Fecal carriage of *Escherichia coli* and *Klebsiella spp.* as major reservoirs of clinically important resistance markers

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ABSTRACT

Intestinal normal flora can become reservoirs of antibiotic resistance genes present among the strains responsible for nosocomial infections. It is suggested that gram negative intestinal bacterial flora have increased capacities to obtain antibiotic resistance genes and therefore can act as main reservoirs for transfer of resistance genes to other pathogenic bacteria. This study aimed to compare fecal carriage of clinically important resistance markers for more frequent members of enterobacteriacae between nondiarrheal and community associated diarrheal patients (control group) versus their counterparts from the patients with nosocomial infections (case group). 261 stool and 190 clinical samples were collected from outpatient and hospitalized patients from 6 hospitals in Tehran, Iran. The samples were cultured on MacConkey agar plates and colonies were identified by standard biochemical methods. Antibiotic sensitivity testing of the isolates against 13 antibiotics was performed according to the CLSI guideline using the disk diffusion method. Among stool and clinical samples, more frequent identified enterobacteriaceae bacteria were included *E. coli* (58.99/ 3.15%), *Klebsiella spp*. (22.61/7.36%), and other members of enterobacteriaceae (8.86/1.06%), respectively. Overall, resistance against four of the main antibiotics (3^{th} and 4^{th} generation cephalosporins, gentamicin, imipenem, and ciprofloxacin) was significantly higher among the case group (50-75% versus 10-14%). Analysis of these results showed similar dissemination of resistance phenotypes among the isolates from the control group in ranges of 1.5-7.6% and 4.4% for *E. coli* and *Klebsiella* spp., respectively. Our results suggested that the fecal carriage of resistant phenotypes related to the β-lactam antibiotics in *E. coli* and *Klebsiella* spp. in compare to the clinical isolates is rapidly increasing. This may be caused by dissemination of β-lactamase producing *E. coli* in the community from the hospitals. There were no significant correlations between the two groups of the samples, as the clinical samples had shown 3 to 7 folds excess resistance phenotypes. Surveillance studies of the resistance patterns among the samples from different regions will provide awareness about dissemination of these bacteria within the community as reservoirs of main resistance markers.

Key words: Fecal carriage; *E. coli*; *Klebsiella* spp; antibiotic resistance.

INTRODUCTION

The most complex ecosystems known in microbial ecology is the human gastrointestinal tract, with over 10^{11} bacteria per gram of stool [1]. The intestinal normal flora have several significant presence in the digestion of food, metabolism of endogenous and exogenous compounds, immunopotentiation and prevention of colonization by pathogens in the gastrointestinal tract and hence is involved in society of human health. The intestinal normal flora can become reservoirs of antibiotic resistance genes [2]. Bacterial resistance to antibiotics is a growing problem that results in prolonged hospital stays and increased morbidity, mortality, and treatment cost. Inappropriate antimicrobial use is the main factor in development of resistance. In society with high antibiotic use, or use of antibiotics without prescription, the risk for carriage of resistant bacteria often increases. Selective pressure of the antibiotics will amplify numbers of resistant bacteria in the community. It is suggested that gram negative fecal bacterial flora have an increased capacity to obtain antibiotic resistance genes and might act as reservoirs for transfer of resistance genes to other pathogenic bacteria in the communities or

hospitals [3]. Resistance-encoding genetic materials are often "clonal" (identical across a wide range of bacteria) and can readily move between bacteria of the same species and different species or genera. *Escherichia coli* and *klebsiella pneumonia* are members of dominant fecal microbiota of the humans. *E. coli* is a major enteric pathogen, particularly in developing countries and is the premier nosocomial pathogen. *Klebsiella* spp. are rarely associated with intestinal diseases, but recognized clinically as responsible agents for pneumonia, urinary tract infections, sepsis, and infections of surgical wounds. During the past decade, resistance of these bacteria to cephalosporin antibiotics has accelerated because of the appearance of plasmid-mediated horizontally transferrable resistance gene markers in these bacteria.

