

Transient *in vivo* selection of a constitutively cephalosporin resistant *Enterobacter cloacae* causing ventriculitis

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A JOFFE, A KABANI, K RAMOTAR, W KRULICKI, G CADRAIN, T JADAVJI. Transient *in vivo* selection of a constitutively cephalosporin resistant *Enterobacter cloacae* causing ventriculitis. *Can J Infect Dis* 1995;6(1):44-48. A case of neonatal ventriculitis complicating a ventriculoperitoneal shunt and caused by one strain of *Enterobacter cloacae* (as shown on pulsed field gel electrophoresis) is presented. Daily ventricular fluid cultures from day 1 to 9 revealed inducible cephalosporin resistance in all isolates except on days 3, 4 and 5 of therapy when isolates were constitutively resistant. This emergence of resistance due to constitutive Bush class 1 beta-lactamase production is an excellent example of the rapid emergence of a predominant strain of bacteria depending on antibiotic selection pressures *in vivo*. The transient nature of the predominant resistant phenotype may have been due to missing a dose of cefotaxime on day 5 or *in vivo* factors allowing persistence of sensitive organisms in antibiotic protected sites. Caution is advised in the use of cephalosporins alone for serious *Enterobacter* species infections. Repeat culture and sensitivity should be done in severe infections that are slow to respond to cephalosporin therapy.

Key Words: *Constitutive resistance, Enterobacter cloacae*

Sélection transitoire *in vivo* d'un *Enterobacter cloacae* constitutionnellement résistant à la céphalosporine et provoquant une ventriculite

RÉSUMÉ : Un cas de ventriculite néonatale compliquée d'un shunt ventriculopéritonéal et causé par une souche d'*Enterobacter cloacae* (visualisée à l'électrophorèse sur gel par champ pulsé) est présenté ici. Des cultures de liquide ventriculaire quotidiennes des jours 1 à 9 ont révélé une résistance inductible aux céphalosporines chez tous les isolats, à l'exception des jours 3, 4 et 5 du traitement où les isolats étaient constitutionnellement résistants. L'émergence de cette résistance due à la production d'une bêta-lactamase Bush de classe 1 constitutionnelle est un excellent exemple de l'apparition rapide d'une souche bactérienne prédominante selon les pressions de sélection de l'antibiotique *in vivo*. La nature passagère du phénotype à prédominance résistante peut avoir été due à l'omission d'une dose de céfotaxime au jour 5 ou à des facteurs *in vivo* ayant permis la persistance d'organismes sensibles dans des sites protégés de l'antibiotique. La prudence est recommandée lors de l'emploi de céphalosporines seules dans des infections à *Enterobacter* graves. Il faut répéter la culture et l'antibiogramme dans les infections graves qui répondent lentement au traitement par céphalosporines.

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BACTERIA EXPRESS BUSH CLASS 1 CHROMOSOME MEDIATED beta-lactamases in two ways: low 'basal' level production (eg, *Escherichia coli*, *Salmonella* species and *Shigella* species) and 'inducible' expression (1). Inducible expression occurs transiently in the presence of an inducer antibiotic (eg, cefoxitin, imipenem) in a predictable group of bacteria, including *Pseudomonas aeruginosa*, *Enterobacter* species, *Citrobacter* species, indole-positive *Proteus* species, *Providencia* species, *Morganella morganii* and *Serratia* species (1,2). These bacteria with inducible expression also have a significant number (10^{-6} to 10^{-7} wild-type cells) of mutant 'stable derepressed' cells that produce constitutively large amounts of beta-lactamase (1,2). These resistant variants can be quickly selected as the predominant bacteria by labile weak inducer antibiotics both in vitro (3,4) and in vivo (1,2,5-12). The emergence of resistance by this mechanism during therapy has been well described and has resulted in a high rate of treatment failures (1).

We describe a case of neonatal meningitis due to *Enterobacter cloacae* that developed resistance to beta-lactam antibiotics during therapy and again became susceptible while the patient was still on therapy. This is the first case of which we are aware of transient selection of a derepressed mutant enterobacter described in the literature.

