Clinical Application and Interpretation of Antimicrobial Susceptibility Testing

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Deaths cloud Illinois firm's skydive record

Critics question safety, location

By Maurice Possley and Jon Hilkevitch
Tribune staff reporters

OTTAWA, Ill.—As the sport's top official looked on, three skydivers under high-performance parachutes lined up their approach to a tiny swimming pond late last Sunday afternoon at Skydive Chicago.

They were "pond swooping," a crowd-pleasing stunt where a skydiver skims across the water, much like a water-skier, and then walks ashore. There was little room for error, with a dock jutting into the water on one side, a swimming platform in the center and trees all around.

The first two made it, but Ronald Passmore Jr., 33, a skydiver with more than 1,300 jumps, was in trouble. He made a dangerous low turn, slammed the water chest first and was killed.

Passmore's death, the sixth at Skydive Chicago in the past 19 months—eight times higher than the national average—suggested an atmosphere that fails to adequately emphasize safety. Since 1993, 13 skydiving deaths have occurred at Skydive Chicago, which is run by Roger Nelson.

At the same time, skydivers, pilots and air traffic controllers report that skydiving operations in the busy flight route into O'Hare International and Midway Airports have caused near misses between commercial jets and the jump planes disgorging divers into dense cloud banks.

"Skydiving in Chicago is an accident waiting to happen," one pilot declared in a report to federal authorities of a near miss in 2000. After Passmore's death, Nelson said he banned "pond swooping" at the busy drop zone here. And Chris Needels, the executive director of United States Parachute Association, who witnessed the accident, said it...
Case series of 6 patients with pandrug-resistant Gram-negatives
- *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*
- Resistant to:
  - Antipseudomonal penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides, polymyxins
- 4 of 6 patients treated successfully with colistin and beta lactams
Review

Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era?

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IDSA Position Paper on New Antimicrobial Development

• “Bad bugs, no drugs” (2004)
  – Resistance is rising
  – The antibiotic pipeline is drying up
  – Federal policy and action are needed
  – Recommendations for Congress, FDA, NIAID

• “Without innovative public policy and additional financial support, fewer and fewer antibiotics will be available to treat the increasing number of drug-resistant and dangerous microbes that threaten Americans and the global community.”
IDSA Position Paper on New Antimicrobial Development

• There are 33 recommendations in the report
• There has been no commitment, response, or acknowledgement of the recommendations by the pharmaceutical industry, the FDA, or Congress
New Resistance Challenges

- Community-acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA)
- Decreased vancomycin susceptibility in Staphylococcus aureus
- Extended-spectrum beta-lactamase (ESBL) in enteric Gram negatives
- Klebsiella pneumoniae carbapenemases (KPC)
- Beta-lactam agent resistance in Streptococcus pneumoniae
Community-Acquired Methicillin-Resistant *Staphylococcus aureus*

- A new strain that has emerged in England, Europe and the US
- Causes a distinctive skin lesion and a necrotizing pneumonia
- Has a unique antibiogram; resistant only to beta-lactams
- Abscesses require drainage
- Skin lesions can often be treated outpatient using sulfamethoxazole or doxycycline
Physiology and Resistance Mechanisms of S. aureus

- **S. aureus** is an adaptive and successful human pathogen, with the ability to elaborate a range of virulence factors and toxins

- Resistance to methicillin first appeared in 1961, attributed to inheritance of a *mecA* gene found on the mobile staphylococcal cassette chromosome *mec* (SCCmec)

- Genetic analysis suggests that *mecA* has been transferred to *S aureus* over 20 times, resulting in 5 major lineages

- Transfer of the *mecA* gene into *S aureus* strains already well adapted to survival in hospital or community setting has given rise to 2 major MRSA categories:
  - Health Care–Associated MRSA
  - Community–Acquired MRSA

Community–Acquired MRSA

In contrast to the rise in nosocomial MRSA from 1990 to the present (attributed to traditional risk factors in health care facilities), growing awareness of community-acquired MRSA has occurred through published reports of MRSA outbreaks over the past 2 decades, including skin and pneumonia outbreaks for which traditional risk factors were not identified.

Outbreak in Detroit, Mich
- 2/3 of patients were IVDU

Children
- w/o identifiable risk factors

1980

Mid 1990s

Late 1990s

2000

Prison and jail populations

Necrotizing pneumonia, United States and Europe

1998 - Athletes/sports teams
1999 - Native Americans

1998 - Athletes/sports teams
1999 - Native Americans

IVDU=intravenous drug users.


