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An Ileal Perforation Associated with Extended Spectrum β-Lactamases-Producing *Escherichia Coli*: Medical Case Report

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Abstract

Background: Intestinal perforation is one of the leading fatal causes of death among individuals mostly in developing countries. Although many reports have associated perforations with typhoid infections, reports on the role of other bacterial pathogens especially resistant strains in causing ileal perforations are limited. We report a rare case of ileal perforation associated with extended-spectrum beta-lactamase (ESBL) - producing *Escherichia coli* (*E. coli*) in Ghana.

Case presentation: A 9-year-old female child presented to the Agogo Presbyterian Hospital with a case of worsening abdominal pain and generalised abdominal tenderness. She was found to have ileal perforation upon surgical operation and her blood culture and swabs from the perforated sites all yielded *E.coli* which was resistant to all routine antibiotics except meropenem. Molecular and phenotypic analysis showed that the isolated bacteria had *blaCTX-M* and *blaTEM-* associated resistance genes. The patient responded well with meropenem administration and was discharged upon full recovery.

Conclusion: ESBL producing bacteria could be associated with ileal perforation in children. Physicians should be mindful of this and administer evidence based therapy when encountered with similar situation.

Keywords

ESBL, Ileal perforation, Resistance, Antimicrobial, Infections

Abbreviations

ESBL: Extended spectrum B-lactamase

Background

Gastrointestinal perforation occurs when a hole forms all the way through the stomach, large bowel, or small intestine as a result of trauma, appendicitis, foreign bodies, parasitic infections, tuberculosis and tumours [1-4]. The ileum is one of the commonest sites for perforations and has been associated with typhoid disease [5]. The infection occurs when the typhoid bacterium accesses the human host through ingested food or drink and infects the Peyer's patches in the ileum leading to necrosis, ulcerations and perforations. Complications associated with perforations are usually fatal and could result in death if early surgical intervention is not offered. Treatment is often dependent on resuscitation with fluids, electrolytes and administration of broad spectrum antibiotics such as quinolones, third generation cephalosporins and metronidazole.

Although many reports, especially in developing countries, have associated perforations with typhoid infections, reports on the role of other bacterial pathogens, especially resistant strains, in causing ileal perforations are limited. Resistant bacterial strains associated with intestinal perforation are the one of the most life threatening conditions which could often result in death if the appropriate antibiotics are not administered. A group of resistant bacteria which seem to be recognized in causing severe disease are the extended spectrum beta lactamase (ESBL) - producing bacteria (ESBL), of which *E.coli* is a member. The emergence of ESBLs followed the introduction into clinical practice of expanded-spectrum cephalosporins, which are acknowledged to be the most powerful selectors for these resistance determinants. Extended-spectrum beta-lactamase (ESBL) - producing *E.coli* cause a significant therapeutic challenge since they hydrolyze all β-lactams except carbapenems and cephamycins. Since 1960 when the first mutant form (TEM) was isolated, novel resistant genes including SHV, CTX-M and OXA families have been found [6-9].

In Ghana and in many other African countries, most reported ileal perforation cases are managed as typhoid and hence treated with *β*-lactam antibiotics and quinolones as part of routine patient

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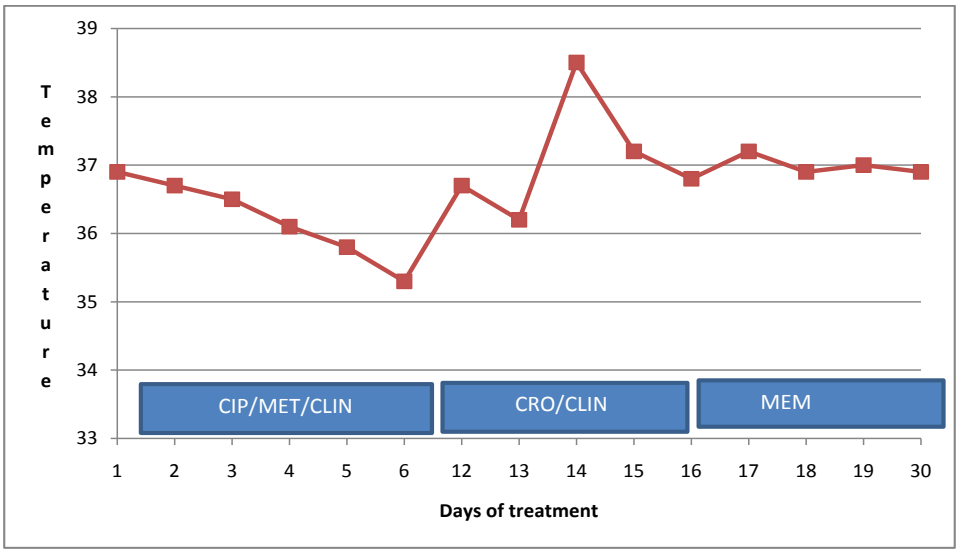