CASE PRESENTATION

This eight-day-old Caucasian female was seen by the infectious disease consultant for irritability and fever of one day's duration. She was delivered by caesarean section (for failure to progress) with birth weight 3800 g to a well mother after a normal full-term pregnancy; membranes were ruptured for 5 h without evidence of chorioamnionitis, and Apgars were 9 and 9 at 1 and 5 mins, respectively. At birth she was found to have a sacral myelomeningocele and hydrocephalus; surgical repair and ventriculoperitoneal shunt insertion were done on day 2 of life. She was transferred out of the intensive care unit to the neonatal ward on day 4 of life.

On examination the neonate was very irritable. Vital signs showed a heart rate of 160/min, respiratory rate 45/min, blood pressure 84/42 mmHg and temperature 38.6°C tympanic. Head circumference was 38.7 cm (greater than 90th percentile) and weight 3740 g. The anterior and posterior fontanelles were large and full, and the skin over the entire ventriculoperitoneal shunt reservoir and tubing was erythematous, tender and swollen. She was hemodynamically stable, and the spinal wound was healing well. The rest of the examination was noncontributory. Laboratory investigations revealed a white blood cell count of $7.9 \times 10^9/L$ with 14% polymorphonuclear leukocytes, 52% band forms and 27% lymphocytes, hemoglobin 105 g/L and platelet count $444 \times 10^9/L$. Electrolytes, urea, creatinine, glucose, liver enzymes and urinalysis were all normal. After blood, urine and cerebrospinal fluid (CSF) cultures were drawn she was started on intravenous cefotaxime (150 mg/kg/day divided every 8 h) and vancomycin (45 mg/kg/day divided every 12 h), and operated for removal of the ventriculoperitoneal shunt and insertion of an externalized ventricular drain. CSF from the shunt reservoir showed a white

TABLE 1
Susceptibility of daily ventricular fluid *Enterobacter cloacae* isolates by the Vitek Auto-Microbic System

Antibiotic	Susceptibility (MIC $\mu\text{g/mL}$)		
	Day 1-2	Day 3-5	Day 6-9
Ampicillin	≥ 32 R	≥ 32 R	≥ 32 R
Cefazolin	≥ 32 R	≥ 32 R	≥ 32 R
Ceftazidime	≤ 8 S	≥ 32 R	≤ 8 S
Ceftriaxone	≤ 8 S	≥ 64 R	≤ 8 S
Cefotaxime	≤ 8 S	≥ 64 R	≤ 8 S
Cefuroxime	16 MS	≥ 64 R	16 MS
Gentamicin	≤ 0.5 S	≤ 0.5 S	≤ 0.5 S
Tobramycin	≤ 0.5 S	≤ 0.5 S	≤ 0.5 S
Ticarcillin	≤ 16 S	≥ 256 R	≤ 16 S [†]
TMP-SMX	≤ 10 S	≤ 10 S	≤ 10 S
Amoxicillin, clavulanic acid	$\geq 16/8$	$\geq 16/8$	$\geq 16/8$

M Moderately sensitive; MIC Minimal inhibitory concentration ($\mu\text{g/mL}$); R Resistant; S Sensitive; TMP-SMX Trimethoprim-sulfamethoxazole. [†]Confirmed by the Sceptor Microdilution method and the agar dilution method for cefotaxime, ceftazidime and gentamicin. [†]On day 5 the MIC was 128 R

blood cell count of $45,900/\text{mm}^3$, red blood cell count $50,000/\text{mm}^3$, glucose less than 0.62 mmol/L and protein 1400 mg/L with Gram-negative bacilli on Gram stain. Culture of CSF and blood (both sets) grew *E cloacae*, urine was sterile, abdominal ultrasound was normal, and computed tomography (CT) scan of the head showed evidence of hydrocephalus and ventriculitis.

The following day the patient looked well, was afebrile, and the skin along the previous shunt tubing appeared normal and nontender. Cefotaxime was continued and gentamicin was added (7.5 mg/kg/day divided every 8 h). Vancomycin was discontinued. Daily CSF cultures were done (twice on day 7), which became sterile on day 10 of therapy. Blood culture was sterile when first repeated on day 5. CT scan of the head with contrast on day 6 and day 10 of therapy showed no change. In view of the resistant isolate (see below), therapy was changed to intravenous gentamicin and trimethoprim-sulfamethoxazole (TMP-SMX) (20 mg/kg/day divided every 6 h) on day 7 and continued until day 40. Intraventricular gentamicin (1 mg every 24 h) was given from day 12 to 16 (CSF gentamicin levels 3 h post-dose were 19.2 mg/L and 12 h post-dose were 14.0 mg/L). The CSF bactericidal titre on day 25 was 1:64.