Although carriers of resistant organisms in the community are suspected, in general practice this condition has rarely been reported. Previous history of hospitalization and antibiotic usage can help persistence and dissemination of the resistance bacteria in the community and also their transmission into the hospitals. It appeared that resistance against some antibiotics, including third-generation cephalosporins, gentamicine, vancomycin, imipenem, and intravenous fluoroquinolones have a higher risk for management of diseases [4, 5].

This study aimed to compare fecal carriage of clinically important resistance markers for more frequent members of enterobacteriacae between nondiarrheal and community associated diarrheal patients (control group) versus their counterparts from the patients with nosocomial infections (case group).

MATERIALS AND METHODS

Sample preparation and identification: Total of 261 stool samples from outpatients with or without diarrhea (control group) between the periods of 1 June - 30 October 2010 and 190 clinical samples from patients with nosocomial infections from ICUs of 6 hospitals during the period from September 2010 – August 2011, were collected during the study. Records of the antibiotic usage, and demographic characteristics (age, sex), for the control group and hospitalization within the last three months, antibiotic prescription, and types of diseases in the case group were collected according to the standard criteria for nosocomial infections. Each homogenized stool sample was cultured on MacConkey agar. The microorganisms that grew were identified by standard biochemical methods. *Klebsiella pneumonia* strain ATCC 13883and *E. coli* strain ATCC 25922 were used as control bacteria in all the assays.

Antibiotic susceptibility testing: Bacterial isolates related to species of *Klebsiella* and *E. coli* were tested for susceptibility to cephalothin (CF, 30µg), tetracycline (TE, 30µg), ampicillin (AM, 10µg), ceftazidime (CAZ, 30µg), gentamicin (GM, 10µg), amoxicillin–clavulanic acid (AMC, 30µg), trimethoprim sulfamethoxazole (SXT, 300µg), chloramphenicol (C , 30µg), ciprofloxacin (CP, 5µg), cefepime (FEP , 30µg) , carbenicillin (CB , 30µg), oxacillin (OX , 10µg), imipenem (IMP , 10µg), and EDTA (0.5 M) by Kirby–Bauer disk diffusion method and results were recorded according to the guidelines recommended by the CLSI [8]. Interpretation of results for resistance patterns related to the ESBLs and carbapenemase producing bacteria was done according to the recommendations by Bush et al. [12].

Statistical analysis: All data were analyzed by SPSS software version 11.0 (SPSS Inc, Chicago, IL, USA). Comparative statistics were calculated using the two-tailed $\chi 2$ test and Fisher's exact test, when appropriate. A p-value of ≤ 0.05 was considered to be significant.

Approval by ethics committee: The Ethics Committee of Research Center for Gastroenterology and Liver diseases, Shahid Beheshti University of Medical Sciences, approved the study.

RESULTS

Total of 266 isolates of *E. coli*, 102 isolates of *Klebsiella* spp. and 40 isolates of other members of enterobacteriaceae family were identified from 451 samples from 6 hospitals in Tehran, Iran. Proportion of the isolation rate for the stool (diarrheal and non-diarrheal) and clinical samples were 99.58% and 100% in the case of *E. coli* and 36.6% and 9.5% in the case of *Klebsiella* spp., respectively (Table 1). Among the studied stool samples (92.3% diarrheal and 7.7% non-diarrheal), most of these isolates were related to the diarrheal samples. Respiratory samples had the highest isolation rate of *E. coli* and *Klebsiella* spp. among the clinical samples. Figure 1 summarizes the

antibacterial susceptibilities of all the isolates against the tested antibiotics. Most of the isolates from the clinical samples showed multi-drug resistance phenotypes (MDR), especially against more important antibiotics of cefepim, ceftazidime, imipenem and gentamicin. Comparison of the results for the isolates from the control group also showed presence of these resistance phenotypes in lower frequencies (1.5% to 7.5%). No significant differences were found among the isolates from the diarrheal and non-diarrheal samples. In general, resistance against the tested antibiotics was greater among the *Klebsiella* spp. isolates in the clinical samples, but was relatively higher among the *E. coli* isolates in the control samples against cefepim and ceftazidime. Seventy five percent of the *Klebsiella* spp. clinical isolates showed resistance to ciprofloxacin in compare to 10 % for the isolates from control group. Analysis of the resistance patterns among the *E. coli* isolates did not support existence of phenotypes related to extended spectrum β-lactamase (ESBLs).

