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Faecal carriage of extended-spectrum β-lactamase (ESBL)-producing enterobacteria in liver disease patients from two hospitals in Egypt and France: a comparative epidemiological study

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SUMMARY

This study aimed to assess and compare the epidemiology of faecal carriage of extended spectrum β-lactamase-producing enterobacteria (ESBL-E) in Hepatology departments of two hospitals specializing in liver diseases, Theodor Bilharz Research Institute (TBRI) in Cairo (Egypt) and Beaujon Hospital (Bj) in Clichy (France). CTX-M groups were identified by PCR, and TEM and SHV derivatives with the check-point system. Phylogenetic groups of *E. coli* were determined by multiplex PCR, and clone ST131 by PCR of gene *pabB*. Prevalence of ESBL-E was 77·6% (45/58) in TBRI and 6·5% (13/199) in Bj ($P<10^{-7}$). Previous hospitalization was more common ($P=0·003$) in Bj patients (93%) than in TBRI patients (45%) suggesting high prevalence of ESBL-E in the Egyptian community. The presence of *E. coli* B2 ST131 among ESBL-E faecal *E. coli* in Egypt confirms its pervasiveness in the community and raises concern regarding this highly virulent and resistant clone.

Key words: Antibiotic resistance, *Escherichia coli* (*E. coli*), hepatitis C, typing.

INTRODUCTION

Since the 1990s, nosocomial outbreaks due to extended spectrum β-lactamase-producing enterobacteria (ESBL-E) have been increasingly reported worldwide. Knowing that intestinal carriage is the main reservoir of these organisms, it has been suggested that gut colonization is associated with a high risk for developing self- and cross-infections with ESBL-E producers [1, 2]. Although carriers of ESBL-E producers are expected to be present in general practice, there are few studies conducted in the community or in hospital settings during non-outbreak situations [3]. In developing countries, prevalence studies on the faecal carriage of ESBL-E are scarce, whereas the burden of linked infections is increasing [4, 5]. Bacterial infections are much more frequent in cirrhotic patients than in the general population [6], with episodes of infection reaching up to 40% of hospitalized patients [7]. In a previous study [8] on ESBL-E isolates causing infection in liver disease patients the proportion of *E. coli* ESBL producers did not differ significantly between hospitalized and outpatients (20% *vs.* 17%) which showed that ESBLs were equally pervasive in both hospital and community settings and responsible for the possible high prevalence of ESBL faecal

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carriers in the community [8]. This study aimed to assess and compare the epidemiology and molecular types of ESBL-E in digestive carriage of patients hospitalized for more than 24 h in the Hepatology department of two hospitals specializing in liver disease in Egypt and France, i.e. Theodor Bilharz Research Institute (TBRI), Cairo, Egypt and Beaujon Hospital (Bj), Clichy, France.

METHODS

Rectal swabs were collected in parallel over a period of 30 and 40 days from all patients admitted to the Hepatology departments of two hospitals: TBRI (Cairo, Egypt) and Beaujon hospital (Clichy, France), respectively. Samples were obtained in the first 48 h of hospitalization.

Microbiological detection of ESBL-E

TBRI

At TBRI rectal swabs were cultured on Drigalski agar containing 0·5 mg/l cefotaxime. Growing colonies were tested by the double disc synergy test on Muller–Hinton agar. Briefly, amoxicillin-clavulanic acid (AMC) disc was placed in the centre and discs of ceftazidime (CAZ), cefotaxime (CTX) and cefipime (FEP) were placed 1·5 cm and 2·5 cm, respectively, distal from the AMC disc. All samples showing positive synergy were identified by API 20E (bioMérieux, France).

Beaujon Hospital

At Bj rectal swabs were cultured on chrom ID^{IM} ESBL agar (bioMérieux). In case of growth of pink colonies, an indole-positive test confirmed the diagnosis of *E. coli*. For non-pink colonies or pink colonies with negative indole test, bacterial identification was performed using API 20E (bioMérieux). ESBL detection was tested by double disc diffusion test performed on Muller–Hinton agar using two discs of FEP placed at 1·5 cm and 2·5 cm, respectively, distal from the AMC disc. The appearance of synergy between AMC and FEP confirmed ESBL.

Antibiotic susceptibility testing was applied to all identified ESBL isolates according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [9]. ESBL-producing strains were conserved on conservation agar medium (Bio-Rad, France) for molecular study.

