sylvana Nady Gaber
M.B.B.Ch, M.Sc.

Under supervision of

Dr. Ahmed Ashraf Wegdan
Professor of Medical Microbiology and Immunology
Faculty of Medicine
Fayoum University

Dr. Rasha Hamed Ahmed Bassyoni
Assistant Professor of Medical Microbiology and Immunology
Faculty of Medicine
Fayoum University

Fayoum University

2013

Summary of MD

Prevalence of Extended Spectrum-β-Lactamases (ESBLs) in Faecal *Escherichia coli* among Healthcare Workers in Fayoum University Hospital

Introduction:

ESBLs are plasmid-mediated enzymes that hydrolyze the 3rdGCs and the monobactams (aztreonam) but have no effect on the cephamycins (cefoxitin, cefotetan) and the carbapenems. They have been found to be inhibited by clavulanic acid, sulbactam and tazobactam. This is the property which helps their detection in the laboratory. The plasmid mediated ESBLs can be grouped into three main types: TEM, SHV or CTX-M. Another class of β-lactamases, the AmpC β-lactamases, confers resistance to 3rdGCs and cephamycins and they are not inhibited by clavulanic acid. The expression of the AmpC β-lactamases can be generated by chromosomal or plasmid genes. ESBL and AmpC producing pathogens have increased in hospitals and community settings. Currently, the antimicrobial therapeutic options for treating infections caused by ESBL-AmpC producers are limited. The fecal flora of healthy individuals in hospitals and in the community can represent a reservoir for ESBLs, pAmpC β-lactamases.

Aim of work:

To determine the prevalence of faecal carriage of ESBLs, Ampc producing *E. coli* among HCWs at Fayoum University Hospital by conventional microbiological methods and on molecular basis.

Subjects and Methods

-Stool samples were collected from 200 HCWs and cultured on MacConkey agar plates within 4 hrs of sampling, and incubated aerobically at 37°C for 24 hrs

-Identification of *Escherichia coli*:

-Colony appearance, Microscopic examination of Gram stained preparation, and Biochemical reactions

-Disc diffusion antibiotic susceptibility testing**:** *E. coli* isolates were tested for β-lactamases production using 9 antibiotics, which helped in screening for ESBL and AmpC β-lactamases done by a Kirby- Bauer disc diffusion method on MHA plates.

-Dilution antimicrobial susceptibility test: using (broth microdilution): The *CLSI (2012*) has proposed dilution methods for screening for ESBL production by *E. coli*.

-Phenotypic Screening for ESBLs, AmpC Enzymes –

I. The combined discs method

II. Double Synergy Differential Test (DSDT): (phenotypic confirmatory detection of the hidden AmpC β-lactamases or combined ESBL and AmpC β-lactamases)

-Multiplex PCR amplification was performed by group-specific primers to search for β-lactamase-encoding genes belonging to *bla*TEM, *bla*SHV, *bla*CTX-M group1 and *CIT* group.

Results:

-The prevalence of fecal carriage of ESBLs producing *E. coli* among HCWs was 21% (42/200).The prevalence of fecal carriage of pAmpC β-lactamases was 1% (2/200) and 2% (4/200) carry combined ESBL and AmpC producing *E. coli*.

-Fecal carriage of ESBLs producing *E. coli* among HCWs from different wards were as follow; 26.1% (12/46) of HCWs in internal medicine wards and ICUs, followed by 22.5% (9/40) of HCWs in operating theaters, 18.5% (5/27) in outpatient wards, 15.4% (6/39) of HCWs in surgery, 13.6% (3/22) in pediatric wards, 11.8% (2/17) in obstetrics, and 11% (1/9) in laboratory ward.

-Fecal carriage of plasmid AmpC producing *E. coli* was detected in operating theaters. While fecal carriage of combined ESBLs and AmpC β-lactamases producing *E. coli* detected in internal medicine wards and ICUs**.**

-All 200 isolates were susceptible to Imipenem. Also Amikacin have an excellent susceptibility to *E. coli*. Isolates exhibited higher resistance to Ceftazidime.

-Phenotypic screening for ESBL enzymes detection by disc diffusion test and MICs revealed; 29.5% showed resistance to one or more of 3rdGCs, among the 3rdGCs used in the screening, Ceftazdime had a better sensitivity (100%), while Cefotaxime lacked sensitivity (52.3%).

-Phenotypic AmpC screening test by Cefoxitin resistance; detects 8% isolates positive for AmpC β-lactamases with sensitivity 100%, and accuracy 83% when compared with PCR.

-Phenotypic conformation for ESBL by combined discs test detected 62.7% of 59 ESBL screening test positive isolates, with sensitivity 86%, high positive predictive value 100% and accuracy 90% when compared with PCR.

- DSDT for AmpC and combined ESBL, AmpC enzymes confirmation detected; 22.7% produce AmpC β-lactamases, 18.2% produce both ESBL and Ampc β-lactameses and 59.1% were negative isolates. Sensitivity of DSDT: 100% and accuracy 86% when compared with PCR.

-Molecular analysis revealed that; the prevalence of genes encoding ESBLs, AmpC among HCWs as follows: 19% had pure ESBL genes, among these isolates, 94.1% were *bla (SHV)*, 18.4% *bla (TEM)* and 5.3% *bla (CTX-M*). Only 7.9% had two types of ESBLs. 1% of isolates had pure AmpC genes (*CIT group)*, while 2% of isolates with combined AmpC and ESBL genes. It was found that: *bla (SHV)* are the dominant ESBLs among the *E. coli* resistant strains in HCWs.

Recommendation:

-We recommended detection of ESBLs and AmpC β-lactamases production by confirmatory DSDT (using BA and clavulanic acid as inhibitory for AmpC, ESBL enzymes respectively) which is simple and any microbiology laboratory can do it along with the routine antibiotic susceptibility testing (for screening ESBLs and AmpC β-lactamases production).

-A threatening epidemiological problem is the dissemination of ESBL-AmpC producing organisms to the patients in hospitals and healthy people in the community. This requires sound infection control measures including improving hygiene and regular detection of ESBL- AmpC producing isolates.