The patient's recovery was uneventful apart from generalized seizures on day 6. In retrospect, it was found that on day 5 the intravenous line was out and therapy held for 13 h 20 mins until one dose of intramuscular ceftriaxone (50 mg/kg) was given. Cefotaxime was restarted intravenously 18 h after the last dose.

MICROBIOLOGY

The susceptibilities of the *E cloacae* isolates in CSF are shown in Table 1; the day 3, 4 and 5 isolates were resistant to second- and third-generation cephalosporins and ticarcillin,

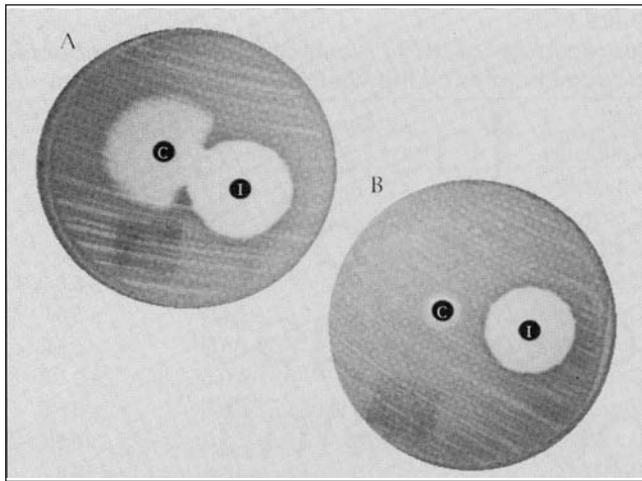


Figure 1) The inducible beta-lactamase disk approximation test. A Day 1, 2 and 6 to 9 isolates. Imipenem (I) induces beta-lactamase production and thus a zone of consequent ceftriaxone (C) resistance, shown as growth around the C disk only in closest proximity to the I disk. B Day 2 to 5 isolates. There is constitutive resistance to ceftriaxone shown as growth all around the C disk, regardless of any proximity to the I disk

whereas all other initial and later isolates were susceptible. Each day at least four colonies were 'picked' from the culture plate to use for sensitivity testing; however, from day 6 to 9 there were very few colonies on the culture plate to 'pick' (day 7: one colony; day 8: one colony; day 9: three colonies) for sensitivity testing.

The results of the inducible beta-lactamase disk approximation test are shown in Figure 1. As described by others (8), the ceftriaxone and imipenem disks were placed on the Mueller-Hinton agar such that they were separated by exactly one half the sum of the zone size around each disk when used alone. The day 3, 4 and 5 isolates had constitutive resistance to ceftriaxone, whereas all other isolates (including the initial blood cultures) had only inducible (with imipenem and cefoxitin) resistance. The nitrocefin assay was positive on all isolates. Clavulanic acid did not change the sensitivity to ampicillin (Table 1) and thus did not inhibit beta-lactamase. Disk-diffusion sensitivity testing showed sensitivity of all isolates to imipenem (diameter 25 mm) and resistance of all isolates to cefoxitin (diameter 6 mm).

Pulsed field gel electrophoresis: Genomic DNA in agar plugs from one blood isolate and the nine CSF isolates from day 2 to 9 were prepared essentially as described by Haertl and Bandlow (13). Restriction endonuclease digestion of genomic DNA was done with Xba I (New England Biolabs, Massachusetts) using a 4 mm slice of the agarose plug incubated with 20 units of enzyme in a total volume of 225 µL at 25°C overnight. Digested DNA was electrophoresed in a 1% agarose gel (Fastlane agarose, FMC Bioproducts, Maine) in 0.5 x TBE buffer (0.05 M Tris, 0.05 M boric acid, 1 mM EDTA) with the use of the contour-clamped homogeneous electric field apparatus (CHEF Mapper, BioRad, California). Lambda concatemers (New England Biolabs) were run as size controls. Electrophoresis was carried out for 27 h using ramped pulse-time beginning with 3 s and ending with 40 s at an applied voltage of 6