Figure 1: Response to antibiotic administration.
CIP: Ciprofloxacin; CRO: Ceftriaxone; MET: Metronidazole; CLIN: Clindamycin; MEM: Meropenem.

care. Here, we report a rare case of ileal perforation associated with *blaCTX-M-* and *blaTEM*-β-lactamase *Escherichia coli.*

Case Presentation

A nine-year-old-female was referred from a rural health centre to the Agogo Presbyterian Hospital in the Asante Akim North district of the Ashanti region as a case of worsening abdominal pain with a 10-day history of fever. Her referred medical record showed she had severe abdominal pains 5 days before and was being treated with intravenous (IV) ciprofloxacin and metronidazole. On examination, the patient was pale, looked very ill and was thus haemo-transfused with 1 unit of blood before being referred to the Agogo Presbytarian Hospital (APH).

Her respiratory rate was 36 cycles per minute with a reduced bilateral air entry. The first and second heart sounds, blood pressure and pulse rate were all normal. There was, however, generalised abdominal tenderness, guarding and rebound tenderness. A preliminary diagnosis of generalised peritonitis secondary to perforated hollow viscus was made with differential diagnosis of ruptured appendicitis and typhoid ileal perforation. The patient was admitted at the intensive care unit and investigations including full blood count, liver function test, malaria test, sickling and chest x-ray were requested. Blood for culture and susceptibility testing was also collected and transported to the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) for analysis. The patient was further administered with IV ciprofloxacin 300 mg twice daily for 72 hours and IV metronidazole 250 mg three times daily for 72 hours.

A kidney challenge with 500 ml of ringers lactate and 500 ml of normal saline was given over 30 mins in addition to 30 mg of IV Lasix. The urine output was assessed to monitor adequacy of the kidneys and the patient was prepared for laparotomy.

Laparotomy was performed on the 2nd day of admission and showed an ileal perforation at about 30 cm from the ileocecal junction, respectively. Both perforations occurred along the anti-mesenteric border. The diameter of the ileal perforation was 7 mm. The feculent peritoneal fluid was suctioned and the ileal perforation was sutured with vicry l2.0. Two swab samples were taken at the perforated site. Swab 1 was collected at the perforated site and swab 2 was collected a day after laparotomy at the sutured site. Both samples were transported to the bacteriology laboratory of KCCR for culture and antimicrobial susceptibility testing.

The vitals were monitored and metronidazole was replaced by clindamycin 300 mg three times daily for 7 days and temperature monitored for stability and improvement. The patient was still unwell two days after laparotomy and showing spiky temperature

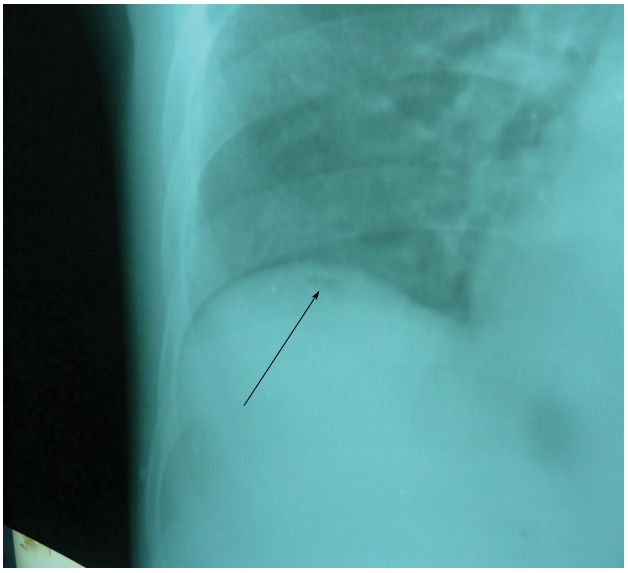

Figure 2: The right side of the chest X-ray showing small air bubble (arrow) below the right hemidiaphragm.

with respiratory distress. The IV ciprofloxacin was replaced with IV ceftriaxone 2 g daily for 72 hours; however the clinical condition of the patient did not improve.

