

Prolonged febrile illness due to CTX-M-15 extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* infection in Nigeria

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Dates:

Received: 25 June 2011
Accepted: 19 Jan. 2012
Published: 04 June 2012

How to cite this article:

Aboderin OA, Adefehinti O, Odetoyin BW, Olotu AA, Okeke IN, Adeodu OO. Prolonged febrile illness due to CTX-M-15 extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* infection in Nigeria. Afr J Lab Med. 2012;1(1), Art. #16, 4 pages. <http://dx.doi.org/10.4102/ajlm.v1i1.16>

We report on an 8-year-old patient with septicaemia unresponsive to therapy for five weeks. Undetected, extended-spectrum β -lactamase (ESBL) production by the infecting *Klebsiella* strain was regarded as responsible for treatment failure. Intravenously administered imipenem during the sixth week led to sustained resolution of fever. Resource-limited hospitals can incur prohibitive costs from ESBL-producer infections because of diagnostic limitations and consequent treatment failure involving prolonged supportive therapy.

Introduction

The first report of plasmid-encoded β -lactamase capable of hydrolysing the extended-spectrum cephalosporins was published in 1983.¹ Since then, extended-spectrum β -lactamases (ESBLs) have become an increasingly important resistance mechanism among Enterobacteriaceae worldwide. A 2006 report of the Infectious Diseases Society of America listed ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* among drug-resistant microbes for which new therapies are urgently needed.² Reports show that the ESBL problem is rapidly evolving and increasing in severity and scope with the discovery of new ESBLs, particularly the CTX-M types, which have become the most prevalent.³ The β -lactamases are the greatest threat to the usefulness of β -lactam antibiotics such as the penicillins and cephalosporins. Of all the different types of β -lactamases, ESBLs currently have the greatest clinical impact in terms of diversity and distribution as well as the ability to hydrolyse expanded-spectrum third generation cephalosporins. The earliest variants of ESBLs originated as a result of point mutations in the genes for broad-spectrum β -lactamases whilst newer ones, including the most successful such as CTX-M, arose by acquisition from the environmental metagenome through horizontal gene transfer.⁴ Cephalosporins as bactericidal, cell wall-active β -lactam agents were introduced in the 1980s and as a result of effectiveness against broad-spectrum β -lactamases became standard for treatment of severe conditions such as bloodstream infections, pneumonia and intra-abdominal infections, until ESBLs started compromising usefulness in response to overuse and selective pressure. Organisms that produce ESBLs are an important reason for therapy failure with cephalosporins and have serious consequences for infection control. Furthermore, CTX-M ESBL enzymes have been associated with coresistance to other agents including trimethoprim-sulphamethoxazole, tetracycline, gentamicin, tobramycin and ciprofloxacin.⁵ It is essential that clinical microbiology laboratories rapidly and reliably detect and report ESBL-producing organisms.

Case report

An eight-year-old girl presented with a two-week history of fever, abdominal pain, passage of watery stool and recurrent vomiting. There was also a history of frequent micturition with occasional dysuria but neither haematuria nor passage of dark-coloured urine. Prior to presentation in the teaching hospital, she had been admitted to a distant private hospital for five days, where she was treated with amoxicillin, ciprofloxacin and artesunate (doses and duration of treatment are unknown). She was discharged from that hospital because her state of health was not improving significantly and also because of the need for better family support. There was no other history of hospital admission or blood transfusion and no history suggestive of haemoglobinopathy. Immunisation and nutritional history were essentially normal.

General physical examination revealed a conscious but ill-looking, somewhat pale, febrile (temperature 38.5 °C) girl. She was moderately dehydrated and jaundiced. Her weight was 22 kg (86% of expected weight for age). There was no facial or pedal oedema. The respiratory rate was

Note: This report was presented as a poster (abstract C-119) at the 111th General Meeting, American Society for Microbiology, May 21–24, New Orleans, Louisiana, United States.

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32 cycles per min and breathing was regular; pulse regular at 120 beats per min and with good volume. Her blood pressure was 90/50 mmHg. The significant systemic findings on examination at admission were severe suprapubic tenderness, moderate hepatosplenomegaly (firm, not tender) and negative renal angle tenderness. All other systems were normal.

Conventional blood cultures were done at five different times after admission. There was a growth of *Klebsiella* sp. on three occasions. Once, the isolate was sensitive to gentamicin and ceftriaxone but resistant to all available antibiotics tested on the other two occasions. Urine and stool cultures did not yield growth of any pathogens. Screening for human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B surface antigen (HBsAg) was negative.

