Updating Antimicrobial Susceptibility Testing Challenging Cases

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LEARNING OBJECTIVES:

1. Consider the effect of *Acinetobacter baumannii* on the underlying comorbidities of the 2 patients.
2. Define and discuss necrotizing fasciitis and its treatment.
3. Summarize the events leading to the transmission of *Escherichia coli* ST131.
4. Discuss the effect of treatment with a fluoroquinolone and the patient outcome.

ABBREVIATIONS: MDR-multi-drug resistant; HAI-hospital-associated infection; CAI-community-associated infection; WBC-white blood cell; VRE-vancomycin-resistant *Enterococcus* species; MRSA-methicillin-resistant *Staphylococcus aureus*; PCR-polymerase chain reaction; UTI-urinary tract infection; GNB-gram-negative bacilli; MIC-minimal inhibitory concentration; ICU-intensive care unit; ESBL-extended-spectrum beta-lactamase enzyme; MLST-multi-locus sequence typing; PFGE-pulse-field gel electrophoresis

INDEX TERMS: *Acinetobacter baumannii*, *Escherichia coli*

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A series of cases is presented to demonstrate the tenacity of organisms known for acquired resistance through mechanisms of mutation or selection as a result of antibiotic exposure. The cases describe the devastating effects and frightening speed with which multi-drug resistant (MDR) organisms may overwhelm a patient by increasing morbidity and mortality. Also apparent is the frustration and powerlessness felt by physicians who expeditiously attempt to diagnose and treat patients at risk for hospital-associated infection (HAI) and community-associated infection (CAI).

Acinetobacter baumannii, 2 cases of necrotizing fasciitis

In 2007 and 2008, two unusual cases of necrotizing fasciitis were seen at a regional medical center. These cases were notable for necrosis of leg muscle and intercostal tissue caused by *A. baumannii*. Etiologic agents commonly associated with this type of infection are streptococci mixed with anaerobic organisms.[1]

The first patient was a 21 year old male with medical problems including end-stage renal disease, systemic lupus erythematosus, thrombotic thrombocytopenic purpura and mesenteric vasculitis. Treatment with corticosteroids, intermittent rituximab, plasmapheresis, blood transfusions and hemodialysis preceded his admission to the hospital for pulmonary edema and rectal passage of bright red blood. His blood cultures grew vancomycin-resistant *Enterococcus faecium* (VRE) and *Candida albicans*, which were treated with daptomycin and fluconazole. Although subsequent blood cultures were negative, his stool tested positive for *Clostridium difficile*-associated colitis requiring the addition of metronidazole to his regimen.

Gastrointestinal bleeding continued 3 weeks after his admission, prompting performance of an exploratory laparotomy. The surgical findings mandated an ileocecal resection and ileostomy. Although he appeared to be recovering 5 days post-surgery, blood culture reports showed he had become bacteremic with *Klebsiella pneumoniae* and *Citrobacter freundii*. Further treatment with imipenem followed.

Two weeks later, the patient experienced pain along the left flank and thigh where physical exam revealed

erythema and tenderness. Within 9 hours, the symptoms escalated dramatically. Erythema was noted with a line of infection progressing > 2 cm/hour beyond the originally marked area. The new area was extensive - from left to right flank, across his back to both thighs - and included bullae. The appearance of necrotic fibroconnective tissue and muscle prompted physicians to immediately perform an incision and drainage with frozen sections collected at bedside.

When the laboratory reported growth of >100,000 GNB from quantitative biopsy specimens, emergency surgery for debridement was performed. Pending culture and susceptibility testing reports, empiric treatment with amikacin, vancomycin and clindamycin were added to the regimen. Surgical debridement was far more extensive than the physical exam had predicted. As much as 40% of the patient's body surface area required removal of necrotic tissue, which appeared along the anterior abdominal wall, both flanks, cephalad to scapulae and down to both knees. Appearance of GNB on Gram stains mandated debridement of the perineum, lower extremities, deep intercostal muscles and the entire abdominal wall.

