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Brief Original Article

Fecal carriage of extended-spectrum β-lactamase- and AmpC- producing *Escherichia coli* among healthcare workers

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Abstract

Introduction: Commensal *E. coli* can be considered a reservoir of genes coding for antibiotic resistance that may be transmitted in hospitals by healthcare workers (HCWs). This study aimed to determine the fecal carriage rate of extended-spectrum β-lactamase (ESBL)-producing *E. coli* among HCWs.

Methodology: Stool samples were collected from 200 HCWs. Phenotypic screening for ESBL and AmpC β-lactamases was performed using disk diffusion and minimum inhibitory concentration methods followed by the combined disks test and double synergy differential test for confirmation. Multiplex polymerase chain reaction (PCR) was used to detect bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and CIT groups for AmpC genes.

Results: Of 200 *E. coli* isolates, 100% were susceptible to imipenem, and 59 (29.5%) were resistant to one or more third-generation cephalosporins. By molecular analysis, 21% (42/200) were colonized by ESBL-producing *E. coli*, and 3% (6/200) were colonized by AmpC-producing *E. coli.* The bla_{SHV} gene was the predominant ESBL gene, detected in 81.8% of the resistant *E. coli* isolates.

Conclusions: These findings highlight the increase in fecal carriage of *E. coli* carrying ESBL and AmpC genes among HCWs, which may be one of the causes of the spread of ESBL-producing bacteria in hospitals and requires sound infection control measures. This is the first study of the fecal carriage rate of *E. coli* carrying AmpC genes in HCWs.

Key words: *E. coli*; ESBL; AmpC; HCWs.

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Introduction

E. coli-producing extended-spectrum β-lactamases (ESBLs) are considered one of the most multidrug resistant (MDR) pathogens in hospitals. Infections caused by ESBL-producing organisms have resulted in reduced rates of clinical and microbiological responses, longer hospital stays, and great hospital expenses. Gastrointestinal colonization is one of the most frequent reservoirs of infection [1].

The importance of detecting carriers of antimicrobial-resistant bacteria in patients and healthy people has recently increased. The increase in the proportion of carriers heightens the risk that other individuals will become carriers as a consequence of human-to-human transmission of resistant bacteria. Also, environmental enriching by the resistance gene pool may facilitate the acquisition of resistance mechanisms by susceptible bacteria [2].

Several studies have evaluated fecal carriage of ESBL-producing isolates from the stools of healthy individuals in community settings [3-5], but aside from one recent study [6], they did not specifically investigate ESBLs in healthcare workers (HCWs).

E. coli that produces both ESBLs and AmpC has been increasingly reported worldwide. The AmpC-producing organisms can act as a hidden reservoir for ESBLs; the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs [7].

The present study aimed to determine the prevalence of fecal carriage of ESBL-producing *E. coli* among HCWs by conventional microbiological methods and on a molecular basis, and also to determine the prevalence of fecal carriage of AmpC β-lactamases and combined ESBLs including AmpC β-lactamases.

Methodology

The present study was conducted at Fayoum University Hospital. Fecal samples were collected from 200 HCWs accepted to participate in the study who had not been recently hospitalized and had no recent exposure to antibiotics (≥ 3 months); 46 (23%) isolates were from internal medicine and intensive care units (ICUs), 40 (20%) were from operating theaters, 39 (19.5%) were from surgical wards, 27

(13.5%) were from outpatients clinics, 22 (11%) were from pediatric wards, 17 (8.5%) were from obstetrics, and 9 (4.5%) isolates were from laboratory staff. The study was approved by Fayoum University's ethics committee.

Bacterial isolates

Fresh stools samples were collected (between January 2013 and July 2013) and transported to the Department of Medical Microbiology and Immunology. The samples were cultured on MacConkey agar plates (Oxoid, Basingstoke, UK) and then incubated for 18–20 hours at 37°C. *E. coli* was isolated and identified according to conventional microbiological methods [8].

Phenotypic screening for ESBL- and AmpC β-lactamase-producing strains

E. coli isolates were tested for β-lactamase production using the Kirby-Bauer disk diffusion method. The antibiotics used in the study included a second-generation cephalosporin (cefoxitin, 30 µg); third-generation cephalosporins (ceftazidime, 30 µg and cefotaxime, 30 µg); a fourth-generation cephalosporin (cefepime, 30 µg); monobactam (aztreonam, 30 µg); β-lactam-β-lactmase inhibitor (amoxicillin-clavulanic acid, 20/10 µg); carbapenem (imipenem, 10 µg); a quinolone (ciprofloxacin, 5 mg); and an aminoglycoside (amikacin, 30 µg) (Oxoid). Amoxicillin-clavulanic acid was placed in the center and on either side of the disk, ceftazidime and cefotaxime were used for detection of any synergy, cefoxitin was used for AmpC β-lactamase screening, and imipenem was also used to detect any carbapenem-resistant isolates [9,10]. For quality control, *E. coli* ATCC 25922 was used.

