Carriage prevalence of carbapenem-resistant *Enterobacteriaceae* in stool samples: A surveillance study

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BRIEF REPORT

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Abstract

Background

With more people being exposed to antibiotics, intestinal microflora faces constant pressure of antibiotic selection, which has resulted in the emergence of multidrug resistant strains. This may pose a severe problem as intestinal *Enterobacteriaceae* members are commonly implicated in human infections.

Aims

This surveillance study was undertaken to investigate the carriage of carbapenem-resistant *Enterobacteriaceae* (CRE) in the gastrointestinal tract among patients attending the outpatient clinic in a tertiary care center of East Delhi, India.

Method

We performed a prospective surveillance study to screen 242 *Enterobacteriaceae* isolates for carbapenemase production from the stool samples of 123 outpatients attending a tertiary care hospital in East Delhi over a four-month period.

Results

Twenty-four (9.9 per cent) isolates demonstrated carbapenemase activity among 242 screened *Enterobacteriaceae* isolates. Four stool samples had two isolates of different species, both eliciting this feature and therefore indicating presence of multiple carbapenem-resistant *Enterobacteriaceae* (CRE) isolates in a single sample.

Conclusion

Screening for carriage of CRE in stools of patients undergoing elective or emergency gastrointestinal surgical procedures, with haematological malignancies taking chemotherapy, or those planned for bone marrow transplantation can guide clinicians about gut colonisation of multidrug-resistant *Enterobacteriaceae* as these groups of patients are at risk of possible endogenous infection.

Key Words

Carbapenem resistant *Enterobacteriaceae*; gut colonisation; prophylactic antibiotic

What this study adds:

1. What is known about this subject?

To our knowledge, this is the first Indian study investigating the prevalence of CRE in stool samples from an urban area.

2. What is the key finding of this report?

This type of surveillance study can guide clinicians and clinical microbiologists about colonisation of such multidrug-resistant strains in the human gastrointestinal tract, a major reservoir of *Enterobacteriaceae* isolates that can act as source of infection, particularly in immune-compromised patients.

3. What are the implications for policy, research, and practice?

Active surveillance is a key part in preventing the spread of drug-resistant strains as gastrointestinal carriers may serve as the reservoir for cross-transmission in the healthcare setting as well as the community.

Background

The human gastrointestinal tract is a reservoir of pathogens causing infections such as urinary tract infections (UTI), skin soft tissue infections (SSI), and nosocomial infections.[1] Bacterial translocation is the invasion of indigenous intestinal bacteria through the gut mucosa to normally sterile tissues and the internal organs. Bacterial translocation occurs more frequently in patients with intestinal obstruction and in immunocompromised patients and is the cause of subsequent sepsis. Factors that can trigger bacterial translocation from the gut are host immune

deficiencies and immunosuppression, disturbances in the normal ecological balance of gut, mucosal barrier permeability, obstructive jaundice, or stress.[2] With an increase in the number of people being exposed to antibiotics, the intestinal microflora faces constant pressure of antibiotic selection, which has resulted in the emergence of multidrug-resistant strains including carbapenem-resistant strains. This may pose a severe problem as intestinal *Enterobacteriaceae* are most commonly implicated in human infections and antibiotic options in infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) may be limited to colistin, tigecycline, and polymyxin B. Routine laboratory culturing of stool samples for diagnosing common clinical pathogens may often overlook commensal *Enterobacteriaceae* that can harbour resistant phenotypes. Antibiotic overuse and improper sanitation and hygiene in urban slum areas can lead to the rapid spread and large scale carriage of multi- or pan-drug-resistant isolates in the intestinal microbiota that can be a potential cause of endogenous and exogenous infections.[3] The US Centers for Disease Control and Prevention (CDC) has issued guidelines for hospital surveillance of CRE;[4] however, there are no surveillance studies on screening of CRE in stool isolates in an Indian cohort of patients. With this in mind we performed a prospective surveillance study for four months to evaluate the carriage of CRE from *Enterobacteriaceae* isolates from stool samples of patients attending the outpatient clinics in a tertiary care centre of East Delhi. This was undertaken to look for carriage of CRE isolates over one time period, irrespective of the patient symptoms or any history of present/previous antibiotic treatment.

