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Early Detection of Colonization by VIM-1-Producing *Klebsiella pneumoniae* and NDM-1-Producing *Escherichia coli* in Two Children Returning to France▽

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Rapid identification of metallo-β-lactamase-producing Gram-negative species is crucial for the timely implementation of infection control measures. We describe two pediatric cases in which colonization by VIM-1- and New Delhi metallo-beta-lactamase 1-producing *Enterobacteriaceae* was rapidly detected by phenotypic and genotypic methods. Phenotypic methods can be useful for routine detection of carbapenemase production.

CASE REPORTS

Case 1. In November 2010, we detected fecal carriage of health care-associated metallo-β-lactamase (MBL)-producing *Klebsiella pneumoniae* together with extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, *Enterobacter cloacae*, and *Citrobacter freundii* in a 4.5-month-old infant having traveled to France from Egypt.

The child was born in Egypt in July 2010. He was treated for an occlusive syndrome in Benha Children's Hospital, Benha, Egypt, and was discharged with a rectal drain at 10 days of age. He arrived in Paris, France, 2 months later, where Hirschsprung rectosigmoid disease was diagnosed. On admission to our institution, culture on ChromID ESBL medium (bioMérieux) was used to screen his stool flora for cefpodoxime resistance. MBL and ESBL producers exhibit high-level resistance to cephalosporins and are easily detected with this medium (16, 25). A carbapenem-nonsusceptible *K. pneumoniae* strain was recovered from his feces at a density of 10^2 CFU/g. After 2 days of cefotaxime prophylaxis (100 mg/kg/24 h) for abdominal surgery, the *K. pneumoniae* fecal count rose to 10^8 CFU/g. In addition, ESBL-producing *E. coli* (3 different strains), *E. cloacae*, and *C. freundii* were recovered from his stool at densities of 10^9 CFU/g, 10^8 CFU/g, 10^7 CFU/g, 10^9 CFU/g, and 10^6 CFU/g, respectively. He was discharged from the hospital 10 days later.

Case 2. In February 2011, we detected community-acquired fecal carriage of MBL-producing *E. coli* together with ESBL-producing *E. coli* in a 21-month-old boy having returned to France from India who was admitted to our institution for asthma exacerbation. He was born in France to parents originating from Sri Lanka.

Ten days prior to his admission, he had returned to France after a 1-month stay in India (Chennai), where no health problems or hospitalizations were reported. On admission, he had respiratory distress related to asthma but no other symptoms. Carbapenem-nonsusceptible *E. coli* and ESBL-producing *E. coli* were recovered on ChromID ESBL culture medium (bioMérieux) from an admission stool sample, both at a density of 10^8 CFU/g (16). He was discharged from the hospital after 7 days.

The carbapenem-nonsusceptible *K. pneumoniae* and *E. coli* isolates were resistant to all beta-lactams agents, with the exception of aztreonam for the *E. coli* isolate by the disk diffusion method. Both isolates were also resistant to aminoglycosides, quinolones, nitrofurantoin, and cotrimoxazole. They were susceptible to colistin and tigecycline, with respective MICs determined by Etest of 0.125 μg/ml and 1.5 μg/ml (*K. pneumoniae*) and 0.094 μg/ml and 0.094 μg/ml (*E. coli*), and also susceptible to fosfomycin (5, 6, 7). The MICs of ertapenem, meropenem, and imipenem were >32 μg/ml, >32 μg/ml, and 8 μg/ml, respectively, for the *K. pneumoniae* isolate and 1.5 μg/ml, 0.75 μg/ml, 0.75 μg/ml for the *E. coli* isolate (6). The modified Hodge test (MHT) (3) was strongly positive for carbapenemase production by the *K. pneumoniae* isolate and weakly positive for carbapenemase production by the *E. coli* isolate.

To identify the resistance mechanism(s), we used combined disk assays with meropenem (10 μg) as the substrate and distinct carbapenem inhibitors, i.e., dipicolinic acid (DPA) for MBL and boronic acid (BO) for *K. pneumoniae* carbapenemase (Rosco, Taastrup, Denmark), in conjunction with the Etest using imipenem/imipenem-EDTA strips (bioMérieux).