Minimum inhibitory concentrations (MIC) of ceftazidime and cefotaxime at concentrations ranging from 0.025 to 500 µg/mL were determined by microdilution method. A MIC of the cephalosporins of ≥ 2 µg/mL is suggestive of ESBL production [11].

Isolates with one or more of the following criteria were considered to be potential ESBLs and were listed for confirmation of ESBL production by the combined disks method. The criteria included an inhibition zone to ceftazidime ≤ 22 mm, cefotaxime ≤ 27 mm, aztreonam ≤ 27 mm, extension of the zone of inhibition of any cephalosporins towards amoxicillin-clavulanic acid by the disk diffusion method, or MIC of ceftazidime or cefotaxime ≥ 2 µg/mL [10]. Isolates resistant to cefoxitin (inhibition zone < 18 mm), in addition to being resistant to one or more of the third-generation cephalosporins and being intermediate or resistant to amoxicillin-clavulanic acid were considered as putative AmpC β-lactamase producers [9,12].

Phenotypic confirmatory tests for ESBL production

The combined discs method was used; Cefotaxime (30 µg) and ceftazidime (30 µg) disks with or without clavulanate (10 µg) were used for confirmatory phenotypic detection of the ESBL-producing strains [11].

Isolates with negative results by the combined disks method were tested using the double synergy differential test (DSDT) for detection of AmpC β-lactamases or combined ESBL and AmpC β-lactamases [12].

Detection of ESBLs and plasmid AmpC genes

Automated DNA extraction was performed with MagNA Pure-DNA Isolation and Purification Kit I using a MagNA Pure LC 2.0 Instrument (Roche Lifescience, Penzberg, Germany). Multiplex polymerase chain reaction (PCR) amplification was performed as described previously by group-specific primers to bla_{TEM}, bla_{SHV}, bla_{CTX-M} group 1 and CIT group for the AmpC gene [13, 14] (Table 1).

Statistical analysis

All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science) version 15 for Microsoft Windows. Data were statistically described in terms of frequencies and percentages. P values less than 0.05 were considered statistically significant.

Results

Antibiotic susceptibility and phenotypic screening for ESBL and AmpC β-lactamase-producing strains

All 200 isolates were susceptible to imipenem. The rates of resistance (to β-lactam antibiotics) were as follows: ceftazidime (19.5%), cefotaxime (13.5%), cefepime (10%), aztronam (9%), amoxicillin-clavulanic acid (8%), and cefoxitin (8%). In non-β-lactamase antibiotics, the resistance rates were 2.5% to ciprofloxacin and 1.5% to amikacin. Screening tests for ESBL and AmpC production revealed that the prevalence of third-generation cephalosporin resistance among *E. coli* was 29.5% (59/200) (16 isolates screened positive for AmpC production). Of these, 7/59 isolates were MDR, 56/59 were resistant to ceftazidime, and 49/59 were resistant to cefotaxime. The MIC_{50} of cefotaxime and ceftazidime was 500

μg/mL, and the MIC_{90} of cefotaxime and ceftazidime was > 500 μg/mL.

Phenotypic confirmatory tests for ESBL production

Out of 59 isolates (with third-generation cephalosporin resistance), 37 (62.7%) were positive for ESBLs by the combined disks method. The remaining 22 (37.3%) negative isolates were examined by DSDT, which detected five isolates (22.7%) that produced AmpC β-lactamases and four isolates (18.2%) that produced combined ESBL and AmpC β-lactamases.

Detection of ESBL and plasmid AmpC genes

Among 59 isolates resistant to third-generation cephalosporins, 44 were positive with multiplex PCR to one or more of the tested genes. All 44 ESBL- and AmpC-producing *E. coli* isolates were susceptible to imipenem, and 100%, 52.3%, 43.1%, 41%, 29.5%, 27.3%, 13.6%, and 9.1% were resistant to ceftazidime, cefotaxime, amoxicillin-clavulanic acid, cefepime, cefoxitin, aztreonam, ciprofloxacin, and amikacin, respectively. The prevalence of genes encoding ESBLs and AmpC among HCWs is illustrated in Table 2, while the distribution of tested genes among the 44 resistant isolates is illustrated in Table 3.

Discussion

The evolution of antibiotic resistance is a continuous threat to human health worldwide [4]. *E. coli* may act as a reservoir of genes coding for antibiotic resistance and may also be responsible for a group of endogenous infections. Large unrecognized reservoirs of HCWs with ESBL- and AmpC-producing *E. coli* may lead to transmission of antimicrobial-resistant strains in hospitals from HCWs to patients, especially if the HCWs do not adhere to infection control measures at their hospitals [7].