Multiple units of blood and vasoactive agents to preserve hemodynamic stability were given. However, no further surgery was considered and the patient died when he returned to the ICU 36 hours after first noting pain in his flank. The organism cultured from his blood was resistant to all antimicrobial agents tested (Table 1).

A second patient, who experienced a similar case scenario, was a 47 year old woman with a history of HIV infection and end-stage renal disease, whose previous exploratory laparotomy for ovarian torsion was complicated by the lysis of succeeding adhesions. She left the hospital prior to approval, was readmitted a day

Table 1. Antimicrobial Susceptibility Results for MIC† and S/I/R**

Acinetobacter baumannii Case 1 2 patients (blood and wounds)			**Escherichia coli Case 2 2 patients, younger sister only (blood and urine)**		
Antibiotic	MIC†	S/I/R**	Antibiotic	MIC†	S/I/R**
Amikacin	>32	R	Amikacin	<16	S
			Aztreonam	16	R
			Cefotaxime	>32	R
			Ceftriaxone	>32	R
Ceftazidime	>16	R	Ceftazidime	<8	R*
Gentamicin	>8	R			
TMP-SMX[1]	>2/38	R	TMP-SMX[1]	<2/38	S
Imipenem	>2	R	Imipenem	<4	S
Ciprofloxacin	>2	R	Ciprofloxacin	>2	I-R
Pip/Tazo[2]	>64/4	R	Pip/Tazo	<16	S
Tic/Clav[3]	>64/4	R			
Meropenem*	>8	R			
Levofloxacin	>4	R			
Cefipime	≥16	R	Cefipime	>16	I-R
Tigecycline[4]	1/0.5	NA[5]			
			Tobramycin	>8	I-R
Colistin[6]	≤2	S			
Ampicillin-sulbactam	NA	R/S[7]			

† MIC—minimal inhibitory concentration in µg/ml
** S/I/R—susceptible, intermediate or resistant
[1] TMP-SMX Trimethoprim-sulfamethoxazole
[2] Pip/Tazo Piperacillin-Tazobactam
[3] Tic/Clav Ticarcillin-Clavulanate
* CLSI 2010 interpretation
[4] CLSI breakpoints not available
[5] NA--not available
[6] Patient # 2 only
[7] Patient #2, wound & abdominal fluid only

later, and diagnosed with cellulitis of her surgical wound. Wound culture reports of coagulase-negative staphylococci prompted empiric treatment with amoxicillin-clavulanate. Unfortunately, susceptibility testing proved the organism was a methicillin-resistant *Staphylococcus* sp. and she went on to develop a pelvic abscess a week later.

After a percutaneous abdominal drain was inserted in the abdomen, she was treated again empirically, this time with piperacillin-tazobactam, vancomycin and clindamycinin in an attempt to treat both gram-negative and gram-positive pathogens. Amikacin was added to the regimen when she did not respond. Because the laboratory was unable to identify the organism, standard method susceptibility test results were delayed while the blood culture isolate was submitted to a reference laboratory for DNA sequencing. In the interim, the patient had a resection of the abscess via another exploratory laparotomy after which she developed blisters on her right thigh. Biopsied specimens showed the presence of GNB in the dermal layer and underlying fat tissue.

Following her blood culture report indicating growth of *Acinetobacter baumannii* susceptible only to ampicillin-sulbactam, another surgical procedure was required to remove the necrosis in the right thigh and buttock. Matching the blood culture report, *A. baumannii* susceptible only to ampicillin-sulbactam, was the only organism isolated from the many cultures of debrided tissue. Subsequent specimens from the blood and Jackson-Pratt drain grew *Pseudomonas aeruginosa*. Similar to the first patient, however, only *A. baumannii* was isolated from the wound cultures. Although colistin was added to her extensive regimen, she did not improve and died of septic shock 18 days after the appearance of the blisters.[1]

Escherichia coli, strain ST131

The case of two sisters with alpha-1-anti-trypsin deficiency is presented as the last of the challenging cases. The older sister had a history of chronic UTI caused by *Escherichia coli.* Additionally, the older sister's urinary isolates had increased in antimicrobial resistance until a recent isolate, a MDR GNB tested positive for an extended spectrum beta-lactamase (ESBL) enzyme. This patient had been caring for her younger sister in her home for several months because of the severity of the sister's advancing alpha-1-anti-trypsin deficiency disease.