Characterization of ESBLs

CTX–M enzyme groups were characterized as described previously [10], TEM and SHV derivatives were identified using the check-point system/KPC® (Check-points, The Netherlands, info@check-points.com).

Molecular analysis of *E. coli* strains

Phylogenetic groups of *E. coli* were determined as described previously [11]. Molecular typing of strains was performed by using ERIC-2 PCR [12].

Representatives of previously published *E. coli* clones were used as controls in the ERIC-2 PCR analysis: for group A (ST709, ST10, ST744, ST167, ST606); for group B2 isolates (ST95, ST131, ST141) and for group D isolates (ST117, ST393, ST69-CGA). In order to confirm that *E. coli* group B2 belonged to clone ST131 a PCR specific for clone ST131 (*pab B* gene) was performed on group B2 *E. coli* isolates as described previously [13].

Epidemiological data

Demographic and clinical data were collected on the designed sheet from all patients in the study including: age, sex, date of admission and of sample collection, previous hospitalization, underlying disease, ascites and comorbidity.

Statistical analysis

Statistical analysis was performed by analytical epidemiological software Epi Info v. 6 (CDC, USA). Statistical data were analysed using χ^2 test and Fisher's exact test.

RESULTS

Prevalence of digestive carriage of ESBL-E in TBRI and Bj patients

In TBRI, the period of study, initially designed for 2 months, was shortened as the prevalence of ESBL-E carriers was significantly higher than in Bj. During the study period, 58 patients were screened and 45 (77·6%) were ESBL carriers, four of whom carried two different strains. Therefore for the study performed in TBRI, 49 strains were isolated from 45 patients. In Bj, 199 patients were screened and 13 patients (6·5%) were ESBL-E carriers. Of these patients, one was a carrier of two different ESBL-E strains. These 14

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Table 1. *Prevalence of patients with faecal ESBL-E at admission*

Hospital	No. of patients		Prevalence (%)	ESBL-producing isolate (*n*)
	Screened	With ESBL-E		
TBRI	58	45**	77·6	49
Beaujon	199	13	6·5	14

ESBL-E, Extended spectrum β-lactamase-producing enterobacteria.
** $P < 10^{-7}$.

strains isolated from 13 patients were retained for the study. The difference in prevalence of digestive carriage observed in the two study populations was statistically significant ($P < 10^{-7}$) (Table 1).

Species and ESBL enzyme distribution

Bacterial species distribution was not statistically different between the two hospitals ($P = 0{\cdot}06$), CTX-M was the most frequent ESBL identified in ESBL-producing *E. coli* and *Klebsiella* spp. isolates detected in the gut of the studied patients. PCR and the checkpoint system showed that the CTX-M-1 group was the most prevalent enzyme produced by ESBL-E in both TBRI and Bj (59% and 79%, respectively). No significant difference was found in the relative distribution of CTX-M-1 and SHV groups between the two populations studied, but CTX-M-9 group enzymes were significantly more common in TBRI than in Bj ($P = 0{\cdot}027$) whereas TEM-derived ESBLs were significantly more common in Bj than in TRBI ($P = 0{\cdot}047$) (Table 2).

Table 2. *Species and molecular type distribution of ESBLs in TBRI and Beaujon hospitals*

Bacterial species	TBRI isolates, *N* (%)	Beaujon isolates, *N* (%)
E. coli	44 (90)	10 (72)
K. pneumoniae	4 (8)	2 (14)
K. oxytoca	—	1 (7)
E. cloacae	1 (2)	1 (7)
ESBL		
CTX-M-1 group	29 (59)	11 (79)
CTX-M-9 group	14 (29)*	—
TEM-derivative	—	2 (14)*
SHV-derivative	6 (12)	1 (7)

ESBL, Extended spectrum β-lactamase.
* $P \leqslant 0.05$.

Antibiotic susceptibility of ESBL-producing *E. coli* in TBRI and Bj

ESBL-producing *E. coli* isolates had high resistance rates to tested antibiotics. The resistance rates for amikacin were the lowest in both hospitals (18% and 10%). Statistical analysis of the antibiotic susceptibility showed no significant difference regarding the global sensitivity of *E. coli* isolates to antibiotics between the two hospitals (Table 3).