On the 4th day of admission, the blood culture result was communicated to the hospital and the attending physician. The isolated bacterium was an ESBL producing *E.coli* which showed resistance to all antimicrobials except meropenem. The physician was therefore advised to administer meropenem to the patient. Intravenous meropenem 500 mg three times daily for 7 days was administered, and the patient's condition thereafter improved clinically and she was discharged upon full recovery. Stool samples were collected from the patient three days after discharge and sent to KCCR for culture and antimicrobial susceptibility testing. Figure 1 describes patient's response to antibiotic administration.

Diagnostic Investigations

Basic investigations

Baseline full blood count investigations showed a haemoglobin concentration of 11.3 g/dl, total white blood cell count of 6900 cells/µl (lymphocyte 11.5%, neutrophil 80.3% and mixed cells of 8.2%). Malaria parasite and sickling status proved negative. Alanine transaminase (ALT) and aspartate transaminase (AST) were

Table 1: List of primers and their product size.

Target gene	Sequence		Product size (bp)	
	SHV-F	CTTTACTCGCCTTTATCG		[22]
bla_{SHV}	SHV-R	TCCCGCAGATAAATCACCA	827	
	TEM-F	GTATCCGCTCATGAGACAATA		[23]
bla_{TEM}	TEM-R	TCTAAAGTATATATGAGTAAAC	966	
	CTX-M-F	TTTGCGATGTGCAGTACCAGTAA		[24]
bla_{CTX-M}	CTX-M-R	CGATATCGTTGGTGGTGCCATA	590	

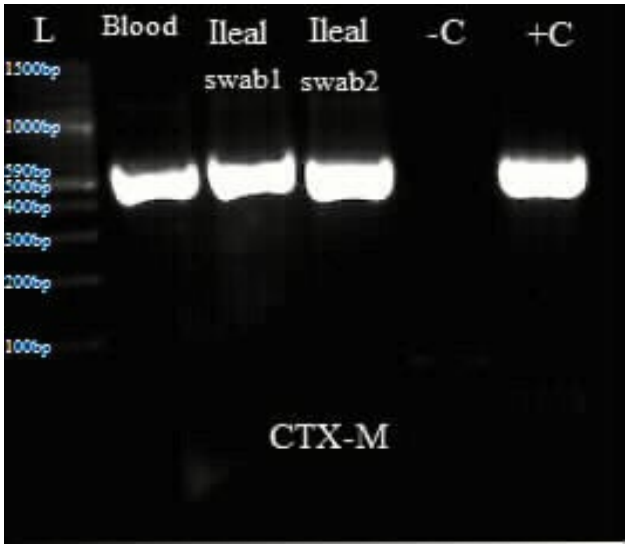

Figure 3: Represents PCR results for CTX-M.

L: Ladder; +C: Positive controls; –C: Negative controls; Blood: Isolate from blood sample; Ileal swab 1: Isolate from perforated site; Ileal swab 2: Isolate from sutured site.

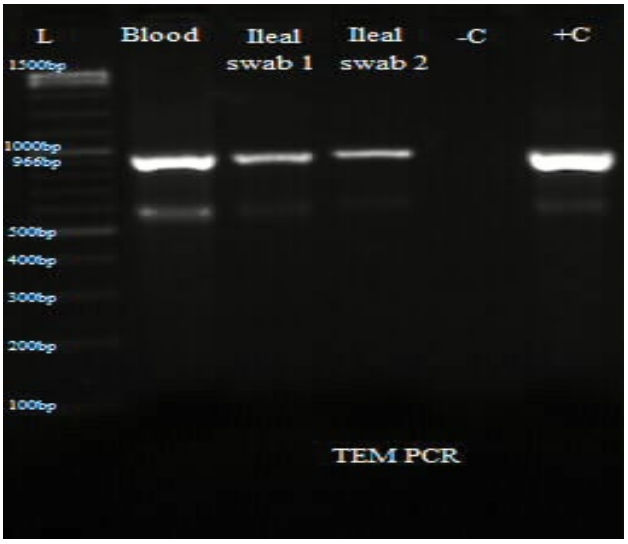

Figure 4: PCR results for TEM.

L: Ladder; +C: Positive controls; –C: Negative controls; Blood: Isolate from blood sample; Ileal swab 1: Isolate from perforated site; Ileal swab 2: Isolate from sutured site.

marginally raised and the total protein was slightly low although serum albumin was markedly reduced (22.0 g/dl). Chest X-ray showed minute pneumoperitoneum below the right diaphragm (Figure 2).