Gilette et al.
Decreased Vancomycin Susceptibility in *Staphylococcus aureus*

- Vancomycin is still the standard therapy for serious methicillin-resistant *S. aureus* infections
- Most wild-type *S. aureus* strains used to show vancomycin MICs of 0.25 or 0.5 ug/ml
- MICs are now 1 or 1.5 ug/ml by E-test
- We now have seen mutant *S. aureus* strains with intermediate and resistant MICs (VISA, VRSA)
  - Vancomycin resistance tends to involve highly altered cell wall structure and/or synthetic enzymes
Glycopeptide-Resistance Transposons

• In *E. faecium* and *E. faecalis*, vanA and related genes are located on a transposon (Tn1546) which resides on a plasmid
• Resistance is associated with a number of genes (vanHAX gene cluster, vanS, vanR, vanX and vanZ) on the transposon
• These transposons have been transmitted to *S. aureus* creating VRSA
Decreased Vancomycin Susceptibility in *Staphylococcus aureus*

- Of more concern is that the entire population of *S. aureus* has higher MICs to vancomycin.
  - Tested 6002 isolates collected between 2000 and 2004
  - Mean MICs increased since 2001
  - 70% of *S. aureus* now have an MIC of 1.0
  - MICs of 2 are still very rare, but for how long?
  - More common in MSSA than MRSA
Decreased Vancomycin Susceptibility in *Staphylococcus aureus*

- Two studies have suggested that vancomycin success against *S. aureus* infections decreases when the MIC is 1 or 2; increasing vancomycin dose causes more toxicity
- Should we consider a vancomycin MIC of 1 to be resistant?
- What is the alternative antimicrobial agent to vancomycin for therapy of MRSA infection?
Treatment Options – 2008

• Currently Available
  – Linezolid (Zyvox)
  – Quinupristin/dalfopristin (Synercid)
  – Daptomycin (Cidecin)
    • a cyclic lipopeptide, cell membrane damage
  – Tigecycline
    • A glycyclcycline, protein inhibition

• In the Pipeline
  – Ramoplanin (a lipoglycodepsipeptide)
    • has toxicity; may be developed for topical use
Extended-Spectrum Beta-Lactamases (ESBLs)

- ESBLs are a class of beta-lactamases in Gram-negative bacilli that inactivate the more advanced penicillins and cephalosporins
- They are hard to detect because the MIC is not always at the resistant level
- They are clinically significant because they cause treatment failures
- Physicians need to know if they are suspected so they can use alternative therapy, usually carbapenems
Extended-Spectrum Beta-Lactamases (ESBLs)

- US labs have been detecting ESBLs in *E. coli, Klebsiella, and Proteus mirabilis* now for several years
- The dilemma is that ESBLs are also found in other members of the *Enterobacteriaceae*
- Some commercial susceptibility systems flag potential producers
- CLSI has not yet recommended screening or confirming of other species, but many experts are recommending it
- Cefpodoxime is very susceptible to ESBLs
Testing for ESBLs

Amoxicillin/Clavulonic acid

Ceftazadime

Ceftazadime

Clavulanate

Reduction $\geq$ 3 dilutions
Treatment of ESBL Infections

• Most organisms with ESBLs are often multidrug resistant
• Carbapenem frequently used as first line therapy in ESBL infections
• Carbapenem resistance does occur
  – AmpC expression and loss of outer membrane proteins associated with resistance in *K. pneumoniae*
  – *K. pneumoniae* carbapenemases (New York)
• Tigecycline and Polymyxins are last active agents available
  – Polymyxin resistance has been reported
**Klebsiella pneumoniae**

**Carbapenemase (KPC)**

- Carbapenemases are the most versatile of all beta-lactamases; they break the beta-lactam bond of the carbapenems, which is hard to do

- Two classes
  - Metallo-carbapenemases
    - Contain a zinc atom, removed by EDTA
    - In Gram positives, Pseudomonas and Acinetobacter
  - Serine carbapenemases, Class A, includes KPC
    - Contain a serine at the active site
    - In *Enterobacter*, *Serratia* and *Klebsiella*

- KPCs inactivate all beta-lactam antibiotics
- Activity is ertapenem<meropenem<imipenem<doripenem
The Next Step in Resistance–Pan-Resistant *K. pneumoniae*

- **KPC β-lactamase (*K. pneumoniae* carbapenemase)**
  - Confers resistance to all β-lactams!
    - R to all penicillins, cephalosporins, monobactams, inhibitor combo drugs, carbapenems
    - Inhibited by clavulanic acid (but not consistently)
  - Bush group 2f/Class A
  - Serine carbapenemase
  - Plasmid-mediated
    - Plasmid usually contains other resistance mechanisms as well (fluoroquinolones, trimethoprim/sulfamethoxazole)
KPCs: The Potential for Global Dissemination is Significant

- Several outbreaks in New York were followed by rapid spread in Eastern USA (KPC-2 and KPC-3)
  - First reported N. Carolina 2001. Since, reported from 20 other states, and Europe, Asia, S. America
  - First report in an institution requires immediate attention by infection control
  - High all-cause attributable mortality (up to 47%)
  - Therapy = polymyxin B and/or tigecycline (possibly add an aminoglycoside). Labs should be prepared to test these agents
KPC Producers

• Members of family *Enterobacteriaceae*
  – *Klebsiella pneumoniae* (most common)
  – *K. oxytoca*
  – *E. coli*
  – *Enterobacter* spp.
  – *Citrobacter freundii*
  – *Salmonella* spp.
  – *Serratia* spp.