The sensitivity of the isolates in TBRI to augmentin (AMC), cotrimoxazole (SXT), nalidixic acid (NAL), gentamicin (GEN) and amikacin (AMI) was not significantly affected by the molecular type of the ESBL. However, a significant difference was observed for ciprofloxacin (CIP) ($P = 0{\cdot}005$) between *E. coli* isolates producing CTX-M-1 group and SHV enzymes in comparison to *E. coli* isolates producing CTX-M-9 group enzymes (Table 4). No statistical significance was observed in ESBL-E in Bj in relation to the type of ESBL produced.

Molecular analysis of ESBL-producing *E. coli* isolates

Molecular analysis of all isolates was performed in the microbiology laboratory of Bj. The genetic background of 44 *E. coli* isolates from TBRI showed that 59% (26/44) belonged to phylogenetic group A, 27% (12/44) to group D, 7% (3/44) to group B1 and 7% (3/44) to group B2. Among the 10 *E. coli* isolated in Bj, 40% belonged to group A, 40% to group B1 and 20% (2/10) to group D.

Globally, there was a significant difference between the phylogenetic group distribution of the *E. coli* isolates from TBRI and Bj ($P < 0{\cdot}05$). Moreover, group B1 *E. coli* isolated at Bj (40%) were significantly higher than those isolated at TBRI (7%) ($P = 0{\cdot}017$). Molecular typing by ERIC PCR of *E. coli* isolates from Egypt showed that for 26 group A isolates, two had a profile identical to that of ST709, four had an identical profile (Pf1) to each other and to one isolate from France, two had another identical profile (Pf2), four had an identical profile to ST167

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Table 3. *Antibiotic susceptibility of ESBL-producing* E. coli *in TBRI and Beaujon hospitals*

	Number (%) of *E. coli* isolates resistant to				
Hospital (no. of isolates)	SXT	NAL	CIP	GEN	AMI
TBRI ($n=44$)	42 (95)	33 (75)	23 (52)	24 (55)	8 (18)
Beaujon ($n=10$)	8 (80)	7 (70)	6 (60)	2 (20)	1 (10)

ESBL, Extended spectrum β-lactamase.

Table 4. *Antibiotic resistance of ESBL-producing enterobacteria in relation to ESBL type in TBRI*

	Number (%) of resistant isolates					
ESBL	AMC	SXT	NAL	CIP	GEN	AMI
CTX-M-1 group ($n=29$)	19 (66)	26 (90)	25 (86)	19 (66)*	17 (59)	6 (21)
CTX-M-9 group ($n=14$)	10 (71)	14 (100)	8 (57)	2 (14)	6 (43)	1 (7)
SHV ($n=6$)	3 (50)	5 (83)	4 (67)	4 (67)*	2 (33)	2 (33)
P value	0·65	0·37	0·1	0·005*	0·41	0·34

ESBL, Extended spectrum β-lactamase.
* $P \leqslant 0.05$.

and one had an identical profile to ST10; while 13 isolates each had a unique profile (Fig. 1). Therefore, 50% of the *E. coli* isolates of group A had clonal relationship to seven representatives of the clonal complex ST10 including ST167, ST709 and ST10. Three of the four *E. coli* isolates from France belonging to group A showed a unique profile. The two French strains of group D had a unique profile, while in the 12 Egyptian isolates of this group, three had the same profile PFA, two had the same profile PFB, and two had the same profile PFC. The five remaining strains each had a unique profile. In total 58% of the Egyptian strains of group D were clonally related. For the isolates of group B1, unique profiles were observed for three of the four French isolates, and for all of the Egyptian isolates. All three Egyptian isolates belonging to group B2 had a profile identical to the representative of ST131. This was confirmed by the amplification of *pabB* recovered from those three isolates.

Demographic features of patients colonized by ESBL-E in the two hospitals

No significant difference was found between the two study populations concerning the mean age ($P=0·21$) or in the distribution of sexes ($P=0·75$). Previous history of hospitalization was more common in patients harbouring ESBL-E in Bj (92%) compared to those from TBRI (40%) ($P=0·007$). Regarding the associated pathology in patients colonized by ESBL-E, the two studied populations showed significant difference ($P<0·05$). Chronic HCV infection was significantly higher in TBRI patients, found in 35/45 patients (78%) compared to 4/13 patients (4%) in Bj. Notably 57% of patients affected by chronic HCV were previously treated by parenteral antischistosomal therapy during the campaign of eradication of this endemic parasitic disease in Egypt ($n=20$), this phenomenon was not observed in France ($P=0·002$). No cases of alcoholic hepatitis were found in TBRI; however, chronic alcoholism represent an important part (31%, $n=4$) of the aetiology of chronic hepatitis in France ($P=0·002$). No significant difference was seen in the distribution of cirrhotic and non-cirrhotic patients between Bj and TBRI. Statistical analysis of gravity score (model for end-stage liver disease; MELD) and different biological parameters (prothrombin, creatinine, international normalized ratio, aspartate transaminase, alanine transaminase, total bilirubin) did not show significant difference between the two populations (Table 5).