Given the resistance profiles for the clinical isolates, in compare to these frequencies among the isolates from control group (7.6% and 4.4% for *E. coli* and *Klebsiella* spp., respectively), it was cleared that 50% and 31% of these isolates had resistance patterns related to carbapenemase producing bacteria. Based on these data, it appeared that the isolates in both of the groups have highest resistance rates to ampinicillin and oxacillin. History of antibiotic usage among patients from the control group did not show significant correlation with the resistance rates (P value $\leq$ 0.05).

Table 1. Frequency of *E. coli* and *Klebsiella spp.* isolates among the case and control groups samples. (P value $\leq$ 0.05)

	Inpatients	Outpatients		P value
		Diarrheal	Non-diarrheal	
E. coli	3.15%	99.58%	100%	0.000
Klebsiella spp.	6.36%	36.6%	9.5%	0.001
Other enterobacteriaceae	1.05%	15%	9.5%	0.01

Table 2. Antimicrobial resistance phenotypes of MDR *Klebsialla spp.* isolates from case and control group samples.

Antimicrobial resistance phenotype	% MDR isolates in inpatients	% MDR isolates in outpatients
Cefepim, Gentamicin, Imipenem, Ceftazidime	31%	4.4%
Cefepim, Gentamicin, Ceftazidime	23%	4.4%
Cefepim, Ceftazidime	15.3%	4.4%

Table 3. Antimicrobial resistance phenotypes of MDR *E. coli* isolates from case and control group samples.

Antimicrobial resistance phenotype	% MDR isolates in inpatients	% MDR isolates in outpatients
Co-amoxiclav, Ceftazidime, Cefepime, Imipenem	50%	7.6%
Co-amoxiclav, Ceftazidime, Imipenem	0%	1.5%
Co-amoxiclav, Ceftazidime, cefepim	0%	4.2%
Co-amoxiclav, Ceftazidime	16.6%	4.6%

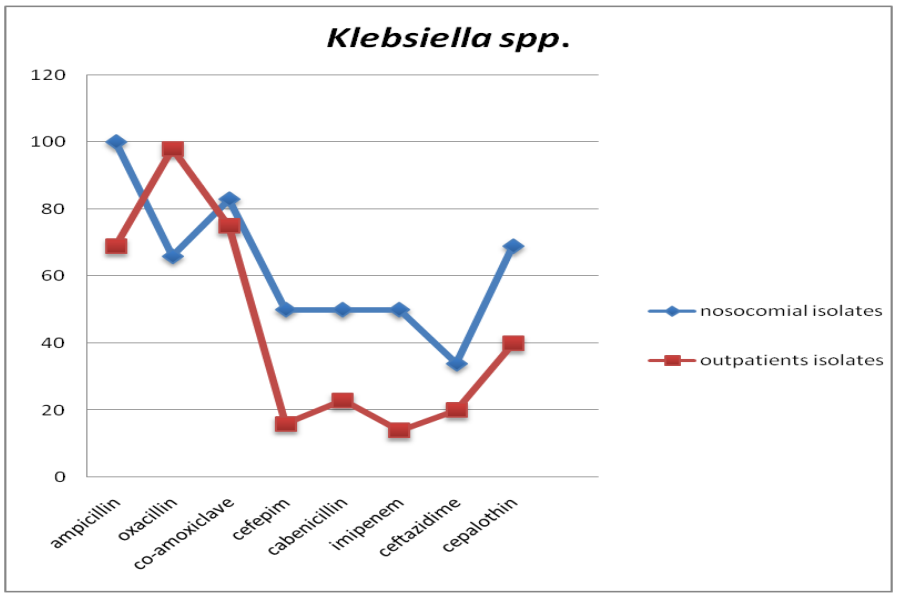

Figure1. Percentage of drug resistance in *Klebsiella* spp. isolates.