Identification Procedure of Microorganisms and Antimicrobial Tests

Blood culture

The blood culture sample was incubated in Bactec 9050 at 37 ^{0}C. The blood culture flagged positive after 24 hours of incubation and was also sub-cultured on blood and Mackonkey agar media. The Growth characteristics showed pure lactose fermentation on Mackonkey agar and non-haemolytic colonies on blood agar. The colonies were inoculated in Citrate agar (BD, France), Kliegler iron agar (BD, France) and Urea agar and biochemical characteristics showed features consistent with *E.coli.* The isolate was subjected to analytical profile index 20E (Biomerieux, France) testing and a profile of 5144572 was generated giving an identification of *Escherichia coli*.

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method following the Clinical Laboratory Standards Institute guidelines (CLSI) [10]. The antimicrobials used were ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), amoxicillin (25 µg), ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg) and tetracyclin (30 µg). The isolates were resistant to all antimicrobials. The isolate was subjected to a second line testing using meropenem (10 µg) and this time they were susceptible. Based on the resistance pattern of ceftriazone and ceftazidime, confirmatory tests for ESBL production were performed using the double disc synergy method as described in the CLSI guidelines [10]. Briefly, 0.5 McFarland suspensions of the isolates were inoculated in Mueller Hinton agar. Antibiotic disc of ceftazidime (30 µg) and cefotaxime (30 µg) were placed beside ceftazidime/clavulanate (30/10 µg), and cefotaxime/clavulanate (30/10 µg) respectively. After incubation, inhibition zone diameters were measured and interpreted. A zone difference of ≥ 5 mm was observed between the single (cefotaxime and ceftazidime discs) and corresponding combination disks (cefotaxime/clavulanate and ceftazidime/clavulanate) and hence noted as ESBL positive. The ATCC strains used for antimicrobial susceptibility testing and phenotypic confirmation of ESBL were *E.coli* ATCC 25922 and *Klebsiellapneumoniae* ATCC.

Swab cultures

The two swab samples taken from the perforated site were also sub-cultured on blood agar and Mackonkey agar (BD, France) and incubated at 37 ^{0}C overnight. Direct examination of the swab samples yielded Gram negative rods. After overnight incubation, pure lactose fermenting bacteria were isolated and further biochemical and API 20E investigations identified the bacteria as *E.coli*. The antimicrobial susceptibility test yielded the same pattern as those of the blood isolate and phenotypic typing for ESBL showed positive.

Stool cultures follow-up

Follow up stool samples collected after patient has been discharged was sub-cultured directly on Mackonkey agar and Xylose lysine deoxycholate agar (XLD). Analytical profile index of the isolate yielded *E.coli* with similar colony morphology as previous isolates. The isolate was however susceptible to all antimicrobials and phenotypic testing for ESBL was negative.

ESBL typing of *E.coli* strains

Total DNA was extracted from the swab and blood isolates using Spherolyse DNA extraction kit (Hain Life science GmbH, Germany), following the manufacturer's instructions. The extracts were subjected to single plex conventional PCR analysis targeting the *blaSHV*-, *blaTEM*- and *blaCTX-M*-encoding genes. Fifty(50- µl) reaction mixes consisted of 5 µl of 10X reaction buffer, 4 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 1 µl of forward primer, 1 µl of reverse primer, 0.25 µl of 5 units Taq DNA polymerase and 5 µl of DNA template. The final concentration of each primer was 0.2 µM.

Amplifications were carried out with the following thermal cycling profile: initial denaturation for 3 mins at 94 ^{0}C, followed by 35 cycles of amplification consisting of 94 ^{0}C for 45 s, annealing at 56 ^{0}C for 35 s and primer extension at 72 ^{0}C for 30 s. A final extension cycle at 72 ^{0}C for 10 mins with a holding step at 4 ^{0}C was included to finish the entire PCR amplification. In the case of CTX-M, an annealing temperature of 62 ^{0}C for 45 s was used. Primers sequences are shown in table 1. PCR results showed the three bacterial isolates were positive for TEM and CTX-M (Figure 3, Figure 4 and Figure 5).

Discussion

ESBL-producing organisms pose a serious therapeutic challenge because of their ability to hydrolyse B-lactams and other associated antibiotics including quinolones [11,12]. Although some studies have demonstrated the presence of ESBL-mediated resistance in bacteria causing infections in patients in Ghana and other developing countries, few reports have shown the real impact of this bacterium in patients with ileal perforation.

Both molecular and phenotypic investigations confirmed the blood and swab isolates as being β-lactamase-producing *E.coli* harbouring a*blaCTX-M* gene and a*blaTEM* gene. Reports on the association between ESBL-producing *E.coli* and ileal perforations are quite rare and perhaps under-reported in developing countries because of the general lack of laboratory capacity for identifying the causative agents involved.