Clonal relationship of strains and previous hospitalizations

The majority of patients harbouring clonal strains in TRBI had no history of hospitalization. This is the

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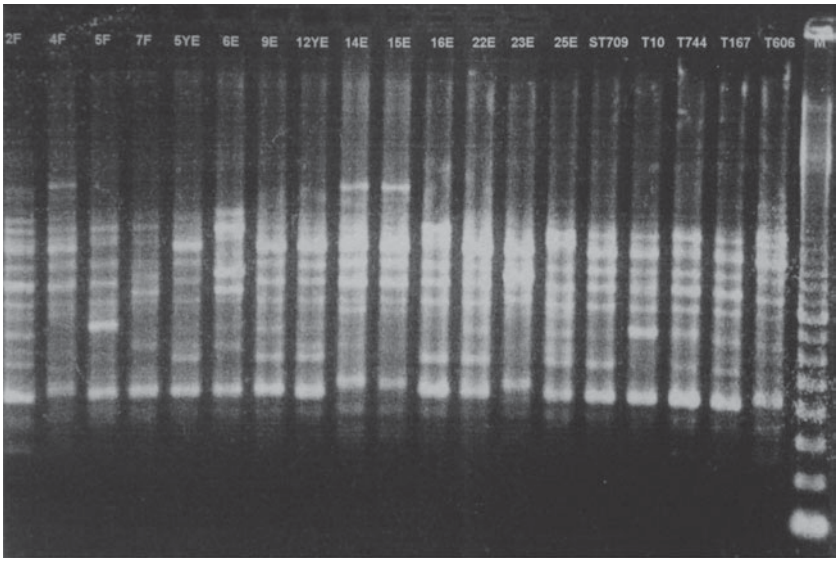

Fig. 1. Molecular typing by ERIC-2 PCR of *E. coli* isolates of phylogenetic group A.

case of the four patients harbouring strains of group A which had an identical profile (PF1), of the two patients harbouring the three strains of group D that had an identical profile (PFA), and the two patients harbouring strains of group D having the same profile (PFB). Two of the three Egyptian patients harbouring strains of group B2 related to clone ST131 had a previous history of hospitalization, one in 2002 and the other one in 1995.

DISCUSSION

This comparative epidemiological study demonstrated a very high prevalence (77·6%) of faecal colonization with ESBL-E in liver disease patients at the time of their admission to the TBRI Hepatology unit. As patients presenting to the two hospitals in our study are liver disease patients so high ESBL faecal carriage rate could be expected as liver disease has been described previously among the risk factors for colonization with ESBL [14]. Moreover the treatment for complications of liver cirrhosis such as hepatic encephalopathy, spontaneous bacterial peritonitis and variceal bleeding with antibiotics is believed to impact directly or indirectly on the composition of gut microbiota which may enhance faecal carriage of ESBL [15]. Prevalence in TBRI was 10 times higher than that found in patients presenting in the same period to Bj (6·5%). Gender difference can be a further contributing factor to high ESBL carriage rate. It is of note that 27 (60%) TBRI patients were female. Female sex has been described as one of the multivariate risk factors for rectal carriage of ESBL-producing enterobacteria at hospital admission [16]. Our results are also consistent with the findings of a study performed in Sweden that investigated the occurrence of ESBL-E in patients with traveller's diarrhoea. They reported that out of the patients who had travelled in Europe, 3% (2/63) were found to be ESBL-E carriers in comparison to 36% (50/138) of those who had travelled outside Europe. ESBL-producing *E. coli* was especially common in patients returning from India (11/14, 79%), Egypt (19/38, 50%) and Thailand (8/38, 22%) [17]. Similarly Wickramasinghe *et al.* found a statistically significant difference between ESBL-producing faecal *E. coli* carriers from Europe (8·1%) and the Middle East/South Asia (22·8%) with a higher rate of CTX-M-15-producing *E. coli* carriage in the latter group [18].