Full blood counts showed a haematocrit ranged between 14% and 30%, white cell counts of $8 \times 10^9/L$ – $9.2 \times 10^9/L$ and an essentially normal platelet count ($260 \times 10^9/L$). The erythrocyte sedimentation rate was 80 mm/hr (Westergreen method) and the haemoglobin phenotype (by electrophoresis) was AS. Serum biochemistry parameters were all normal except for conjugated hyperbilirubinaemia. Repeated abdominal ultrasonography showed findings that are consistent with hepatosplenomegaly in a septicemic patient.

Whilst in hospital and when fever was uncontrolled and persistent, the patient was given fresh whole blood transfusions thrice and exchange blood transfusions twice, amongst other forms of treatment.

Fever remained persistent for five weeks following admission, despite different courses of antibiotics involving ciprofloxacin, gentamicin, ceftazidime, ceftriaxone and amoxicillin-clavulanic acid (Table 1). It was only at this point that the possibility of infection with an ESBL-producing organism was considered. ESBL-producers are not routinely sought in the diagnostic laboratory. During the sixth week, the *Klebsiella* sp. isolate from the patient was tested and confirmed to be producing an ESBL. Immediately following this test result, treatment was commenced with imipenem (not routinely available in the hospital) and there was dramatic resolution of fever. The patient remained free of fever for one week after receiving imipenem and was subsequently discharged. Two weeks later, when she reported for follow-up, she was still fever-free and healthy.

The *Klebsiella* sp. isolate was identified as *Klebsiella pneumoniae* subspecies *pneumoniae* using the API 20E identification strips for Enterobacteriaceae (bioMérieux, Marcy-l'Étoile, France). Presumptive ESBL phenotypic testing and confirmation in the organism was performed by disc diffusion tests on Mueller Hinton agar by employing ceftazidime (30 µg) and cefpodoxime (10 µg) alone and in combination with clavulanic acid as ceftazidime-clavulanic acid (30/10 µg) and cefpodoxime-clavulanic acid (10/1 µg) respectively. Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria for disc diffusion.⁶ Antimicrobial susceptibility testing for the organism was carried out by the disc diffusion technique according to the guidelines and recommendations of CLSI.⁶ The isolate was resistant to streptomycin, gentamicin, chloramphenicol, tetracycline, nalidixic acid, ciprofloxacin, ampicillin, trimethoprim, sulphamethoxazole, ceftriaxone, cefepime and amoxicillin-clavulanic acid, but susceptible to imipenem.

Genomic DNA was extracted from the isolate using the Wizard genomic extraction kit (Promega) according to the manufacturer's directions and used as template for PCR reactions targeting resistance elements and genes. Platinum PCR Supermix (Invitrogen) was used for all reactions, and PCR cycle conditions were as recorded in the original articles describing the primers (Table 2). We employed oligonucleotides that prime the conserved ends of the cassette regions of class 1 and 2 integrons respectively to screen for these elements (Table 2).^{7,8} As shown in Figure 1, we were able to determine that the strain harboured a class 1, but not a class 2 integron. Sequencing of the 1.6 kb amplified class 1 cassette region revealed that it was identical to the cassette region of plasmid pIP1206 (Genbank Accession number NC_010558), containing two integrated cassettes: a *dfrA17* cassette encoding resistance to trimethoprim, and an *aadA4* aminoglycoside resistance cassette.⁹ Since the integron did not contain an ESBL cassette, we screened the isolate for *bla*_{CTX-M} type genes, employing primers that amplify an internal fragment from multiple *bla*_{CTX-M} alleles (Table 2)¹⁰. The resulting 550 bp product shown in Figure 2 was sequenced and found to be identical to the corresponding region of *bla*_{CTX-M-15}.

The cost of repeated investigations (Table 3) was N14 000.00 (\$90.92), which is more than a tenfold increase on projected diagnostic expenses, had a diagnosis estimate been made immediately on admission. Antibiotics and

TABLE 1: Treatment interventions.

S/No.	Period	Treatment	Cost	
			NGN	USD
1	First week	I/V ciprofloxacin & I/M gentamicin	3780.00	24.55
2	Second week	I/V ceftazidime & I/M gentamicin	10 920.00	70.91
3	Third/Fourth week	I/V ceftriaxone & I/M gentamicin	8500.00	55.19
4	Fifth week	I/V amoxicillin/clavulanate & I/M gentamicin	6800.00	44.16
5	Sixth week	I/V imipenem/cilastatin	50 400.00	327.27
6	-	Blood transfusions	9500.00	61.69
Total cost			89900.00	583.77

S/No., Serial number; NGN, Nigerian Naira; USD, United States Dollar; I/V, intravenous; I/M, intramuscular.