Method

This surveillance study was conducted on stool samples from 123 patients attending the outpatient departments of a tertiary care hospital of East Delhi that caters for populations belonging to the urban slum areas and resettlement colonies of East Delhi and Western Uttar Pradesh. The samples were received in the Hospital Laboratory Services (HLS) for four months in 2011 for routine examination. These samples were cultured on MacConkey's medium (HiMedia, India). Multiple isolates of family *Enterobacteriaceae* were purified from a single sample and subsequently one to three different *Enterobacteriaceae* isolates per sample were tested for carbapenemase activity by the conventional Modified Hodge Test (MHT) as per CLSI guidelines.[6] A Re-modification of the MHT (RMHT) was performed to increase its sensitivity and demonstrate zinc dependency.[7]

Results

Among the 123 stool samples cultured, 242 *Enterobacteriaceae* isolates were identified using conventional methods,[8] purified, and screened by the MHT and the RMHT. MHT and RMHT were read as negative, slight indentation, definite indentation, and strong indentation of the ATCC 25922 *E. coli* control strain in the background. Interpretation of this reading was classified as negative, indeterminate, and positive as per CLSI guidelines.[6] Among the 242 isolates, 208 were negative by either of the tests. To rule out the subjective nature of the test, 10 isolates with slight indentation (Table 1) were interpreted as indeterminate. The remaining 24 isolates interpreted as positive were further identified and subjected to antimicrobial susceptibility testing by the Microscan WalkAwayR Plus (Siemens, Mumbai, India) automated system. All the 24 isolates showed MIC ≥ 4µg/ml for imipenem and meropenem. Four samples had at least two isolates, both being different species, which were positive for both MHT and the RMHT indicating presence of multiple CRE isolates in a single sample. Among the 24 isolates, 16 demonstrated zinc dependency on the RMHT indicating presence of metallo beta-lactamases. The Microscan WalkAwayR Plus indicated presence of ESBL (Extended Spectrum Beta-Lactamase) in all 24 CRE isolates on the basis of difference in the MIC's of Cefotaxime/Cefotaxime + Clavulanate and Ceftazidime/Ceftazidime + Clavulanate.

Discussion

The emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) producing acquired carbapenemases have created a global public health crisis. [9]

Knowledge about the prevalence of CRE and other drug-resistant organisms in the intestine can help in formulating antibiotic policy in management of sepsis as a complication of extensive gut surgery or patients with haematological malignancy under chemotherapy or bone marrow transplantation. Das et al. mention that neonates with Gram negative bacilli in the gut had a higher incidence of clinical sepsis than those without.[10] In 50 per cent of cases, the genotypes of the organisms found in the blood were indistinguishable from their gut counterpart.

Our study had a number of limitations, including short duration and, due to lack of funding, source molecular typing of the isolates could not be performed. However, phenotypic methods were employed for the detection of CRE and, by a phenotypic confirmation method, it was found that 9.9 per cent *Enterobacteriaceae* isolates were CRE.

The drug-resistant organisms we have identified may remain for months in the gut of the carrier without causing any symptoms or translocate through the gut epithelium, induce healthcare-associated infections, undergo cross-transmission to other individuals, and cause limited outbreaks.[11] Active surveillance of drug-resistant strains, including extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and carbapenem-resistant *Enterobacteriaceae* is an important component of any infection control program, and more surveillance studies need to be performed in India to provide a better understanding of the prevalence of drug-resistant strains as gut colonisers. Screening of drug-resistant *Enterobacteriaceae* can help in formulating antibiotic policy for a hospital, particularly for oncology and critically ill patients in ICUs, as these organisms can act as sources of endogenous infections.

Conclusion

Screening for carriage of CREs in stool in patients undergoing elective or emergency gastrointestinal surgical procedures, in patients with haematological malignancies taking chemotherapy, or patients with planned bone marrow transplantation can guide treating clinicians about gut colonisation of multi-drug resistant *Enterobacteriaceae* as these groups of patients are at risk of possible endogenous infection. This can also help in starting appropriate prophylactic antibiotics if required. Treating clinicians as well as microbiologists must be aware of the prevalence of CRE isolates in the human intestinal tract as these types of drug-resistant strains are potential sources of endogenous infections.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

Table 1: Isolates with slight indentation

Serial No	Reading of		No of isolates	Organism	Interpretation
	MHT	RMHT			
1.	Negative	Negative	208	102 EC, 80 KP, 24 CF, 1 CK, 1EA	Negative
2.	Negative	Slight indentation(+)	4	1EC,3KP	Indeterminate
3.	Slight indentation(+)	Slight indentation(+)	6	3EC,3KP	Indeterminate
4.	Negative	Definite indentation(++)	3	3EC	Positive
5.	Slight indentation(+)	Definite indentation(++)	11	6EC, 2KP, 2CF, 1EA	Positive
6.	Definite indentation(++)	Definite indentation(++)	8	3EC, 2KP, 3CF	Positive
7.	Definite indentation(++)	Strong indentation(+++)	2	2EC	Positive
Total positive			24		

EC: *Escherichia coli*, KP:*Klebsiella pneumoniae,* CF: *Citobacter freundii*, CK*: Citrobacter koseri* ,
EA: *Enterobacter aerogenes*