Soon after entering her older sister's home, the younger sister developed symptoms of UTI with low grade fever. As the symptoms progressed, she was admitted to a hospital with diagnosis of presumptive pyelonephritis. Her physical findings included right-side flank pain, temperature 37.6ºC, pulse 52 bpm, blood pressure 153/82 mm Hg and respirations of 24 per minute. Her oxyhemoglobin saturation was 92% on 4 liters of oxygen. The laboratory report indicated signs of infection including an elevated WBC of 17,800 cells/µL (normal range: 4,500-10,000 cells/ µL) with 88% neutrophils. A urinalysis defined marked pyuria and bacteriura. Although she was treated empirically with ciprofloxacin of 400 mg intravenously every 12 hours and a stress-dose of corticosteroids, she soon progressed to septic shock and pyelonephritis, requiring transfer to the ICU. Her WBC count increased to 18,400 cells/µL with 29% band forms. The serum creatinine level increased to 2.7 mg/dL (normal range: 0.5-1.4 mg/dL). Mechanical ventilation and vasopressor therapy commenced, followed by placement of a right percutaneous nephrostomy tube via a fistula into the renal pelvis.

Cultures of both blood and urine at this time grew *E. coli* leading physicians to treat empirically again with piperacillin-tazobactam based on the hospital's antibiogram. The patient continued to fail as the septic shock worsened, requiring vasopressor support for hemodynamic stability. The susceptibility report confirmed the isolate was resistant to fluoroquinolones and ESBL cephalosporins, but susceptible to piperacillin-tazobactam, amikacin, carbapenems and trimethoprim-sulfamethoxazole. Despite a change of therapy to meropenem, the patient died within a few hours.

Results of the isolates from both sisters (urine from the older, blood and urine from the younger) had identical susceptibility profiles and their multi-locus sequence typing (MLST) gave allelic profiles consistent with *E. coli* ST131 and 99% similar pulse-field gel electrophoresis (PFGE) profiles. Because the younger sister had no recent exposure to antibiotic treatment, physicians assumed she had contracted the organism while living with her older sister. Both isolates exhibited

bla$_{CTX-M-15}$, which encodes the ST131 strain associated CTX-M-15 ESBL variant. *E. coli* ST131 strains are known for both morbidity and mortality and are community-acquired.

Fluoroquinolone therapy was recommended according to standardized guidelines. However, the global susceptibility of *E. coli* to these drugs has dramatically declined. As would be expected from excessive antibiotic usage and clonal spread, virulent strains of ESBL-producing *E. coli* exist as CAI and HAI. This case is presumed to be the first report of a fatality resulting from within-household transmission, i.e. from sister to sister.

The failed effect on two occasions of empiric treatment (ciprofloxacin followed by piperacillin-tazobactam), adding to the delay in susceptibility reports from the laboratory, was attributed to the demise of this patient. Pleas from physicians stated the desperate need for rapid molecular methods to detect MDR pathogens or resistance elements, specifically targeting infection from the beginning.[2]

An additional case study that documents the challenges of a MDR GNB may be accessed at: www.medscape.org /viewarticle/718328/

The case studies presented here were used with permission from the *Journal of Clinical Microbiology*.

CONCLUSION

The two organisms presented here, *Acinetobacter baumannii,* and *Escherichia coli,* have evolved in antimicrobial drug resistance as causes of both HAI and CAI from relative susceptibility to multi-drug resistance, leading to high rates of morbidity and mortality in many cases. In the final article, Present and Future Relevance, questions addressing the effect (if any) of early intervention with molecular detection assays versus AST are posed for consideration. The next article in the series, Methods, is a discussion of antimicrobial agents and breakpoint determination as well as an overview of phenotypic (qualitative and quantitative procedures) and genotypic methods of determining antimicrobial susceptibility.

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