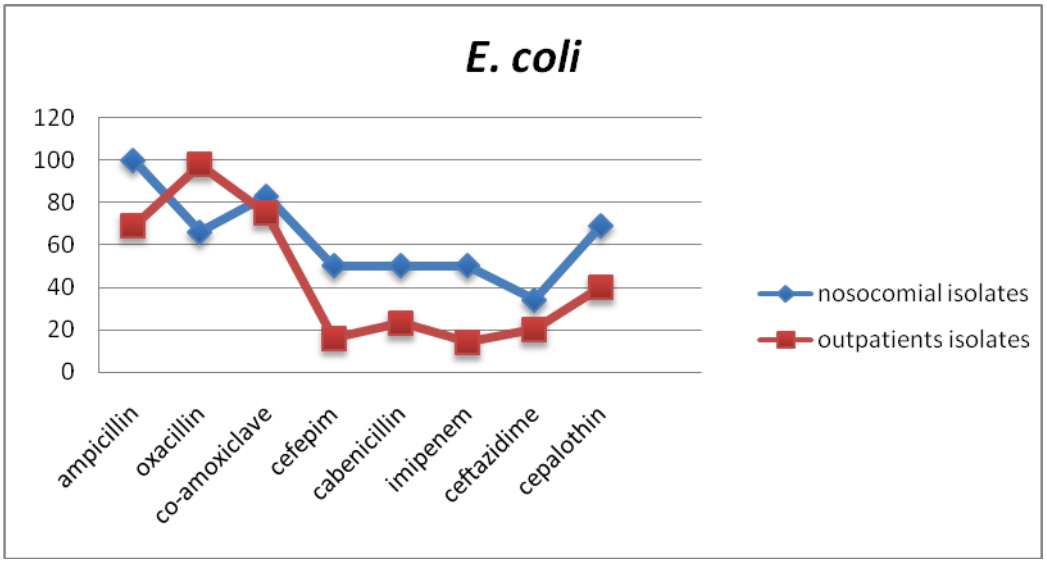

Figure2. Percentage of drug resistance in *E. coli* isolates.

DISCUSSION

Resistant strains of enteric bacteria have been distributed in some regions of the word. Some of these bacteria are responsible for severe infections in the community and hospitals. Determination of resistance patterns among the isolates from each geographic region can predict risk of infections with these bacteria and needs for new antibiotic types against them. Transition of resistance bacteria from the hospitals to the community and vise versa, and also from food products are established in many of studies [6]. Existence of resistance gene markers among the non-pathogenic bacteria in the environment and their transition to the pathogenic bacteria in the community have been considered as one of the most important strategies for emerging new resistance phenotypes in these bacteria. So, healthy member of a community can act as main reservoirs for these resistance genes.

E. coli and *Klebsiella* spp. are members of the enterobacteriacea family that exist in the human intestine and are involve in nosocomial infections [7]. Comparison of clinically important resistance phenotypes among isolates of these bacteria from the non-hospitalized patients and patients with nosocomial infections help us to assess distribution of resistant strains within the community [8]. In this study, by analyzing more important resistant phenotypes, we found fecal carriage of resistant strains of *E.*

coli and *Klebsiella* spp. in a range of 1.5 -7.6 and 4.4, respectively that was 3 to 7 fold lower than the clinical isolates. In contrast to results from some other studies, none of the isolates from both of the groups showed resistance phenotypes related to ESBLs, but all of the imipenem resistant isolates had shown a phenotype for carbapenemase producing bacteria [13]. The fluoroquinolone resistance rate was high in isolates recovered from inpatients (75%) that were similar to previous report by Abdulla Kader et al., while was lower for the isolates in those recovered from outpatients (10%) [14].

CONCULOSION

These finding suggests low frequency of important resistance markers within the isolates from the community. In the case of the tested bacteria in the control group, no significant correlation was found between previous usages of antibiotics and appearances of the resistant phenotypes. Consumption of food products containing resistant bacteria from animal sources, arbitrary usage of antibiotics without prescription and transition of resistant bacteria from medical centers to the community can increase risk of this carriage within the community. Surveillance studies of the resistance patterns among bacterial isolates from different regions will provide awareness about dissemination of these bacteria within the community as reservoirs of main resistance markers. More detailed studies will clear these associations in future.

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