Two studies from Egypt have also reported a high rate of intestinal colonization with ESBL-E in healthy carriers (63·3% and 22·6%) [19, 20]. The prevalence rates reported from Egypt are comparable with the prevalence rate of 65·7% of CTX-M-type ESBL-E found in asymptomatic rural Thai people [21], but higher than that recorded in other countries in the Middle East region: 16% in Lebanon [22], 26·1% of patients hospitalized with diarrhoea in Saudia Arabia [23] and 12·7% of healthy people studied in Saudi Arabia [3].

A factor contributing to this high prevalence may be the high population density in Cairo (38 221 inhabitants/km^2) which may facilitate transmission of ESBL-E in the general population. Easy access to

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Table 5. *Comparison of demographic and biological characteristics of patients colonized by ESBL-E in TBRI and Beaujon*

	Number of patients (%)	
Character	Beaujon ($n=13$)	TBRI ($n=45$)
Sex		
Male	9 (69)	18 (40)
Female	4 (31)	27 (60)
Average age, years (range)	51 (33–67)	53 (19–75)
Previous hospitalization		
<3 months	9 (69)	5 (11)
3 months to 3 years	3 (23)	4 (9)
>3 years	0	9 (20)
None	1 (8)	22 (49)*
Not available	0	5 (11)
Main pathology		
Chronic hepatitis	9	35
HCV	4 (31)	15 (34)*
HCV + bilharziasis	0	20 (44)*
HBV	1 (8)	0
Alcoholic	3 (23)*	0
Mixed (alcoholic + HCV)	1 (8)	0
Other diseases	4 (31)	10 (22)
Comorbidity	6 (46)	9 (20)
Cirrhosis/ascites	6 (46)	29 (64)
Mean MELD (range)	17 (7–25)	16 (5–39)
Mean PT (range)	73 (39–110)	62 (14–100)
Mean AST (range)	55 (14–108)	68 (12–294)
Mean ALT (range)	51 (16–123)	56 (22–207)
Mean total bilirubin (range)	64 (6–181)	81 (9–554)
Mean creatinine (range)	110 (59–265)	103 (44–256)

MELD, Model for end-stage liver disease; PT, prothrombin time; AST, aspartate transaminase; ALT, alanine transaminase.
ESBL-E, Extended spectrum β-lactamase-producing enterobacteria.
* $P \leqslant 0.05$.

antibiotics in Egypt and their availability without medical prescription may also contribute to this high rate of faecal colonization. [8, 20, 24]. The colonized non-hospitalized patients may be considered as community reservoirs that play a role in the epidemiology of ESBL-E infections [25].

Although the prevalence (6·6%) of patients harbouring ESBL-E in Bj seems negligible in comparison to that of TBRI, and less than that observed by Valverde *et al.* (11·8%) in patients admitted in their hospital in Spain in 2003 [26], it is higher than the rate observed in the general ICU of Bj during their admission (2·6%) at the same time of the study but it is very close to that observed in patients admitted to the ICU of Hepato-gastro-enterology (7·5%) (M.-H. Nicolas Chanoine, personal communication). This is not surprising as the latter ICU receives critical patients with liver transplantation that are followed from the Hepatology department in Bj. This is a serious issue as a 10-year study conducted in Bj reported that pre-transplant carriage of ESBL-E was an independent risk factor for infection after liver transplantation. Interestingly, a significant association between faecal colonization status and occurrence of subsequent infection has been suggested. In post-transplant patients, ESBL-E infection developed in ≈45% of carriers and molecular typing showed that the infecting isolate was identical to the isolate from the pre-transplant faecal swab for most of these patients [25].

CTX-M enzymes are the most prevalent ESBL types worldwide in both hospital and community

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settings [24, 25, 27, 28], so it was expected to be predominant in both study groups (88% in TBRI and 79% in Bj). Group-specific PCR typing showed that the majority (79%) of ESBL-positive isolates from Bj belonged to group CTX-M-1. It has been reported that CTX-M-15, which belongs to CTX-M-1 cluster, is the most widely distributed type in Western Europe [26, 27, 29]. Similarly studies from Egypt reported that CTX-M-15 was also the predominant ESBL type [8, 20, 30–33]. In this study we found that the CTX-M-1 group represented 59% of ESBL-positive isolates from TBRI and 29% belonged to CTX-M-9. CTX-M-14, a member of the CTX-M-9 group, has been reported in Egypt in *E. coli* and *K. pneumoniae* and *Enterobacter cloacae* isolates [31, 33]. However DNA sequencing was not performed on our isolates to confirm the presence of this enzyme type.