Most laboratory diagnosis of typhoid infections in developing countries are based on Widal test other than culture confirmed blood or peritoneal fluid. Widal test, however, has been found to be non-specific and difficult to interpret in areas where typhoid fever is endemic [13]. Majority of enteric perforations diagnosed as due to Salmonella organisms could therefore be only based on conjecture. Our report therefore shows a classical example of an *E.coli*-associated ileal perforation occurring about 30 cm away from the ileocaecal junction along the antimesenteric border. This pattern of perforation is similar to the reported cases of typhoid-associated ileal perforations [14,15].

A similar report by Capoor, et al. [16] identified *E.coli* (23.4%) as being the most predominant isolate out of 47 ileal perforations studied in Northern India. In the same report, only 10.5% of the ileal perforations were due to enteric fever. Similar findings were reported by previous studies [17,18].

One important point of note is the resistant pattern exhibited by the isolated bacteria and its likely impact on patient management. In Ghana and many developing countries, the clinical algorithm for managing patients with ileal perforations is to perform early surgery and administer blind therapy, mostly broad-spectrum antibiotics such as cephalosporins and quinolones. This algorithm is based on the fact that most ileal perforations are due to *Salmonella*, which is generally susceptible to these drugs. The isolation of an ESBL-producing *E.coli* is therefore quite significant to public health and attending physicians and calls for the need for laboratory capacity to be improved in at least strategic laboratories working closely with the Ghana Health Service in order to increase diagnosis of these infections. Carbapenems, which are the recommended choice for managing ESBL-producing bacteria, are quite expensive in Ghana and many developing countries. A full 7-day regimen costs around $1,200. Even the most urban dwellers cannot afford this drug, least of all rural peasant farmers as is the case of this patient. Similar cases of invasive ESBL-producing *E.coli,* which are unrelated to ileal perforations, have been reported in the cerebrospinal fluid, ear discharges and blood samples [19,20].

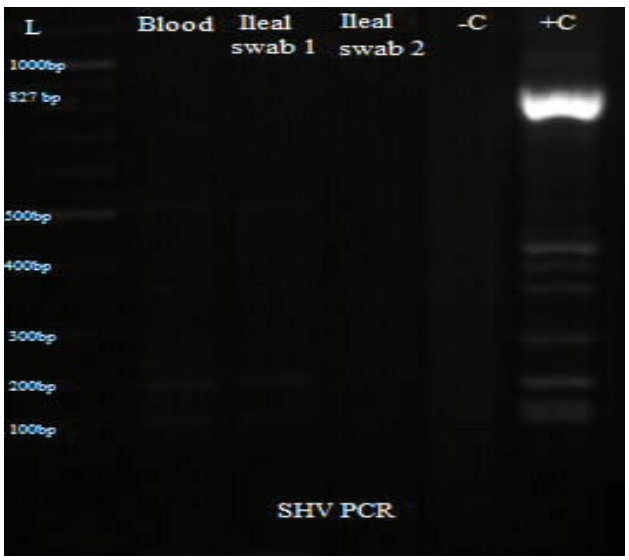

Figure 5: PCR results for SHV.

L: Ladder; +C: Positive controls; –C: Negative controls; Blood: Isolate from blood sample; Ileal swab 1: Isolate from perforated site; Ileal swab 2: Isolate from sutured site.

One question we could not answer was the type of infection the patient had, whether community- or hospital-acquired. This is because we only got opportunity of taking samples for cultures after the patient had spent 10 days at the health centre where she was referred. Literature, however, shows hospitalisation is not a risk factor for intestinal colonisation with *blaCTX-M* type producing *E.coli,* thus possibly suggesting that our patient might have community-acquired ESBL-producing *E.coli* [21].

Although we are also unable to tell the source of this particular bacterium, we do not think it has any relationship with faecal carriage or possible enteric colonisation since the stool sample collected after discharge was negative for ESBL *E.coli*.

Conclusion

Our report highlights a possible association between ESBL producing *E.coli* and ileal perforation. Physicians are encouraged to rely on evidence through appropriate antibiotic susceptibility testing before resorting to the use of antimicrobial agents.

Ethical Approval and Consent to Participate

We obtained consent from the parents of the child before proceeding with publication of this report.

Consent for Publication

Written informed consent was obtained from the parents of the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of Data and Material

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors declare no competing interests. No author has any proprietary interest in any of the products or ideas mentioned in this article.

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Authors' Contributions

KTM, NS, JA and IO reviewed the patient, and contributed to writing the manuscript. KSM, AA and MO performed laboratory analysis of the samples. EOD, YAS and MO documented the clinical

findings, validated clinical and laboratory data and contributed to writing the manuscript. All authors have read and approved the final manuscript.

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