Synergy between EDTA and imipenem (Etest) and between meropenem and DPA was observed for the *K. pneumoniae*

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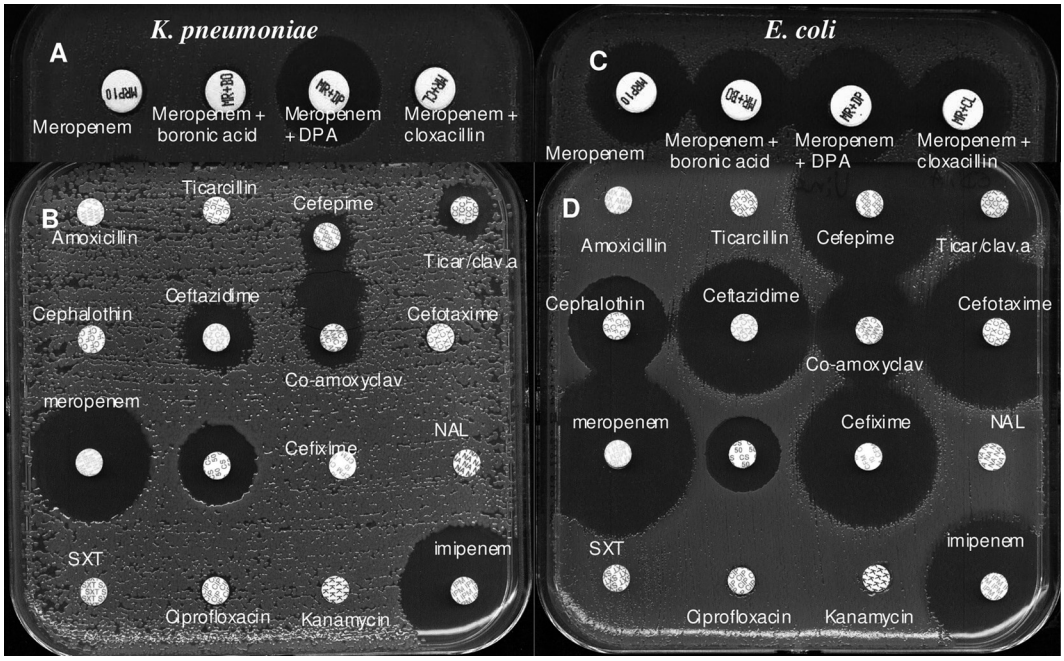

FIG. 1. *K. pneumoniae* is shown in panels A and B. *E. coli* is shown in panels C and D. (A and C) Phenotypic carbapenemase detection by the disk diffusion method using a combination of disks containing meropenem (10 μg), meropenem (10 μg) plus DPA, meropenem (10 μg) plus BO, and meropenem (10 μg) plus cloxacillin. (A) A zone size difference of ≥5 mm between meropenem and meropenem plus DPA points to MBL production. (C) A zone size difference of 3 mm between meropenem and meropenem plus DPA (positive if the difference is ≥5 mm). (B and D) Culture on Mueller-Hinton agar containing 5×10^{-3} M EDTA. (B) Inhibition of MBL by EDTA. Detection of an ESBL is shown. Synergy between the co-amoxiclav and cefepime disks points to ESBL production. (D) Inhibition of MBL by EDTA. Detection of plasmidic class A beta-lactamase 2b (TEM-1/-2, SHV-1) is shown. NAL, nalidixic acid; SXT, sulfamethoxazole-trimethoprim; Ticar/clav.a, ticarcillin-clavulanic acid.

isolate (zone size difference, ≥5 mm), suggesting MBL production (Fig. 1A) (8, 14, 19). In contrast, the synergy between meropenem and DPA (Fig. 1C) (zone size difference, <5 mm) and between imipenem and EDTA for the *E. coli* isolate was equivocal. However, ertapenem could be used instead of meropenem since ertapenem showed the least activity against the New Delhi MBL 1 (NDM-1)-producing *E. coli* isolate.