• *Pseudomonas aeruginosa*
Laboratory Problems

• Automated susceptibility testing systems may not detect imipenem or meropenem resistant isolates (Some are better than others)
  – Imipenem and meropenem MICs may be in susceptible range
  – Ertapenem is most sensitive indicator of KPC (but least specific)
  – Ertapenem is not on all routine testing panels/batteries

• ESBL testing may be positive
  – If KPC not detected, carbapenems reported as susceptible
  – Profound impact for patient therapy
• Note: new carbapenem, doripenem, is also inactivated by KPCs
Modified Hodge Test

1. Swab *E. coli* ATCC 25922 onto plate to create lawn (1:10 dilution of McF 0.5).

2. Place imipenem disk in center.

3. Streak test isolates from edge of disk to end of plate.

4. Incubate overnight.

5. Look for growth of *E. coli* around test isolate streak - indicates carbapenem-hydrolyzing enzyme.

Slide courtesy of Janet Hindler and Jean Patel
Both Types of Carbapenemases are Detected by the Modified Hodge Test

- Metallo-beta-lactamases
  - Class B beta-lactamases
  - Problematic in *P. aeruginosa* and *Acinetobacter*
  - Uncommon in North America
- Serine carbapenemases
  - Class A beta-lactamases
  - Sporadic in *Enterobacter* (IMI & NMC) and *Serratia* (SME)
  - Cause of current epidemic of KPC, primarily in *Klebsiella pneumoniae*
- If the Hodge test is negative, what is most likely alternative mechanism of resistance to carbapenems (in USA)?
  - Combination of ESBL or AmpC + porin loss
The Emergence of Resistance to *Streptococcus pneumoniae*

- Same exposure to penicillin as *S. aureus*
- Yet, there was 25 years without resistance
- In the last 15 years: rapid emergence of penicillin resistance followed by multi-drug resistance has occurred
- The mechanism of penicillin resistance is transformation of DNA from oral streptococci
- 2008: approximately 45% of isolates are penicillin non-susceptible
### PENICILLIN-BINDING PROTEINS OF S pneumoniae

<table>
<thead>
<tr>
<th>PBP</th>
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<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>Alteration confers resistance to penicillins.</td>
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<tr>
<td></td>
<td>b</td>
<td>Not involved in penicillin resistance.</td>
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<tr>
<td>2</td>
<td></td>
<td>Alteration confers resistance to cephalosporins and penicillins. This is the primary resistance determinant.</td>
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<tr>
<td></td>
<td>a</td>
<td>Alteration confers resistance to penicillins.</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>Alteration confers resistance to penicillins such as piperacillin. Cephalosporins do not bind.</td>
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<tr>
<td>3</td>
<td></td>
<td>Only involved with penicillin resistance in the laboratory setting.</td>
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The Strain From Spain

- Iceland had no reports of resistant S. pneumoniae until 1988
- By 1992, 17% of all isolates were multi-drug resistant
- 70% of the isolates were serotype 6B
- This was one of the resistant strains that was common in Spain
- Why and how did it show up in Iceland?
The Strain From Spain

- Icelandic families vacation in Spain
- Icelandic children probably picked up the strains from Spanish children
- High usage of tetracycline and SXT probably contributed to selection pressure
- The majority of resistant strains were from around Reykjavik which houses 57% of the total population of 250,000
- 80% of children, ages 2-6 attend day care centers
Penicillin Resistance with *Streptococcus pneumoniae* in the United States

![Bar chart showing the percent of resistant and intermediate strains from 1979-2003.](chart.png)
Multi-Drug Resistant
*Streptococcus pneumoniae*

Percentage

Year

Fluoroquinolone Prescriptions and the Emergence of Pneumococcal Resistance

Resistance to Levofloxacin and Failure of Treatment of Pneumococcal Pneumonia

- 4 patients in Canada
- Empirical therapy
- In 2 patients the organism acquired resistance during therapy
- In 2 patients the original isolate was resistant

Davidson et al. NEJM.346: 747-750.2002
Emergence of Fluoroquinolone Resistance Among Multi-Drug Resistant Strains of *Streptococcus pneumoniae* in Hong Kong
Conclusions

• Susceptibility testing continues to present new challenges as new resistance mechanisms are described
• Some of these emerging new mechanisms of resistance are not easy to be detected using older test methods
• Very few new antimicrobial agents are being developed
• Physicians need to keep current on these issues so that they can treat their patients effectively as well as so they can communicate with the clinical microbiology laboratory
• The clinical microbiology laboratory continues to attempt to provide accurate and useful susceptibility test results on microorganisms that are constantly changing