The *E. coli* species have been classified into four main phylogenetic groups (A, B1, D, B2), with the commensal strains belonging mainly to A and B1 phylogenetic groups whereas extraintestinal pathogenic strains are essentially from the B2 and D groups [34]. Phylogenetic analysis of ESBL-producing *E. coli* isolates from both hospitals in our study showed that group A was the major group detected in TBRI (59%), whereas group A (40%) and group B1 (40%) represented the majority in Bj. However, groups D and B2 were also detected in faecal carriers in our study (B2: 7% in TBRI) and (D: 27% in TBRI; 20% in Bj) as well as in other studies [21, 35]. ERIC PCR analysis showed that four *E. coli* strains belonging to group A from Bj had a unique profile and were not related to the profile of the known sequence type, whereas 50% of the Egyptian *E. coli* strains of same group A had clonal relatedness to seven strains of the clonal complex ST10 which includes ST10, ST167 and ST709 [36]. This was similar to what was previously found by Fam *et al.*; i.e. 40% of group A *E. coli* clinical isolates belonged to clonal complex ST10 [8]. One strain from Bj (2F), and four other strains from TBRI had a profile identical to clone ST167. In addition two isolates (39E and 40E) from TBRI had a similar profile to a French isolate (PF2). These data may suggest clonal propagation of sequence type complexes worldwide. The *E. coli* O25b-ST131 clone is a highly resistant and virulent clone that has been reported worldwide and represents a major public health concern [30, 37, 38]. The rapid intercontinental spread of this clone was mainly in the form of isolates producing CTX-M-15, but has also been found in isolates free of CTX-M enzymes in 10% of healthy subjects in Paris [12, 18, 36, 39]. In the present study, PCR amplification of the *pabB* gene that is specifically found in isolates belonging to the *E. coli* O25b-ST131 clone [13] confirmed the presence of this clone in three ESBL-producing *E. coli* faecal carriers from Egypt. The three isolates carried three different enzyme groups, i.e. CTX-M-1, CTX-M-9 and SHV. This clone had been detected in our previous study where seven CTX-M-15-producing *E. coli* isolates were found causing urinary tract infections in both community and hospital settings [8]. The presence of this clone with ESBL enzymes in potentially pathogenic *E. coli* (group B2) strains colonizing the lower gastrointestinal tract could represent a potential source for infection with drug-resistant bacteria. So the possibility of infections due to of extended-spectrum cephalosporin-resistant isolates must be taken into consideration in the empirical treatment given to those patients particularly in areas with known high prevalence rates of ESBL in the community. Concerning the adjacent pathology of patients colonized by ESBL-E, the two studied populations showed a significant difference ($P = 0{\cdot}0003$). The proportion of patients affected by chronic HCV was significantly higher in TBRI (78%) than in Bj (30%), whereas chronic alcoholism represented an important part (31%) of the aetiology of chronic hepatitis in France. That was expected as Egypt has the largest epidemic of HCV in the world [40]. The recently released Egyptian Demographic Health Survey (EDHS) that tested a representative sample of the entire country for HCV antibody reported that percentage of people positive for antibody to HCV was 14·7%, of which 10% were positive for HCV RNA [41]. The samples included both urban and rural populations, including all 27 governorates of Egypt and over 11 000 individuals were tested.

As 44% of TBRI patients had associated HCV and history of previous antischistosomal therapy, further studies are needed to assess possible correlation between these factors and the change in faecal flora.

It is noteworthy to mention that some limitations in our study exist, such as the possible effect of the shortened enrolment period of patients to 1 month in TBRI (initially designed for 2 months). However this change in the plan of study duration is not likely to affect the conclusion, as the prevalence of ESBL-E carriers was significantly higher than in Bj. Moreover, the study only covered two hepatology centres, so further multicentre studies are required to apply in other settings.

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CONCLUSION

The prevalence of faecal colonization with ESBL-E in non-hospitalized liver disease patients in TBRI is one of the highest reported worldwide. This raises concern that empirical therapy of liver disease patients presenting with serious Gram-negative infections as spontaneous bacterial peritonitis may need to be modified. The presence of faecal carriage of the *E. coli* B2 ST131 clone in ESBL-producing *E. coli* confirms its pervasiveness in the community

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DECLARATION OF INTEREST

None.

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