On Mueller-Hinton agar containing 5×10^{-3} M EDTA, MBL production by both strains was totally inhibited. Culture on the same medium revealed synergy between co-amoxiclav and cefepime for the *K. pneumoniae* isolate, suggesting ESBL production (Fig. 1B) and plasmidic class A beta-lactamase 2b (TEM-1/-2, SHV-1) production by the *E. coli* isolate (Fig. 1D).

PCR amplification and sequencing were used to identify the beta-lactamase genes. In case 1, the carbapenem-resistant *K. pneumoniae* carried $bla_{\text{VIM-1}}$ and $bla_{\text{CTX-M-14}}$. The three *E. coli* isolates harbored $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-1}}$; $bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M-15}}$; and $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$, respectively. The *C. freundii* isolate carried $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$, and the *E. cloacae* isolate carried $bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M-15}}$ (2, 21).

In case 2, the MBL-producing *E. coli* isolate harbored $bla_{\text{NDM-1}}$ and $bla_{\text{TEM-1}}$ and the ESBL-producing *E. coli* isolate carried $bla_{\text{CTX-M15}}$ and $bla_{\text{OXA-1}}$.

Phylogenetic analysis using a triplex PCR method targeting *chuA*, *yjaA*, and the TspE4 DNA fragment showed that the NDM-1-producing *E. coli* isolate belonged to commensal, less virulent group A (4). The strain was tested for virulence factor genes, revealing the presence of *fuyA*, *papC*, *aer*, and *papGIII* (1).

To our knowledge, this is the first case of imported health care-associated fecal carriage of an Egyptian VIM-1-producing *K. pneumoniae* strain in France, the first isolation of a community-acquired *E. coli* strain harboring the NDM-1 gene from a child in France, and the sixth reported isolation of NDM-1-producing bacteria in France (10, 17, 20, 23).

Discussion. The emergence of MBL-producing bacteria in children is a matter of serious concern because it severely limits treatment options. The most frequent MBLs reported to date belong to the VIM and IMP types, which have been reported extensively worldwide (18). NDM-1, a novel acquired MBL, has recently started to spread (11). The genes that encode these enzymes are a source of concern, as they usually are carried by mobile genetic elements with a high capacity for horizontal dissemination (11, 22). In contrast, carbapenem resistance due to a combination of ESBL or AmpC production and porin loss has a fitness cost and spreads slowly. It is therefore important to determine the mechanism of carbapenem resistance. The MHT has been used to detect carbapenemase producers (3). In contrast to the VIM-1-producing *K. pneumoniae* isolate, the MHT showed only weak production of the NDM-1 carbapenemase by the *E. coli* isolate. However, weak or negative MHT results for NDM-1 have already been re-

ported (15). Molecular testing is recommended to characterize the MBL genes of *Enterobacteriaceae* (16), but PCR is not universally available. Phenotypic methods such as those used in our study can efficiently detect the resistance mechanism.

Invasive infections by carbapenem-resistant strains are associated with high morbidity and mortality rates (24). Early identification of colonized patients on hospital admission is crucial for timely implementation of infection control measures and for rapid adaptation of antimicrobial chemotherapy in case of infection. Indeed, delayed detection of carbapenem-resistant organisms may lead to outbreaks of colonization or infection (12, 13). The French health authorities recommend the screening of all patients hospitalized abroad for carriage of multiresistant bacteria on the day of their admission to any French hospital (9). In our pediatric hospital, all patients arriving from countries where infections with these bacteria are endemic, whether or not they were hospitalized abroad, are screened on admission. Further cases of colonization or infection by the carbapenemase-producing bacteria described here were prevented by reinforcing infection control procedures and by screening all of the patients on the affected ward by rectal swabbing.

In conclusion, infections by carbapenem-resistant bacteria are difficult to treat and rapid identification of MBL-producing Gram-negative species is crucial both for appropriate treatment and for timely implementation of infection control measures. Phenotypic methods can be useful for routine detection of carbapenemase production, particularly when PCR is not immediately available.

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