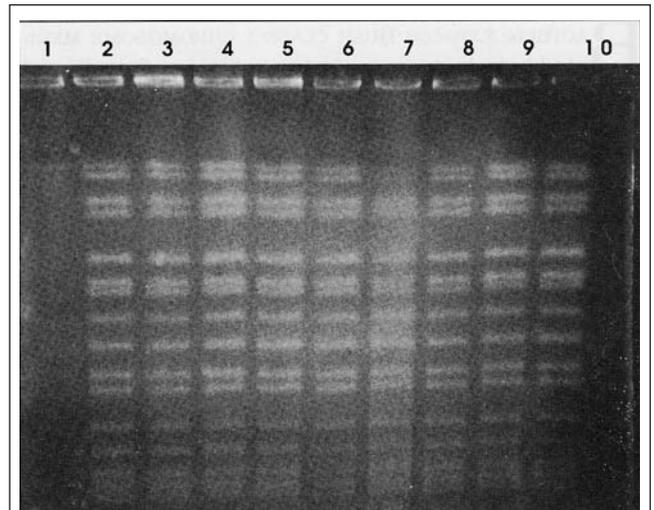


Figure 2) Xba I digested *Enterobacter cloacae* genomic DNA after pulsed field gel electrophoresis and ethidium bromide staining. Lane 1: blood isolate day 1; lanes 2 to 10: cerebrospinal fluid isolates from day 2 to 9. Identical banding is shown for all isolates, indicating a single clone

V/cm. The gels were stained with ethidium bromide and destained with distilled water for up to 12 h and photographed under ultraviolet irradiation. Identical banding patterns were found for all the isolates, with 17 bands ranging from approximately 50 to 700 kb size (Figure 2).

DISCUSSION

Selection of Gram-negative rods with constitutive Bush class 1 beta-lactamase production has resulted in emergence of resistance on therapy (with labile weak inducer antibiotics) in 14 to 56% of cases, with treatment failure or relapse in 25 to 75% of these (1). Emergence of resistance is thus recognized as a significant problem. The potentially causative bacteria accounted for 22% of all nosocomial isolates and 11% of all nosocomial bloodstream isolates in the United States in 1986-1989 (14). There have been reports of nosocomial outbreaks of resistance in a cystic fibrosis centre (15) and in neonatal intensive care units (5,11) shortly after introduction of broad spectrum cephalosporins. There are also increasing reports correlating use of broad spectrum cephalosporins to decreased susceptibility of *P aeruginosa*, *Enterobacter* species and *Citrobacter* species to ceftazidime, cefotaxime and piperacillin in the United States (16), in individual hospitals (17) and in individual patients (18). It has also been reported that cefotaxime use in a neonatal intensive care unit resulted in increased colonization with enterobacter strains and decreased susceptibility of these strains to cephalosporins (19). Re-emergence of sensitive strains of enterobacter has been shown hospital-wide with changes in antibiotic use (16,17).

Emergence of resistance while on therapy in neonates with *E cloacae* meningitis and ventriculitis has been recognized in the past (8,11). The present case adds to the number of documented cases of emergence of resistance while on

therapy with cefotaxime in neonates with serious infections due to *E cloacae*. It clearly demonstrates the rapidity with which the resistant phenotype can be selected as the predominant organism in vivo. This case is unusual in that the resistant phenotype was predominant for only three days.

The isolates from each day were tested by: the Vitek Auto-Microbic System (Vitek Systems, BioMerieux, Missouri); the Sceptor Microdilution method (Becton-Dickinson Diagnostic Instrument Systems, Maryland); and the agar dilution method for ceftazidime, cefotaxime and gentamicin (Dalynn Laboratory Products). These were repeated for all the CSF isolates. Failure to recognize resistance in the initial or later isolates is thus a very unlikely explanation for the transient nature of the resistant phenotype (20). It is also unlikely that from day 3 to 5 isolation of the resistant phenotype was a 'chance' in vitro finding. A chance occurrence of a resistant mutant is expected in about 1×10^{-6} colonies (1,2); thus, with four colonies 'picked' for sensitivity testing each day, the chance of detecting this for three days is: $(4 \text{ colonies}) \times (1 \times 10^{-6}) \times (3 \text{ days}) = 9 \times 10^{-6}$. Similarly, since from day 6 to 9 (for five separate cultures) virtually all colonies growing were 'picked' for sensitivity testing, it is reasonable to assume that the sensitive phenotype was truly predominant again.