In the current study, we determined the prevalence of fecal carriage of ESBLs, AmpC, and combined ESBL and AmpC β-lactamases among HCWs at Fayoum University Hospital and found that the prevalence of fecal carriage of ESBL-producing *E. coli* among HCWs was 21%. This result partially

Table 1. Group-specific primers used in the study

Group-specific primer names	Target	Sequence (5′–3′)	Amplicon size (bp)
Multiplex I TEM, SHV	TEM-like	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800
	SHV-like	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	713
Multiplex II CTX-M group 1	CTX-M group 1 including CTX-M-1, CTX-M-3 and CTX-M-15	TTAGGAARTGTGCCGCTGYA[b] CGATATCGTTGGTGGTRCCAT[b]	688
Multiplex III CIT group	LAT-1 to LAT-3, BIL-1, CMY-2 to CMY-7, CMY-12 to CMY-18, and CMY-21 to CMY-23	CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY[b]	538

Table 2. Prevalence of resistant genes encoding ESBLs and AmpC among HCWs

Test	No. of positive isolates	Percentage of positive isolates
Disk diffusion method	59/200	29.5%
PCR	44/200	22%
ESBLs (by PCR)	42/200	21%
AmpC (by PCR)	6/200	3%

Table 3. Distribution of tested genes among 44 resistant isolates

Gene	No. of positive isolates	Percentage of positive isolates
bla_{SHV}	36	81.8%
bla_{TEM}	7	15.9%
bla_{CTX-M}	2	4.5%
AmpC (CIT group)	6	13.6%
bla_{SHV} & CIT group	3	6.8%
bla_{SHV} & bla_{CTX-M}	2	4.5%
bla_{SHV} & bla_{TEM}	1	2.2%
bla_{TEM} & CIT group	1	2.2%

agrees with a previous study in Korea that reported 20.3% fecal carriage of ESBL-producing Enterobacteriaceae (not only *E. coli*) in healthy Korean individuals [5]. Much lower rates were obtained in different regions of the world: 12.3% in Saudi Arabia [15], 11% in Egypt [4], 7.3% in Tunisia [16], and 1.8% in Portugal [3]. All these studies investigated the prevalence of ESBL-producing Enterobacteriaceae in community settings rather than in HCWs, which may explain the lower rates. A recent study conducted in multiple rehabilitation centers examined colonization with ESBL-producing Enterobacteriaceae in healthcare personnel by rectal swabs and found that the prevalence was 3.5% among the isolated *E. coli* [6]. These results are still lower than ours. This may reflect non-adherence of HCWs to hand hygiene in our region. It could also be due to the fact that we collected stool samples from HCWs serving different hospital wards, not only rehabilitation wards. It should be noted that the percentage of resistant organisms isolated from inpatient wards is usually higher than that from outpatient areas [17].

In the current study, the prevalence of fecal carriage of AmpC genes among HCWs was 3%. To our knowledge, this is the first study investigating the prevalence of AmpC-producing *E. coli* isolated from stool samples among HCWs. These results are similar to those obtained from patients in other works; Naseer *et al*. reported a prevalence of 3.7% of AmpC among outpatients in Norway [18], and Li *et al*. reported a prevalence of 1.9% [19]. These findings are nearly similar to those reported in the Czech Republic and Denmark (rates were1.1% and 2.4%, respectively) [20, 21].

In our study, the distribution of genes encoding ESBLs among 44 isolates was: 36 (81.8%) isolates with bla_{SHV}, 7 (15.9%) with bla_{TEM}, and 2 (4.5%) with $bla_{CTX\text{-}M}$. It appears that bla_{SHV} is the predominant ESBL gene among the *E. coli*-resistant strains in HCWs. This is in agreement with another Egyptian study, which found that the main type of ESBL gene isolated from patients in the ICUs at Assiut University Hospitals was bla_{SHV} [22].

Our results disagree with many studies all over the world that reported $bla_{CTX\text{-}M}$ was the predominant gene among ESBL-producing *E. coli* isolated from fecal samples of healthy subjects [4, 23, 24], which may be explained by the variability in resistance patterns and encoding genes according to different geographic regions.

Conclusions

In conclusion, an alarming high rate of fecal carriage of ESBL-producing *E. coli* among HCWs has been detected, with bla_{SHV} being the most predominant gene. These results emphasize the need for continuous surveillance in hospitals for both patients and HCWs to detect resistant strains by implementing strict guidelines for antibiotic therapy and infection control measures to reduce the increasing burden of antibiotic resistance and to prevent its spread.

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