There are very strong data to show that the mechanism of resistance in these isolates was Bush class 1 beta-lactamase. The nitrocefin test showed that beta-lactamase was produced. Clavulanic acid did not inhibit the enzyme (Table 1), ceftoxitin was a strong inducer of and susceptible to the enzyme, and imipenem was a strong inducer of and resistant to the enzyme (Figure 1). Thus, selection by cefotaxime of a stable derepressed mutant on day 3 is the most likely explanation for the emergence of resistance.

Another potential explanation for the resistant phenotype is that there were two different strains of *E cloacae* with different sensitivities. Although identical morphotypes and biotypes (on Vitek identification) for all isolates are not adequate to prove strain identity, pulsed field gel electrophoresis (PFGE) (Figure 2) does argue very strongly that the infection was caused by only one strain of *E cloacae*. PFGE using *Xba* I to digest genomic DNA has been used successfully to discriminate clearly genetic relatedness of *E cloacae* strains (13). Because of the *Xba* I low G-C rare recognition site TCTAGA, a PFGE gel with up to 20 bands was expected (21), providing a discriminating easy to read gel. This was indeed the case, and the identical banding pattern on PFGE for all the *E cloacae* isolates very strongly suggests a single clone (13). Therefore, the re-emergence of the sensitive phenotype on day 6 must be explained.

One potential explanation is that the lack of a selective advantage for the resistant mutant when one dose of cefotaxime was missed for 18 h on day 5 allowed re-emergence of the sensitive phenotype. However, the ceftriaxone dose given at 13 h 20 mins makes this explanation less likely; ceftriaxone (like cefotaxime) is quite labile to beta-lactamase and offers a selective advantage to resistant mutants. Perhaps a more likely explanation is that in vivo factors allowed some antibiotic sensitive organisms, established with the initial infec-

tion, to persist in a relatively antibiotic protected site and be cultured from day 6 to 9. Antibiotic protected sites such as in ventriculitis or shunt tubing would offer little advantage for resistant mutants, and would be expected to take longer to eradicate. Thus, the resistant mutant may have been eradicated with gentamicin by day 5, and then the combination of a missed cefotaxime dose on day 5 and in vivo protected sites contributed to the isolation of the sensitive phenotype from day 6 to 9. Finally, the change in therapy to TMP-SMX and gentamicin on day 7 may have contributed to the lack of re-emergence of a resistant phenotype a second time.

The present case was not an example of treatment failure. In similar patients without resistant strains CSF has also been culture-positive for 7.2 ± 5.0 days (22). Although cefotaxime for Gram-negative meningitis has not been evaluated in a prospective fashion, available data suggest its safety and efficacy to be very good (22-24). However, caution must be exercised in the use of third-generation cephalosporins to treat serious infections due to organisms such as *E cloacae* where emergence of resistance has been a problem; this is true both in individual patients and hospital-wide. In patients slow to respond, or in treatment failure, emergence of resistance must be looked for with repeat culture and sensitivity testing. Concomitant use of gentamicin has not been shown to reduce emergence of resistance (1) – in this case resistance to cephalosporins developed while on combined gentamicin and cefotaxime therapy.

CONCLUSIONS

We report a case of neonatal ventriculitis due to *E cloacae* that had rapid but transient emergence of a stable derepressed resistant mutant on therapy as a result of changing antibiotic selection pressures and in vivo factors. This is an excellent in vivo example of the mechanism of emerging resistance in Gram-negative rods that is becoming an increasing problem (25). We advise caution in the use of third-generation cephalosporins alone for these infections. Repeat culture and sensitivity testing should be done in severe infections with these bacteria that are slow to respond to cephalosporin therapy.

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